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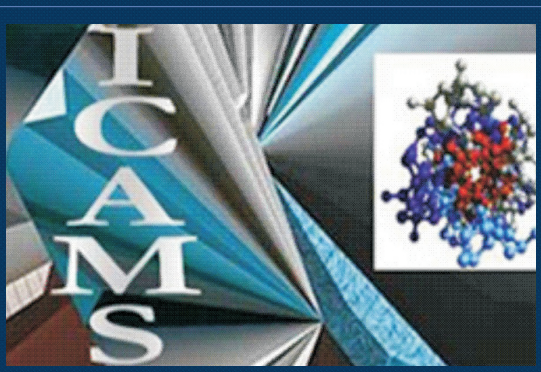

ICAMS 2014

ADVANCED MATERIALS AND SYSTEMS

Proceedings of the 5th International Conference

October 23rd - 25th, 2014

Bucharest, ROMANIA

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Proceedings of

THE 5th INTERNATIONAL CONFERENCE ON ADVANCED MATERIALS AND SYSTEMS

Bucharest, ROMANIA
October 23rd – 25th, 2014
INCDTP - ICPI, ROMANIA

FOREWORD

ICAMS 2014 offers the framework for presenting the latest results in research, focusing on a field of Materials science, which records an impressive dynamics and is recognized as a current national and European priority.
The conference will provide the opportunity for exchanging ideas and experience with researchers, scientists and experts at international level, and for developing new scientific contributions.
The conference topics include, but are not limited to:

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   - *New research opportunities and challenges*
7. **Quality Management and Competitiveness**
   - *National / International Standards*
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   - *Policies and Procedures (on a global, national, regional scale)*

We would like to thank all the participants, the International Scientific Committee, and all the sponsors that made possible this scientific event.
We hope that the Conference will become a tradition in the future, contributing to the advancement of Materials science in academic, social and business environments worldwide.

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CONTENTS

III. SYSTEMS AND TECHNOLOGIES

PLENARY LECTURE

NEW FIBRIN-POLYMER INTERPENETRATING NETWORKS: A POTENTIAL SUPPORT FOR HUMAN SKIN CONSTRUCT

VERONIQUE LARRETA-GARDE[1], MARIE DENEUFCHATEL[1,2], LAURENT BIDAULT[1,2], MATHILDE HINDIE[1], CEDRIC VANCAEYZEELE[2], ODILE FICHET[2]

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Interpenetrating polymer network (IPN) architectures have been conceived to improve the mechanical properties of a fibrin gel. Self-supported biomaterials are synthesized rapidly (one pot – one shot process) and combine the properties of both a protein gel and a synthetic polymer. IPN architectures have been characterized with biochemical (ELISA), chemical (solvent extraction) and physicochemical (rheology, DMA) methods. Mechanical properties of a fibrin gel were improved (viscoelasticity x 100) by associating it with a polymer network (PEO, PVA) inside IPN architecture. The network composition insures the material biodegradability through enzyme hydrolysis. These co-network IPNs are the first ones to be potentially biodegradable through tunable fragmentation, then elimination. They also exhibit the unique feature for a protein-based biomaterial of being non-retractable when used as support for fibroblast culture. The material is biocompatible as demonstrated with human dermal fibroblasts. Adhesion, viability and proliferation of human dermal fibroblasts have been measured for various IPN compositions with Live/dead test and by confocal microscopy. This innovative biomaterials present good potentiality as supports for skin construct.

Keywords: IPN, tissue engineering, biodegradability

INTRODUCTION

Among the large field of soft biomaterials, hydrogels (Slaughter *et al*., 2009) occupy a major position as they are usually biocompatible and occasionally biodegradable. Hydrogels may be synthesized from natural or synthetic compounds or from a mixture of them. Hydrogels made from biomolecules have the advantage to mimic the physiological microenvironment of tissues and to be enzyme-responsive (Khetani and Bhatia, 2006). Fibrin hydrogels show promising biological properties for clinical applications in tissue engineering and damaged tissue regeneration (Anitua *et al.*, 2006; Shevchenko *et al.*, 2010); however, they are very soft and not easily handled when they are synthesized at the physiological concentration (Ahmed *et al.,* 2008).

To confer it good mechanical properties a fibrin network was entrapped inside interpenetrating polymer networks (IPN) architecture. IPN is defined as a combination of two polymer networks that are cross-linked in the presence of the other. This co-network was synthesized by copolymerization of serum albumin (SA) - a protein conferring the network biodegradability - and PVA (polyvinyl alcohol) – a synthetic polymer giving the network rigidity - both modified with methacrylate functions. PVA-SA co-network were then associated with fibrin gel inside IPNs through a one pot – one shot process. Their mechanical properties were evaluated by rheological measurements. Their biodegradability through enzyme hydrolysis was followed and the hydrolysis rate related to the protein content. Finally, fibroblast viability and growth on the different IPN surfaces were examined to demonstrate their biocompatibility.

EXPERIMENTAL PROCEDURES

IPNs were synthesized as follows: PVA and/or SA (total concentration 100 mg), both bearing methacrylate groups were mixed with 5 mg fibrinogen and 0.042% (w/v) Irgacure 2959 all dissolved in 1 mL Tris buffer 50mM at pH 7.4 containing $CaCl_2$ and NaCl, then 0.20 unit thrombin were added. Gelation study was performed at 37°C in a rheometer equipped with U.V source (4.46 mW/cm²) using a cone-plate geometry. Mechanical properties were analyzed with 1% imposed deformation at 1 Hz.

Cell behavior was followed using Human fibroblast from foreskin (FB-BJ, ATCC CRL 2522). Viability tests were performed by staining cells with 0.2µM calcein AM and 0.2 µM ethidium bromide dimer for 30 min at 37°C. The staining of nuclei with DAPI was carried out for cell density evaluation.

RESULTS

Synthesis and Properties of the Different IPNs

While the co-network was carried out by free radical photopolymerization, the fibrin gel was synthesized by the enzymatic hydrolysis of fibrinogen by thrombin at 37°C. Different IPNs were synthesized varying the PVA/SA ratio. The *in situ* synthesis of PVA_{100} /Fb_5, $PVA_{50}SA_{50}/Fb_5$ and SA_{100}/Fb_5 IPNs has been developed. In each case, a solid material was obtained (Figure 1).

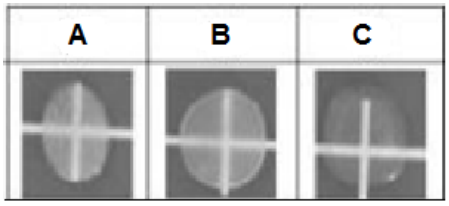

Figure 1. (A) PVA_{100}/Fb_5; (B) PVA_{50}-SA_{50}/Fb_5, and (C) SA_{100}/Fb_5 IPNs just after synthesis

The SA_{100}/Fb_5 IPN (Figure 1, C) is more transparent than IPNs containing PVA (Figure 1, A and B). All the IPNs keep their round shape after synthesis which is indicative of good mechanical properties. In addition, they can be stable for months when immersed in sterile buffer.

The synthesis was thus followed by rheology measurements and gel times determined (Table 1). With every composition, a material was obtained after 15 min.

Table 1. Gel time and storage modulus (G') for various IPNs

Scaffold composition	Fb based IPN	
	Gel time (min)	G' (Pa)
PVA_{100}/Fb_5	3	3240 ± 420
PVA_{50}-SA_{50}/Fb_5	3	640 ± 20
SA_{100}/Fb_5	15	870 ± 420

After 60 min irradiation, the storage moduli were measured and compared to that of the fibrin hydrogel (G' = 80 Pa) which is not easy to handle. All IPNs containing either SA or/and PVA are self-supported (G' > 100 Pa). The choice of IPN architecture has been then validated by a large improvement of the mechanical properties of the fibrin gel at physiological concentration. Indeed, the addition of any synthetic co-network to a fibrin gel leads to a large increase in storage modulus (G') from 8 to 40 fold (Table 1). The partner co-networks well fulfill their role of transforming the fibrin gel into an easily handled gel.

To check the effective formation of the networks in IPN architecture, extractions of soluble fractions of both the synthetic partner and the fibrin of the materials were performed. The part of methacrylate polymers which is not included in the synthetic network is high (from 12 to 16 %), but correct for polymer networks synthesized from a diluted precursor solution. In addition, the presence of PVA-SA co-network does not inhibit the enzymatic formation of fibrin network as all the fibrinogen has been turned to fibrin and included in the material.

Biodegradability

The degradation of IPNs was then tested. In order to quickly prove that SAm confers biodegradability to the materials, they were incubated in a concentrated thermolysin (a zinc endopeptidase)solution for 24 h and macroscopically observed (Figure 2).

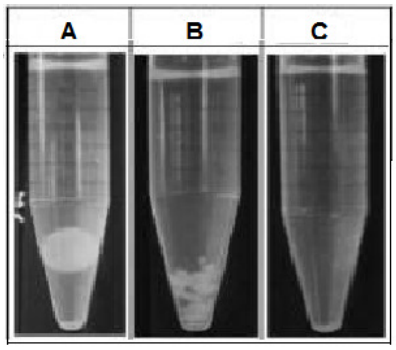

Figure 2. (A) PVA_{100}/Fb_5; (B) PVA_{50}-SA_{50}/Fb_5, and (C) SA_{100}/Fb_5 IPNs after 24 h in a protease solution

As suspected, the PVA_{100}/Fb_5 IPN (Figure 2, A) was not degraded by the protease under those conditions. The protease may degrade the fibrin network inside the IPN, but this protein representing only 5 wt% of the solid fraction of the materials, its proteolysis does not lead to the dislocation of the material; for example PVA_{100}/Fb_5 IPN global integrity is maintained after fibrin hydrolysis When SA was included in PVA network, the IPN can be totally or partly degraded upon enzyme action. Thus, many fragments were obtained after immersion of PVA_{50}-SA_{50}/Fb_5 IPN in thermolysin solution (Figure 2 B), indicating that SA can be degraded inside the IPN and that it was also well distributed in the co-network. Moreover, the SA_{100}/Fb_5 IPN was totally degraded by the protease. This material is thus well biodegradable. The degradation rate depends on the SA weight proportion, ranging from no degradation (no SA) to total one (no PVA).

The degradation kinetics was also measured (Figure 3). The proteolysis was measured for several IPNs all containing 0.5 (w/v) % fibrin and 10 (w/v) % polymers, including from 0 to 10 (w/v) % SAm. The hydrolysis rate depends on the concentration of proteins (fibrin + serum albumin) in the material. A fast hydrolysis was observed for the IPNs containing more than 50% serum albumin in their crosslinked network (PVA_{50}-SA_{50}/Fb_5 and SA_{100}/Fb_5 IPNs) while the hydrolysis was slow when only fibrin may be hydrolyzed by the protease (PVA_{100}/Fb_5 IPN).The absorbance at the equilibrium directly depends on the protein concentration and is well fitted by the relation Abs_{280} = 0.034 total protein concentration (%) + 0.042 (R^2= 0.9775). These experiments show that SA in the material is accessible to the protease and may be hydrolyzed even in the IPN. The complete degradation of the IPNs is possible only if the SA is evenly distributed in the material.

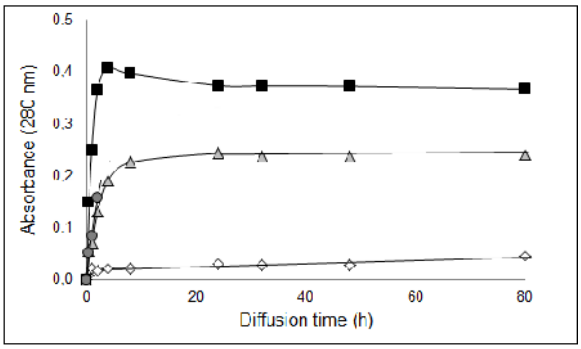

Figure 3. Protein absorbance at 280 nm as a function of immersion time in water for various IPNs. ◇: PVA_{100}/Fb_5 IPN, ▲PVA_{50}-SA_{50}/Fb_5 IPN, ■ SA_{100} /Fb_5 IPN

The degradation rate of the IPNs is depending on the total protein concentration (fibrin + serum albumin). Moreover, the hydrolysis process follows a Michaelis – Menten kinetics, indicating that the diffusion of the protease is not limited inside the solid material. These results show that controlling the protein total concentration allows tuning the IPN degradability.

Cell Viability

Viability of cells in contact with the materials has been measured with BJ fibroblasts. A cell suspension (50 000 cells/ cm^2; confluent density) was spread on the surface of PVA_{100} /Fb_5, PVA_{50}-SA_{50}/Fb_5 and SA_{100}/Fb_5 IPNs. After incubation between 24 h and 3 weeks, a metabolic assay showed the ability of cells to survive on the surface of these IPNs (Figure 4).

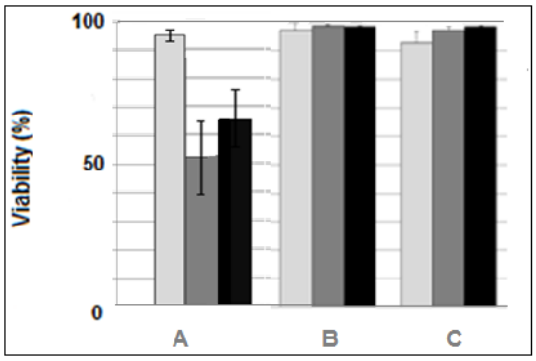

Figure 4. Cell viability on the surface of various materials A: PVA_{100} /Fb_5 IPN; B: PVA_{50}-SA_{50}/Fb_5 IPN; C: SA_{100}/Fb_5 IPN after 24h (▒),1 week (■) and 3 weeks (■) of culture

While cells rapidly die in contact to PVA single network surface [Bidault *et al*., 2013]; the presence of fibrin in the PVA_{100}/Fb_5 IPN slowed down their death. However, this material is not suitable as a cell culture support. When SA was present in the IPNs, cell viability stayed close to 100% after 3 weeks (Figure 4), indicating that the protein environment favored cell viability. In polymer biomaterials, cell growth has been linked to biodegradability, our results are in concordance with this assessment as the biodegradability increased with the protein concentration (Figure 3).

Then the proliferation capability of cells on the surface of these different materials was explored (Figure 5).

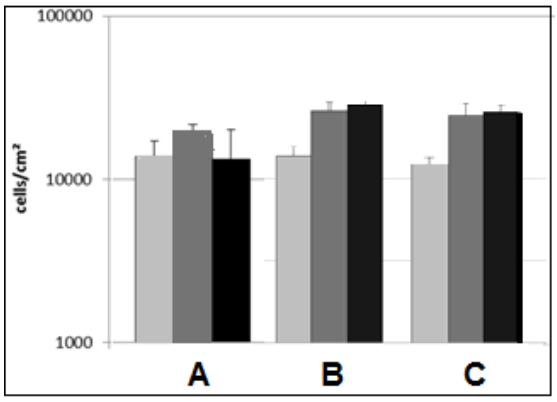

Figure 5. Cell density on the surface of various materials A: PVA_{100} /Fb_5 IPN; B: PVA_{50}-SA_{50}/Fb_5 IPN; C: SA_{100}/Fb_5 IPN after 24h (▒),1 week (■) and 3 weeks (■) of culture

Cells survived but did not proliferate on PVA_{100} /Fb_5 IPN. Contrarily, the introduction of SA into the network allowed fibroblasts to proliferate up to total recovery of the material surface and their number largely increased (Figure 5) when

they were cultivated on either SA_{100}/Fb_5 IPN or PVA_{50}-SA_{50}/Fb_5 IPNs. Comparison with single networks without fibrin showed that the effects of fibrin and SA were synergistic on cell development. This feature is one of the main interests of IPN architectures which allow benefiting of the advantages of each network.

CONCLUSION

Fibrin gel has been associated with different PVA/SA co-networks inside interpenetrating polymer networks architecture. The materials are synthesized rapidly and they are all self-supported because the polymer network allowed improving the mechanical properties of the fibrin gel. These biomaterials are biodegradable by proteases due to SA introduction. This degradability due to the incorporation of SA in the PVA scaffold helped to increase very significantly the bioactivity of materials. When SA was included in PVA-SA/Fb IPNs, the surface of materials can be completely covered by fibroblasts within two weeks. All these properties indicate that the IPNs here synthesized are good supports for cell growth.

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I.
MATERIALS

INFLUENCE OF PARTICLE SIZE AND PHASE DISTRIBUTION ON ADHESION OF ADHESIVE DISPERSIONS BASED ON POLYCHLOROPRENE GRAFTED WITH MMA

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Besides optimum rheological properties and adhesion, adhesives must meet other conditions: not toxic, flammable and does not pollute the environment. Classical adhesives based on volatile organic compounds; do not fully meet these requirements, for which aqueous dispersion adhesives environment have become increasingly used, tending to replace almost completely organic solvent-based adhesives. Through grafting operation of elastomeric chains with methyl methacrylate, of dispersion of their solution in the aqueous environment and the use in the composition of the sodium montmorillonite, was obtained adhesive "water-based" with the characteristics of high initial and final adhesion, comparable with the organic solvents, and low toxicity or even absent. Particle size provides important information on the optical properties, stability, and viscosity of the system. At the same time, can elucidate the kinetic aspects of both during the emulsion the synthesis when obtain composite materials. Particle size distribution of adhesives solutions was determined with equipment Mastersizer 2000 (Malvern Instruments, UK) laser light, the range from 0.02 to 2000 mm, with precision of ± 1%. By measuring the particle size and phase distribution on micron-scale or nanometer, it has been shown that the smaller the particles the better penetrate porous media substrates, and on the other hand, the dispersions can have a high dry matter content, which may lead to an optimum combination of rough media. This class represents the most innovative volatile organic replacement based adhesives products.

Keywords: adhesive dispersions, particle size, phase distribution.

INTRODUCTION

Due to the considerable expansion of the fields it is used in, adhesive production has extended and diversified in the last years. At the same time, adhesives with optimal pre-established properties, both regarding the way they are obtained and the types of blends, have been developed. But aside from the optimal values of the rheological and adherence properties, adhesives must meet other conditions as well: not to be toxic, inflammable and not to pollute the environment. Classical adhesives, based on volatile organic compounds, do not meet these conditions entirely. For reasons such as pollution, fire hazard and economical ones, adhesives with aqueous dispersion medium have become more and more used and they are not far from practically replacing adhesives with organic solvents for good, but they need to have comparable properties in order for this to happen. Adhesive dispersions are emulsions or latex consisting of a stable continuous liquid phase in which a second phase, discontinuous, immiscible with the first one, is present (US Patent 5407993, 1995). Broadly, these can be classified as macro- and micro-dispersions. The classification is based on the size of the dispersed particles: macro-dispersions have dimensions ion the range 0.2-50 μm and micro- or nano-dispersions between 10 and 200 nm (Alexandrescu *et al.*, 2013). Systems having particles with diameters between 10 and 1000 nm are usually called colloidal systems (Knecht *et al.*, 2008). The properties of polymers can be modified to correspond to the

desired applications. Three possibilities to modify the properties of polymers are known: blending, grafting and hardening (Alexandrescu *et al.*, 2011).

Grafting is the method by which macromolecular chains of identical or different monomers are covalently bonded to the backbones of the polymer that must be modified (Zhang *et al.*, 2012). Grafting is controlled by the following factors: nature of polymer, monomer, solvent, nature of initiator, the used additives and the temperature.

EXPERIMENTAL PROCEDURE

Materials

Polychloroprene NEOPRENE AD 20, (DuPont) as polymer, methyl methacrylate (Merck, Germany) as monomer; benzoyl peroxide as initiator, dodecylmercaptan as inhibitor (both Sigma-Aldrich Chemie, Germany), ZnO and MgO (active substance 95%) as cross-linking agent and to consume the hydrochloric acid eliminated during reaction and natural resin colophony (Caroco) as adherence improver were used.

Procedure

The natural clays with modified surface are layered silicates used successfully to obtain nano-composite materials and adhesives due to the properties they impart. The modified sodium montmorillonite have the following characteristics: the chemistry of advanced intercalation, which facilitates the exfoliation of layered structures into nanometric layers, which maximizes the interfacial contact and the capacity to modify the chemistry of the surface by organic and inorganic ionic exchange reactions.

Two types of polychloroprene composites were prepared: with 2, 4 and 7% montmorillonite reported to polychloroprene, as well as containing 4% montmorillonite, which were subjected to grafting on roller with different amounts of methyl methacrylate. The amount of 4% was selected because a higher quantity has as effect the strengthening of mixtures. The producers recommend using of 2-5%.

The technology involves three steps: compounding the polychloroprene rubber or the ones grafted with methyl methacrylate with montmorillonite and ingredients specific to adhesive compounds from Table 1, dissolution of products obtained in the same mixture of solvents and dispersion of solutions obtained following the formulation from Table 2. To increase the grafting efficiency and reduce the formation of block copolymers, benzoyl peroxide was introduced in compositions subjected to grafting.

The dispersion was done into the installation consists of a three-necked thermo-resistant glass flask, 2 L capacity, to which a stirrer and a thermometer were attached, the third one being for introducing the components. A VELP rod stirrer was used, recommended for mixing of materials with a wide variety of viscosities. For an efficient stirring the rod for medium viscous dispersions, with an elongated agitator, two arms and a stationary blade was used, the blades' inclination and the rotation direction being important for the movement of the liquid into the flask.

The compounds were swollen for ½ h in the same solvent mixture, introduced into the dissolution container under stirring (400 rpm) until full homogenized (about 30 min), then the 10% poly(vinyl alcohol) aqueous solution, triethanolamine and de-mineralized water were added. The components were added drop by drop into the rubber solution for 1 h. The obtained adhesive dispersions were first characterized physico-chemical, according to current standards.

Table 1. Formulations for obtaining of polychloroprene compounds non-grafted and grafted with methyl methacrylate containing montmorillonite (parts by weight)

Composite, g/dispersion	C1	C2	C3	C4	C5	C6
Polychloroprene Denka AD 20	200	200	200	200	200	200
Methyl methacrylate	-	-	-	10	20	30
Na montmorillonite	4	8	14	8	8	8
Benzoyl peroxide	-	-	-	1	1	1
Dodecylmercaptan	-	-	-	2	2	2
MgO	8	8	8	8	8	8
ZnO	10	10	10	10	10	10
Natural resin colophony	4	4	4	4	4	4
Total	226	230	236	243	253	263

Table 2. Formulations to prepare dispersions from compounds C1-C6

Composites, g	C1	C2	C3	C4	C5	C6
Compound	47.03	47.87	49.12	50.58	52.63	54.77
Solvents	32.97	32.13	30.88	29.42	27.37	25.23
Polyvinyl alcohol, liquid solution 10%	50	50	50	50	50	50
Triethanolamine	15	15	15	15	15	15
De-mineralized water	80	80	80	80	80	80
KOH, 10% solution, ml	1	1	1	1	1	1
Total	226	226	226	226	226	226

Testing Methods

Particle size distribution was determined by laser light scattering using the Mastersizer Hydro 2000S Particle Size Analyzer, Malvern Instruments Ltd., equipped with Malvern soft, which controls the system during the measurements and processes the information based on standard operation procedure (SOP). The three standard points to read the distribution characteristics are D(v, 0.1), D(v, 0.5) and D(v, 0.9), which represent the fractions from the total particle volume having the size higher than a given value (Ma *et al.*, 2001; Mu and Seow, 2006).

The adherence was measured according to SR EN 1392:2006 standard, test known as "peeling test at (23±2)°C". The following supports were used: (a) mixture of standard rubber, hardness 85°Sh A; (b) leather; (c) split leather; (d) linen; (e) cotton cloth; (f) synthetic leather. The steps followed were: preparing of support by polishing to increase its roughness when standard/standard samples were jointed, application of the adhesive dispersion, drying, jointing of test samples, conditioning and peeling measurements (Zheleva, 2012). The adhesives were applied by brushing as monolayers on rubber and as bilayers on textiles, split and synthetic leather. The drying was done in separated rooms, equipped with devices for vapor absorption. The open time ranged between 15 min and 1-2 h, depending on the absorptive properties of supports. The drying time was reduced by preheating the supports at 70°C (Busato, 2002).

After drying the supports were joined and pressed down for 30 s at 3.5-4.0 atm, then conditioned according to the European standard EN 1391 (24 and 72 h at the standard temperature of 23 ± 2°C; samples conditioned for 72 h heated for 3 h at 50°C or 168 h at 70°C to accelerate the aging, but only for St/St joints). The pealing test was done using a TEBA dynamometer (Timisoara, Romania) after 24 and 72 h for the samples

conditioned at room temperature, immediately for those heated at 50°C and after 24 h for those heated at 70°C using a speed of 100 mm/min.

RESULTS AND DISCUSSION

Particle sizes provide important information on optical properties, stability or viscosity of a system. At the same time they are able to elucidate kinetic aspects taking place during emulsion polymerization or obtaining of some composite materials.

Dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), measures the fluctuations of intensity of scattered light which occur due to the Brownian movement of particles and is time-dependent.

To establish the influence of montmorillonite on particle size distribution, C1-C3 samples, containing various amounts of montmorillonite. To have a general view on sizes and particle size distribution of dispersions containing montmorillonite, non-grafted or grafted with MMA, the curves are represented superposed in Figure 1.

For the sample containing the lowest amount of montmorillonite the distribution is bimodal. This is due, in addition to the method of obtaining the dispersion – mechanical dispersion, to the presence of montmorillonite, with particles ranging between 13 and 16 µm. Increasing the amount of montmorillonite the peaks of fractions with small and medium sizes increases also (it appears as a shoulder at 7-8 µm for sample C1), and the one at larger sizes becomes a shoulder peak. The highest montmorillonite amount used – 7 parts to 100 parts rubber – gives completely different sizes and distribution: peaks at small and medium values disappear practically and very large size particles appear and prevail, ranging from approx. 100 to 700 µm. Tis is explained by the agglomeration of latex particles produced by the high amount of montmorillonite, exceeding the recommended one.

Grafting with methyl methacrylate was done only for polychloroprene rubber having the composition C2, from which compounds C4-C6 were obtained.

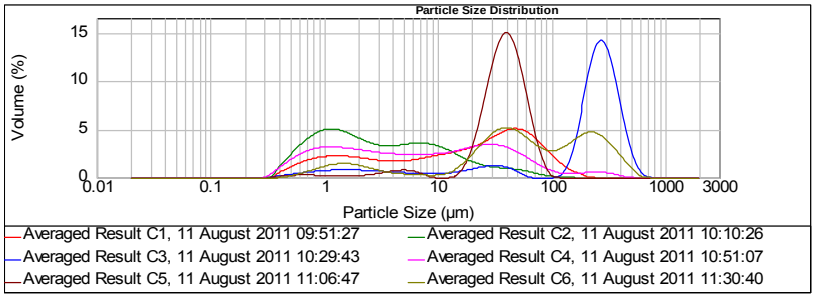

Figure 1. Superposed distribution curves for samples C1-C6

Dispersion C4, with the same amount of montmorillonite as sample C2 and the lowest amount of grafted MMA has a completely different particle size distribution from that presented by C2: particles size range between 0.12 and about 600 µm, the particles with average diameters of about 1.02 and 12 µm respectively prevailing. The weight of large particles, with average diameters of about 260 µm, is very small.

Increasing the amount of MMA, dispersions have different size distribution, very similar in shape but not in size to dispersion C3. Thus, the most particles range between 15 and 100 µm, the fractions ranging between 0.3 and 1.0 µm and between 2 and 10 µm being negligible.

The maximum amount of MMA grafted on polychloroprene has as result again a broad distribution, close to that of dispersion C4, but the fractions of particles have reversed predominant size: the preponderant fraction is the one with large size, ranging between 10 and 100 μm (peak at about 40 μm), followed by the one between 100 and 700 μm and the peak at ca 240 μm, while the one with the smallest size, between 0.4 and 9 μm, is much lower. The values of standard points for samples C1-C6 are given in Table 3.

Table 3. Standard points for reading distribution characteristics for dispersions C1-C6

Sample	D(v, 0.1), μm	D(v, 0.5), μm	D(v, 0.9), μm
C1	1.039	18.351	76.753
C2	0.694	2.703	18.866
C3	4.206	244.891	399.907
C4	0.773	6.892	56.523
C5	20.245	38.204	61.837
C6	2.145	57.269	293.842

It is found that both control chloroprene rubber dispersions containing different amounts of montmorillonite, and those modified by chemical grafting with increasing amounts of MMA have polymodal particle size distribution. Analyzing the size distribution for sample C3, containing 7 parts montmorillonite/100 parts rubber, it can be seen that the largest particle population has sizes larger than 200 μm, while populations with sizes smaller than 100 μm are reduced, so that the distribution curve can be practically assimilated with the unimodal type. The same aspect is found for sample C5, which contains 4 parts montmorillonite and 15 parts MMA reported to rubber, except that the largest particle population has sizes smaller than 100 μm.

The dispersions obtained by chemical grafting of polychloroprene latex are stable for 30 to 40 days but the technology is time and money consuming.

Bonding capacities were determined for the dispersions C1-C6. The peeling resistances for the initial and modified dispersions are given in Table 4.

Table 4. Peeling resistance, N/mm, for C1 - C6 dispersion with additives

Dispersion/ support	24h						72 h	168 h	72h/3h at 50°C, immediate peeling	72h/168hx 70°C, peeling after 24h
	St/ St	St/ L	St/ SL	St/ Le	St/ C	St/ SyL	St/ St	St/ St	St/St	St/St
C1	3.1	0.2	2.4	1.5	2.8	0	3.9	5.1	2.7	5.6
C2	3.6	0.8	2.8	5.2	3.7	0	5.5	7.2	3.5	6.1
C3	2.6	0.5	1.9	2.6	2.7	0.3	3.1	3.9	2.5	5.5
C4	5.9	0.9	3.4	7.3	5.3	1.0	4.3	6.0	3.9	8.0
C5	6.8	2.1	3.9	8.7	6.4	1.8	5.5	7.4	5.2	9.2
C6	8.1	4.2	4.8	9.1	8.2	2.3	6.1	7.5*	7.1	12*

St – standard rubber; L – leather; SL – split leather; Le – linen; C – cotton; SyL – synthetic leather; * - tear rubber

Table 4 emphasizes the following influences of grafting and of additives:

- grafting of methyl methacrylate onto polychloroprene chains increases the adherence with 15-40%, depending on support and monomer amount, their peeling

resistance being very close or even higher than those of the solvent-based adhesives on support as leather, split leather, linen and cotton cloth (higher than 3 N/mm);

- high values of the adherence were obtained at 50 and 70°C, which suggests that the dispersions in which the polychloroprene is grafted with methyl methacrylate give very resistant binding at higher temperature.
- It has been shown that the smaller the particles and have a broader distribution, better penetrate porous media substrates, and on the other hand, the dispersions can have a high dry matter content, which may lead to an optimal combination rough media.

CONCLUSIONS

High performance environmentally friendly aqueous adhesive nanodispersions for shoe manufacture were obtained by mechano-chemical grafting of polychloroprene using benzoyl peroxide as initiator and colophony resin and MMT as adhesion improvers.

Particle size and particle size distribution increase when the polychloropren is grafted, both increasing with the amount of methyl methacrylate introduced.

Peeling resistance is higher for dispersions containing grafted polychloroprene with, it increases with monomer amount and depends on the nature of substrate to which the standard rubber is bonded.

Aging for 168 h at 70°C increases the adherence in all the cases between about 2,7 and 12 times, depending on the degree of polychloropren grafting and the nature of the adhesion improver used.

The obtained adhesives dispersions, and especially C6, are very adequate for manufacturing of footwear resistant to high temperatures.

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INFLUENCE OF SILICA MICRO-PARTICLES LOADING ON THE FLEXURAL PROPERTIES OF DENTAL RESIN COMPOSITES

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The objective of this study was to evaluate the influence of silica (SiO_2) micro-particles loading on the flexural properties of dental resin composites (DRCs). The DRCs were prepared from a resin matrix comprising Bis-phenol A-glycidyl methacrylate (Bis-GMA) as a base monomer and triethylene glycol dimethacrylate (TEGDMA) as a diluent monomer mixed with SiO_2 micro-particles as a reinforcement filler in a ratio of 40, 50 and 60 wt%. The samples were then light-cured using a LED TPC 60. The density (g/cm^3) of the DRC samples was determined according to the ASTM D792-98 standard. The values of flexural strength (FS) and flexural modulus (FM) were determined using the three-point bending test according to the ISO 4049:2009 standard. The results revealed that the density values of the DRCs increased as the SiO_2 loading increased. The FS values decreased as the loading of the SiO_2 increased from 84.52 to 53.2 MPa. In contrast, the FM values increased as the SiO_2 loading was increased from 1.55 to 7.51 GPa. There were significant differences ($p<0.05$) in the values of FS and FM when the composites contained different amounts of SiO_2 micro-particles. The SiO_2 micro-particles used for reinforcement of the resin matrix had an effect on the flexural properties of the DRCs.

Keywords: Flexural properties, Silica, Dental resin composites

INTRODUCTION

For the past three decades, efforts have been directed towards the development of dental resin composites (DRCs) to match not only the chemical and mechanical properties of dental enamel, but its appearance as well. DRCs based on polydimethacrylate monomers together with filler treated with silane are widely used in a variety of dentistry applications. Bisphenol A-glycol methacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) are the most commonly used resins in DRCs. The chemical treatments are frequently added to ensure good polymer-filler adhesion (Benyahia and Merrouche, 2014). The most widely used silane in DRCs is 3-methacryloxypropyltrimethoxysilane (γ-MPS). The coupling agents are known to be effective in improving the properties of the resulting composite system (Chuayjuljit *et al.*, 2014). The mechanical properties of the DRCs basically depend on the filler content, size and morphology; therefore, the filler particles play an important role in determining the mechanical properties of DRCs. Increasing the filler content and reducing the average filler size is an approach in producing DRCs for posterior restorations which require adequate strength and wear resistance to endure mastication forces (Manhart *et al.*, 2000). Several studies documented that DRCs are influenced by several factors such as filler ratio, size and morphology (Kim *et al.*, 2002; Zhang *et al.*, 2005). According to ISO 4049:2009 standards, the flexural strength of DRCs materials must not be lower than 50 MPa. Recently, several studies about flexural properties of DRCs have been studied by various researchers (Rodrigues *et al.*, 2007; Rüttermann *et al.*, 2008; Samuel *et al.*, 2009).

To date, only few data on the effects of the SiO_2 particles on the flexural properties of the DRC have been recorded. Therefore, the aim of the present study was to prepare DRCs reinforced with SiO_2 at different ratios, and evaluate their FS and FM properties.

MATERIALS AND METHODS

In this study, the Bis-GMA monomer was purchased from Esschem, Essington (USA), and TEGDMA monomer, γ-MPS, Camphorquinone (CQ), dimethylaminoethyl methacrylate (DMAEMA) were purchased from Sigma-Aldrich (Germany), and the SiO_2 particles were purchased from Sibelco (Malaysia). The resin matrix was fabricated from a blend of Bis-GMA and TEGDMA (75/25) wt%, respectively. The CQ was added to the resin matrix as an initiator followed by the addition of DMAEMA as an accelerator. The SiO_2 particles were treated by an amount of 10 wt% of γ-MPS relative to the amount of filler. Three different ratios of treated SiO_2 particles 40, 50, and 60 wt% were added into the resin matrix, respectively. The procedure was in accordance to Zandinejad *et al.* (2006). Ten samples of each formulation of the prepared DRC were used to determine the density, apparent porosity, FS and FM. The density and apparent porosity test of the DRC samples were carried out according to the ASTM D792:2008 test method-A for testing solid plastics in water. The FS and FM tests were carried out according to the ISO 4049:2009 standard. The samples were light-cured by using a LED *TPC* 60 unit. The FS and FM tests were performed with a three-point bending test using Instron 3366, at a cross-head speed of 0.75 mm/min.

STATISTICAL ANALYSES

Statistical analyses were conducted with SPSS statistics version 19. The data was subjected to one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc*. The level of statistical significance was considered as $p< 0.05$.

RESULTS AND DISCUSSION

Figure 1 shows the effect of the filler loading on the density and porosity of the DRC. The density of the composites increased proportionally as the loading of the filler particles increased. As the particles of SiO_2 have a higher density relative to the resin matrix, the addition of the filler, therefore, increased the composites' density. These findings are in agreement with Lim *et al.* (2006) who reported that the densities of the high-density polyethylene or ultra-high molecular weight polyethylene/high-density polyethylene blend increased with the increasing loading of the filler. It can be also noted that the DRCs' apparent porosity value was found to be inconsistent as the loading of the filler increased, whereby the DRC samples reinforced with 50 or 60 wt% of SiO_2 particles showed a similar porosity value. Moreover, the results indicated a higher porosity value of DRC reinforced with 50 or 60 wt% of SiO_2 than the porosity value of the DRC reinforced with 40 wt% of SiO_2 particles. It is possible that the increase in the level of porosity is intrinsically linked to the processes taking place during the mixing and the procedure of compression moulding.

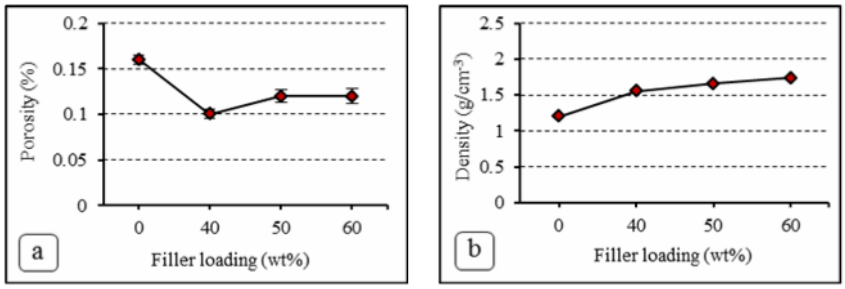

Figure 1. Effect of filler loading on the: (a) density, and (b) apparent porosity of the DRC

Figure 2 shows the effect of SiO_2 particles loading on the FS and FM of the DRCs. The results indicated that when the filler particles' loading increased in the resin matrix, there was a slight decrease in the values of the FS. A statistically significant decrease in the DRC's value of FS was found (p<0.05). This finding was attributed to an increase in the loading of inadequately shaped filler particles. John *et al.* (2001) reported that a polymer which is reinforced has a higher specific strength compared to a non-reinforced polymer. It may also be associated with an increase in the loading of fillers with a large particle size. A number of researchers (Qi *et al.*, 2006; Zhang *et al.*, 2005) claimed that to improve the matrix's mechanical properties the key factor was the dispersion of the small sized particles. These findings are similar to an observation made by Pereira *et al.* (2003) who observed that the composites' FS decreased in comparison to the FS of a pure polymer. According to the ISO 4049:2009 standard, the FS value ought not be less than 50 MPa. In this study, the resin matrix filled with 60 wt% of SiO_2 particles had a FS value which reached 53.2 MPa. This is in line with the ISO 4049:2009 standard which is considered within acceptable limits for applications in dentistry.

The values of the FM improved as the amount of filler loading was increased. Statistically as the filler loading increased the values of the FM significantly increased (p<0.05). The increasing FM value was attributed to an increase in the loading of the filler in the resin matrix. In this study, these findings are similar to the work done by Kim *et al.* (2002) and Alamri and Low (2012) who established that the polymer composites' mechanical properties were associated to the loading of the filler. Several researchers have detected a significant correlation between the percentage of the filler by volume percentage (Ikejima *et al.*, 2003) or the filler by weight (Sabbagh *et al.*, 2002) and the composites' flexural properties.

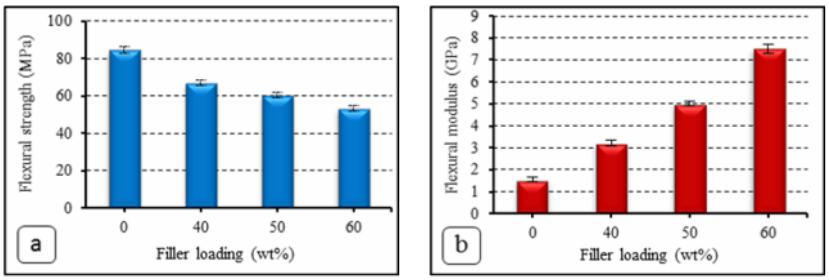

Figure 2. Effect of SiO_2 particles loading on the: (a) FS, and (b) FM of the DRC

CONCLUSIONS

The density of the DRCs increased as the loading of the SiO_2 particles increased, while the value of porosity of the resin matrix when reinforced with different ratios of the SiO_2 particles was found to be inconsistent. It is possible that the increase in the level of porosity is intrinsically linked to the processes that taking place during the mixing and the procedure of compression moulding. However, the FS value of the DRCs decreased as filler loading increased. According to the ISO 4049:2009 standard the FS values of the DRCs in this study are suitable to be used in dentistry. On the other hand, the FM value of the DRCs increased as filler loading increased.

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STUDY REGARDING THE RESISTANCE OF WET-WHITE LEATHER TANNED WITH TITANIUM – ALUMINUM TO THE GROWTH OF FUNGI

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In the tanning industry the problem of micro-organisms able to grow on leather during the different processing phases is well known. Wet-white leathers are excellent substrates for fungal growth: storage temperature, acid pH, presence of water, proteins and fats constitute the most important conditions for the development and growth of a lot of moulds such as *Penicillium* spp, *Aspergillus* spp. and *Trichoderma viride*. The fungal contamination appears as coloured stains on the leather, usually permanent. Microbiological testing has been performed on wet-white leather tanned with Ti-Al based tanning agent using an inoculum with 4 species of fungi. This study revealed that the Ti-Al-tanned wet-white leather is attacked by all types of fungi studied.

Keywords: wet white leather, microbiological analysis, Ti-Al tanning agent

INTRODUCTION

At present, chrome tanning is the most widely used technique for leather tannage, accounting for more than 90% of leathers tanned worldwide. However, chrome tannage involves serious environmental risks resulting from the possible oxidation of chromium to a hexavalent state, although tanners are aware of the carcinogenic effect, in accordance with the International Agency for Research on Cancer (IARC). For this reason, the market has shown a growing demand for "ecological" products, especially regarding the development of tanning processes using alternative tanning agents different from chromium.

Alternative free of chrome (FOC) tanning technologies include the use of tanning agents based of titanium-aluminum, which in combination with other retanning agents of vegetable or synthetic origin, allow for obtaining quality leathers that may be used by footwear and upholstery industries (Kleban, 2004; Adiguzel Zengin *et al.*, 2012; Mutlu *et al.*, 2012).

Wet-white refers to partially processed leathers that have been tanned with titanium and/or aluminium, but not dyed, dried or fat-liquored yet (Crudu *et al.*, 2012a; 2014; 2012b; 2010).

This study aims at testing fungal resistance of wet white leather tanned with titanium-aluminium (Crudu *et al.*, 2013).

The most common fungi found on leather are *Aspergillus niger* and *Aspergillus flavus*, moulds that, in addition to the destructive effect they have on leather objects, are also harmful for human health. *Aspergillus flavus* is dangerous because of the alfatoxin it produces, one of the most carcinogenic substances in the living world, while *Aspergillus niger* may cause aspergillosis in immune-compromised patients.

Aspergillus niger is a widespread mould in the environment which may develop on almost anything: coffee, various foods, textiles, wood, paper and leather goods, which is why this mould was used to test resistance of leather to fungi. Mould is invasive, developing quicker than *Penicillium* or other types of fungi, with the tendency of extending to the detriment of other species.

Aspergillus niger strain ATCC 6275 was first isolated in a laboratory in The United States, from a leather sample. The strain is characterized by the presence of genes for

carboxymethyl cellulase, citric acid, glucosidase, xylanase, lipases and resistance to copper.

Trichoderma viride is a green coloured mould used in the antifungal treatment of soil and seeds due to its ability to inhibit growth of other fungi, but can also be pathogenic for certain plants, such as onion. The mould produces cellulases and chitinases and develops on wood and parasitizing other fungi, which is why it damages mushrooms. This mould may also grow on tanned leather.

Aspergillus oryzae is characterized by the presence of orange globular conidia, and it is used in Asian cuisine for soybean fermentation. The mould produces amylases and carboxypeptidases. Unlike *Aspergillus flavus* and *Aspergillus niger*, *Aspergillus oryzae* has not been identified on leather so far, but the possibility of its development on chromium-free tanned leather samples was taken into account.

Mucor pusillus develops in soils and decomposes organic matter of vegetable origin. No growth of this mould has been yet reported on tanned leather, but this study aimed at discovering whether fungi can damage wet-blue and wet-white tanned leather through the proteases they produce.

MATERIALS AND METHODS

Materials

Samples: specimens of wet white leather were obtained after tanning stage of bovine hides using a tanning agent based on Ti-Al according to Crudu *et al.* (2013).

Biological material: 4 fungi strains were used: *Aspergillus niger* ATCC 6275, *Trichoderma viride*, *Aspergillus oryzae* 153 and *Mucor pusillus.*

Culture medium: Potato Dextrose Agar (PDA) culture medium was used.

Methods

Microbiological tests were performed according to ASTM standard D 4576-08 (2013) - Test Method for Mold Growth Resistance of Wet Blue (Tarlea *et al.*, 2009). Four samples of each type of leather were inoculated with each of the 4 fungi strains and were incubated at 28 ± 1°C for 28 days. Leather samples were evaluated after 7, 14, 21 and 28 days to determine fungi growth, ranking them from 0 to 4, as follows: mark 0 for leathers not covered by mould, mark 0.5 for growth on less than 12% of the sample surface, mark 1 for growth ranging between 12 and 25% of the surface, mark 2 if 50% of the sample surface is covered by fungi, mark 3 if mould grew on 75% of the sample surface and mark 4 if the sample is entirely covered by mould.

Chemical tests of wet-white leathers were performed according to EN ISO standards.

RESULTS AND DISCUSSIONS

Wet- white leathers are excellent substrates for fungal growth: storage temperature, acid pH, presence of water, proteins and fats constitute the most important conditions for the development and growth for a lot of moulds, such as *Penicillium* spp., *Aspergillus* spp. and *Trichoderma viride*. The fungal contamination appears as coloured stains on the leather, usually permanent.

Wet white leathers were characterized for chemical characteristics shown in Table 1.

Table 1. Wet-white leather chemical characteristics

No.	Characteristics	Wet-white Ti-Al tanned
1	Ti/Al oxides, %	3.12
2	Shrinkage temperature, °C	75-78
3	Grease, %	2.1
4	pH	3.6
5	Moisture, %	66.33
6	Ash, %	12.59
7	Total nitrogen, %	13.84
8	Digestibility, %	61.3

Table 2 presents the appearance and assessment (marks) of leather samples inoculated with fungi after 7, 14, 21 and 28 days. According to the working standard, samples were evaluated ranking them from 0 to 4 depending on the growth of mould on the sample (Table 2). The following were found:

After 7 days *Aspergillus niger* grew on less than 25% of the Ti-Al-tanned leather sample.

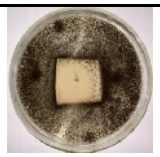
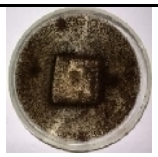
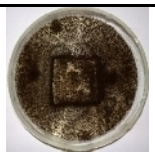
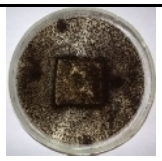
Aspergillus oryzae grew on the surface of the Ti-Al-tanned wet-white leather sample without damaging them. *Trichoderma viride* grew in the culture medium; approximately 10% of the Ti-Al-tanned wet-white leather sample is covered by *Trichoderma*. *Mucor pusillus* only grew in the culture medium around the wet-white leather samples.

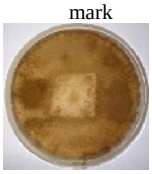
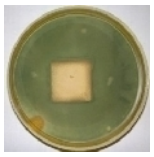
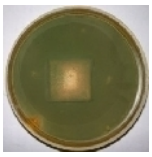
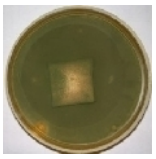
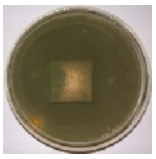
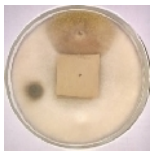
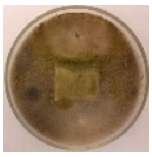
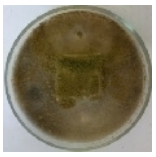
After 14 days, *Aspergillus niger* grew on wet- white leather, while *Aspergillus oryzae* covered the Ti-Al-tanned wet-white samples almost entirely. *Trichoderma viride* grew on 75% of the Ti-Al tanned wet-white leather samples. Wet-white leather samples were entirely covered by *Mucor pusillus*. On the Ti-Al-tanned wet-white leather sample inoculated with *Mucor pusillus*, a colony of *Aspergillus flavus* also appeared. The colony is very invasive, quickly covering the entire surface of the sample.

After 21 days, all leather samples inoculated with *Aspergillus niger* were entirely covered by mould; the situation of samples inoculated with *Aspergillus oryzae* remained unchanged. The appearance of the Ti-Al-tanned wet-white leather sample inoculated with *Trichoderma viride* did not change much.

After 28 days the appearance of samples inoculated with *Aspergillus niger* and *oryzae* did not change.

Table 2. Appearance and assessment of leather samples after 7, 14, 21 and 28 days

Fungus type	7 days / mark	14 days / mark	21 days / mark	28 days / mark
	Ti-Al-tanned wet-white leather			
A. niger	 0.5	 3	 4	 4

Fungus type	7 days / mark		14 days / mark		21 days / mark		28 days / mark	
A. oryzae		2		2		2		3
T. viride		0.5		2		3		3
M. pusillus		0		4		4		4

CONCLUSIONS

Ti-Al-tanned wet-white leather is attacked by all types of fungi studied. *Aspergillus niger* and *Aspergillus flavus* are the most aggressive and invasive species, developing very rapidly, much quicker than *Trichoderma viride*, *Mucor pusillus* and moulds from the *Penicillium* genus, the latter having the slowest growth rate. Although it produces chitinases with antifungal effect, *Trichoderma viride* does not inhibit development of *Aspergillus niger* and *flavus* species.

Acknowledgements

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STUDY REGARDING THE RESISTANCE OF ORGANIC TANNED WET-WHITE LEATHER TO THE GROWTH OF FUNGI

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The paper presents the resistance of wet-white leather organic tanned with oxazolidine and resorcinol to the growth of fungi. Wet-white leather was characterized for microbiological resistance using an inoculum with 4 species of fungi: ATCC 6275 of *Aspergillus niger, Trichoderma viride, Aspergillus oryzae* 153 and *Mucor pusillus*. Wet-white leather samples tanned with resorcinol-oxazolidine are attacked by *Aspergillus niger* and *Mucor pusillus*, but inhibit growth of moulds from *Aspergillus oryzae* and *Trichoderma viride* species. This type of leather can also be damaged by mould from the *Penicillium* genus.

Keywords: organic tanned wet white leather, microbiological testing, fungi

INTRODUCTION

The deep interest of tanners in clean technologies has led to increased efforts to develop chrome-free tanning agents. Most of the leather produced today around the world is chrome tanned and the total leather production system, starting with the beam house and including dyeing, retanning and fat liquoring, has been adjusted and developed to its present high level around chrome tanning. But chromium is considered toxic for human health. The single solution for this problem is finding out an environmentally friendly alternative to chromium tannage. Alternative free of chrome (FOC or wet white) tanning technologies include the use of tanning agents based on organic compounds like oxazolidine, which in combination with other retanning agents of vegetable or synthetic origin, allow for obtaining quality leathers that may be used by footwear industries. Wet-white refers to partially processed leathers that have been tanned with resorcinol/oxazolidine, but not dyed, dried or fat-liquored yet (Lanxess; Padoan, 2006; Adiguzel Zengin *et al.*, 2012; Liu *et al.*, 2010; Platon *et al.*, 2010; Roig *et al.*, 2011; Deselnicu *et al.*, 2014).

Wet-white leathers are excellent substrates for fungal growth: storage temperature, acid pH, presence of water, proteins and fats constitute the most important conditions for the development and growth for a lot of moulds, such as *Penicillium* spp., *Aspergillus* spp. and *Trichoderma viride*. The fungal contamination appears as coloured stains on the leather, usually permanent.

Usually leathers are preserved against fungi with fungicides (Tarlea *et al.*, 2009).

This study aims at testing fungal resistance of wet white organic tanned leather with resorcinol-oxazolidine.

MATERIALS AND METHODS

Materials

Samples: specimens of wet-white leather were obtained after tanning stage of bovine hides using resorcinol-oxazolidine tanning (Deselnicu *et al.*, 2012a; 2012b).

Biological material: 4 fungi strains were used: *Aspergillus niger* ATCC 6275, *Trichoderma viride*, *Aspergillus oryzae* 153 and *Mucor pusillus.*

Culture medium: Potato Dextrose Agar (PDA) culture medium was used.

Methods

Microbiological tests were performed according to ASTM standard D 4576-08 (2013) - Test Method for Mold Growth Resistance of Wet Blue. Four samples of each type of leather were inoculated with each of the 4 fungi strains and were incubated at 28 ± 1°C for 28 days. Leather samples were evaluated after 7, 14, 21 and 28 days to determine fungi growth, ranking them from 0 to 4, as follows: mark 0 for leathers not covered by mould, mark 0.5 for growth on less than 12% of the sample surface, mark 1 for growth ranging between 12 and 25% of the surface, mark 2 if 50% of the sample surface is covered by fungi, mark 3 if mould grew on 75% of the sample surface and mark 4 if the sample is entirely covered by mould.

Chemical tests of wet-blue and wet-white leathers were performed according to EN ISO standards.

RESULTS AND DISCUSSIONS

Chemical characteristics of wet-white leathers are shown in Table 1.

Table 1. Wet-white leather characteristics

No.	Characteristics	Wet-white organic tanned leathers
1	Shrinkage temperature, °C	70-74
2	Grease, %	4.5
3	pH	4.3
4	Moisture, %	55.0
5	Ash, %	10.5
6	Total nitrogen, %	12.9

Table 2 presents the appearance and assessment (marks) of leather samples after 7, 14, 21 and 28 days. According to the working standard, samples were evaluated ranking them from 0 to 4 depending on the growth of mould on the sample (Table 2). The following were found:

After 7 days *Aspergillus niger* grew on the entire surface of the organic tanned wet white leather sample; the organic tanned wet-white leather sample inhibited growth of *Aspergillus oryzae* and *Trichoderma viride*.

Mucor pusillus only grew in the culture medium around the wet-white leather samples.

After 14 days, *Aspergillus niger* grew on the leather, while the growth of *Aspergillus oryzae* was further inhibited by the wet-white leather sample tanned with resorcinol-oxazolidine.

The growth of *Trichoderma viride* colonies was inhibited by the leather sample tanned with resorcinol-oxazolidine, but a colony of *Aspergillus niger* spontaneously appeared on the sample.

Wet-white leather samples were entirely covered by *Mucor pusillus*.

Table 2. Appearance and assessment of leather samples after 7, 14, 21 and 28 days

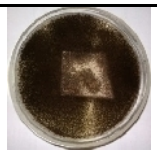
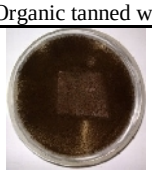
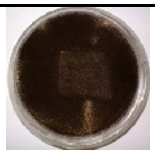
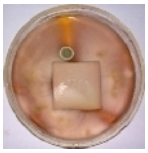
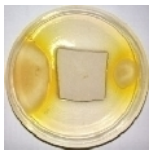
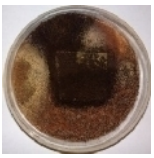
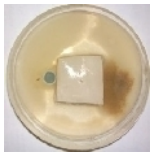
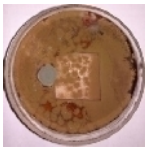
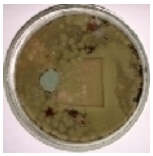
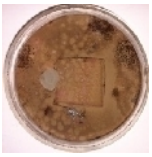
Fungus type	7 days / mark	14 days / mark	21 days / mark	28 days / mark
	Organic tanned wet-white leather			
A. niger	 3	 4	 4	 4
A. oryzae	 0	 0	 0	 0
T. viride	 0	 0	 0	 0
M. pusillus	 0	 3	 4	 4

Figure 1. *Aspergillus niger* and *Aspergillus flavus* moulds spontaneously grown on the wet-white leather sample tanned with resorcinol-oxazolidine after 21 days

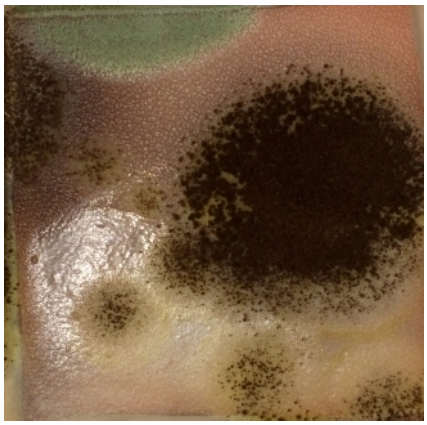


Figure 2. Appearance of the leather sample tanned with resorcinol-oxazolidine and inoculated with *Aspergillus oryzae* after 28 days

After 21 days, the leather samples inoculated with *Aspergillus niger* were entirely covered by mould; the situation of samples inoculated with *Aspergillus oryzae* remained unchanged, but two *Aspergillus niger* colonies and one *Penicillium* colony appeared, while the appearance of samples inoculated with *Mucor pusillus* did not change much. The leather sample tanned with resorcinol-oxazolidine completely inhibited the growth of *Trichoderma viride* colonies, but was entirely covered by *Aspergillus niger* and a colony of *Aspergillus flavus* also appeared (Figure 1).

After 28 days the appearance of samples inoculated with *Aspergillus niger* and *oryzae* did not change, except for the leather sample inoculated with *Aspergillus oryzae* (Figure 2) on the surface of which a *Penicillium* colony also grew, while *Aspergillus niger* colonies developed. The appearance of samples inoculated with *Trichoderma viride* and *Mucor pusillus* did not change much either.

CONCLUSIONS

Aspergillus niger and *Aspergillus flavus* are the most aggressive and invasive species, developing very rapidly, much quicker than *Trichoderma viride*, *Mucor pusillus* and moulds from the *Penicillium* genus, the latter having the slowest growth rate. Although it produces chitinases with antifungal effect, *Trichoderma viride* does not inhibit development of *Aspergillus niger* and *flavus* species.

Wet-white leather samples tanned with resorcinol-oxazolidine are attacked by *Aspergillus niger* and *Mucor pusillus*, but inhibit growth of moulds from *Aspergillus oryzae* and *Trichoderma viride* species. This type of leather can also be damaged by mould from the *Penicillium* genus.

Acknowledgements

This work has been financed by the European Fund for Regional Development and the Romanian Government in the framework of Sectoral Operational Programme under the project INNOVA-LEATHER: «Innovative technologies for leather sector increasing technological competitiveness by RDI, quality of life and environmental protection» –

contract POS CCE-AXA 2-O 2.1.2 nr. 242/20.09.2010 ID 638 COD SMIS – CSNR 12579.

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CHARACTERIZATION OF THE STATE PLANAR ORIENTATION FOR SHORT NATURAL FIBER IN POLYMERIC COMPOSITES BY MEANS OF THE TENSOR ORIENTATION

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This investigation presents a detailed description to evaluate and predict the orientation state of short fibers of "Guadua Angustifolia Kunth" (GAK) as reinforcement in polypropylene (PP) matrix by using the second order orientation tensor. For this, several samples were prepared by injection molding and then metallographically polished. The aim of this work is to predict the state planar orientation for short natural fibers as an initial stage to determine the mechanical behavior of composite materials. The tensor orientation is determined by using digital image processing in selected polished sections of different material samples. For both digital images processing and computational modeling it was possible to find different orientation states over all specimens. This method will allow us to know the real effect of the flow fibers on the main mechanical properties of the biocomposites.

Keywords: Orientation tensor, polymeric composites, image processing.

INTRODUCTION

The averaging method used (Lagzdins *et al.*, 2009) allows for the estimation of elastic properties of a polymeric composite reinforced by short randomly oriented fibers. Such elastic properties were also studied by (Modniks and Andersons, 2010) using the unit cell properties consisting of a fiber of an average length and matrix according to the fiber volume fraction by means of orientation averaging. The stiffness tensor components C_{ijkl} of the composite are calculated by using the formula (Modniks *et al.*, 2011):

$$C_{ijkl} = \iint C^{*}_{ikjl}(\theta,\phi)\psi(\theta,\phi)\sin\theta\, d\theta\, d\phi \tag{1}$$

C^{*}_{ijkl} are the stiffness tensor components of the unit cell, $\psi(\theta,\phi)$ the distribution density of the fiber orientation, θ and ϕ are the elevation and azimuthal angles of a fiber.

The present investigation shows the characterization of the orientation state of short fibers using the orientation tensor. The first approximation used the distribution density of the fiber orientation $\psi(\theta,\phi)$ and second one by means of orientation tensor supported with digital processing images.

THEORETICAL DESCRIPTION

As described (Advani and Tucker, 1987) in earlier reports and (Modniks *et al.*, 2011) recently, the fibers are assumed as rigid cylinders, with an uniform cross-section area and constant length. With these assumptions, the fiber orientation state can be

studied by using the probability distribution function for orientation $\psi(\theta,\phi)$. This function defines the probability of finding a fiber between angles θ_1 and $\theta_1 + d\theta$ and ϕ_1 and $\phi_1 + d\phi$ and described as:

$$P(\theta_1 \leq \theta \leq \theta_1 + d\theta, \phi_1 \leq \phi \leq \phi_1 + d\phi) = \psi(\theta_1, \phi_1) \sin\theta_1 \, d\theta \, d\phi \tag{2}$$

The orientation can be described by associating a unit vector **p** with the single fiber, as shown in figure 1. Thus, the distribution function can be written as a function of **p** vector, $\psi(\bar{p})$.

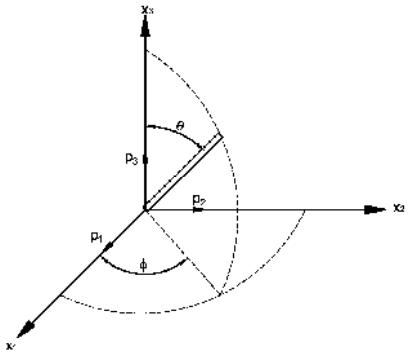

Figure 1. Spherical coordinates system for describing of a single fiber

In according to figure 1, the components of unit vector **p** are related to elevation and azimuthal angles as:

$$p_1 = \sin\theta\cos\phi \qquad p_2 = \sin\theta\sin\phi \qquad p_3 = \cos\theta \tag{3}$$

By denoting the fixed length of the vector **p**, ($p_i p_i = 1$), the set of all possible directions of the vector corresponds to unit sphere. The integral over all possible directions over the surface of the unit sphere can be calculated by

$$\oint d\bar{p} = \int_0^{2\pi}\int_0^{\pi} \psi(\theta,\phi)\sin\theta \, d\theta \, d\phi = \int_0^{2\pi}\int_0^{\pi} \sin\theta \, d\theta \, d\phi \tag{4}$$

The function $\psi(\theta,\phi)$ must satisfy some conditions such as, periodicity, normalization and continuity condition. To denote the orientation state of short fibers in composites, robust processing conditions which is computationally cumbersome is required. Taking into account the symmetrical condition of the distribution function, one set of orientation tensors can be defined by forming dyadic products of the vector **p** (Advani and Tucker, 1987). By integrating dyadic products with the distribution functions only even order orientation tensors are calculated and being the major interest is the second and fourth order orientation tensors.

$$a_{ij} = \oint p_i p_j \psi(\bar{p}) d\bar{p} \qquad a_{ijkl} = \oint p_i p_j p_k p_l \psi(\bar{p}) d\bar{p} \tag{5}$$

By expanding the equation (5), in particular the second order tensor for i,j=1,2 y 3, a symmetrical matrix is obtained. Due to the normalization condition, the second order tensor trace is equal to unity, in other words the summation of the orientation percentages must be 100%. By considering real composites, each discrete fiber sample

is measured where the components of the tensors are calculated by a summation, namely (Lee *et al.*, 2002),

$$a_{ij} = \frac{\sum(p_i p_j) L_n F_n'}{\sum L_n F_n'} \tag{6}$$

where F_n' represents the weighting function for the *n*th fiber,

A planar orientation state considers that all fibers lie on a single plane. This fact is being widely used to study composite materials due to their approximated planar orientation (Advani and Tucker, 1987). The planar orientation of the second order tensor can be defined as,

$$a_{ij} = \int_0^{2\pi} \psi_\psi(\phi) p_i p_j d\phi \tag{7}$$

The planar orientation of the second order tensor is used to describe the orientation state in the main flow direction. By taking the summation for N fibers, a_{ij} is to re-write as follows (Eberhardt and Clarke, 2001),

$$a_{ij} = \frac{1}{N}\left(\sum_{n=1}^{N} p_i^n p_j^n\right) = \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} \tag{8}$$

a_{11} < 0.35 is considered perpendicular to the flow direction.

a_{11} > 0.7 is considered parallel to the flow direction.

0.5< a_{11} < 0.6 is considered a random orientation.

By considering the planar orientation state, the main components of the tensor for the axis x_1 and x_2, as shown in figure 1, can be determined by

$$a_{11} = \frac{1}{N}\sum_{n=1}^{N} \cos^2\varphi \qquad a_{12} = \frac{1}{N}\sum_{n=1}^{N} \cos\varphi \sin\varphi \tag{9}$$

where N is the total number of fibers in the composite, φ is the planar orientation angle of each fiber. To calculate the preferred angle of the fibers Yasuda et al. (2004) used the following equation,

$$\tan 2\alpha = \frac{2a_{12}}{a_{11} - a_{22}} \tag{10}$$

Unlike Yasuda's results and depicted in figure 2, the orientation state is obtained in this report taking into account the a_{11} element of the equation (8) for the two-dimensional case.

EXPERIMENTAL

The orientation tensor method was employed to evaluate the orientation state of short fibers using a micrograph of a polymeric compound of polypropylene and short fibers of GAK (PP+GAK). Homopolymer Polypropylene was supplied by Braskem H-306 and bamboo fiber was supplied by Bamboo House in Ecuador. Fiber lengths of

about 4mm and diameters of 0.2mm according to ASTM E11-95 for mesh 45 were used. The bamboo fibers were chemically modified using NaOH at 5%.

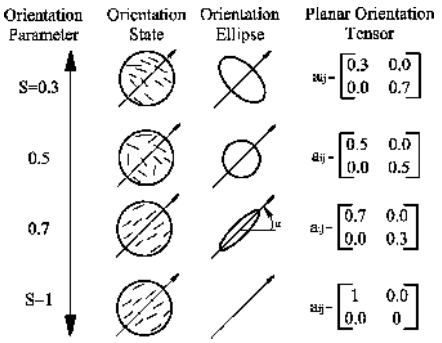

Figure 2. Relation between the orientation parameter, orientation state, orientation ellipse and the planar orientation tensor are shown

ASTM D 638 samples were obtained by injection using a Dr. Boy 35 horizontal injection moulding machine. Metallographic polish was performed on each sample and then unsaturated polyester resin was embedded. Micrograph imaging were recorded by means of a light Olympus BX-41M (5X) microscope. Different positions of the sample were studied by sectioning the sample at 10, 40 and 70 mm along the flux axis. Furthermore, several samples were analyzed at different cutting depths (1, 2 and 3 mm), as shown in figure 3.

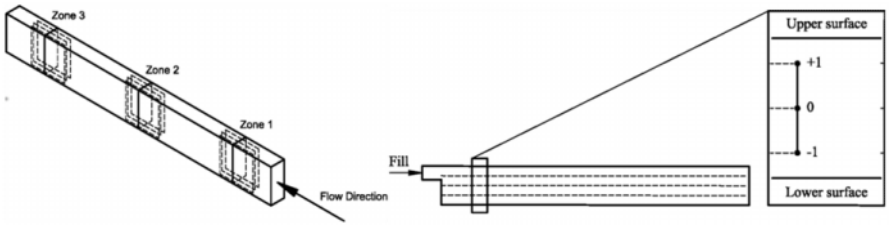

Figure 3. Sectioning of samples along and across the main flux axis

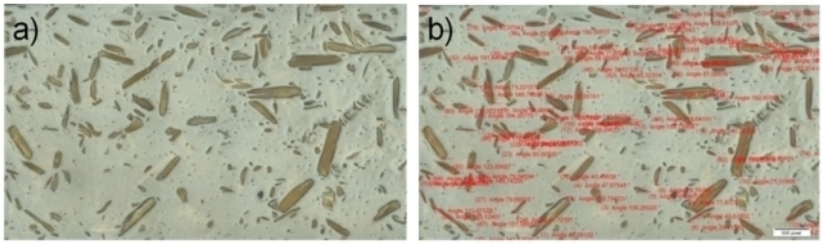

Figure 4. Micrograph recorded in a) zone 1 at 1mm in depth and b) measurements of φ angle

Measurements of the fiber orientation were carried out using imaging processing Olympus Stream® software. Orientation values of each fiber along the main flux axis were measured. Figure 4 shows orientation angles in the plane (φ) of the zone 1 with a depth of 1mm. The measured values from imaging processing were used to obtain the orientation tensor of GAK fibers by means of a MatLab R2012a® script.

RESULTS

Figure 5 shows the trend of the state of orientation (a_{11}) and the preferred angle (α) of the samples with 30% GAK along the flow direction and through thickness z. From the a_{11} calculated element by the second order orientation tensor applied to each one of the areas in each specimen with respect to the direction of entering flow a high orientation of the fibers was measured. The main element of the tensor orientation is above 0.8 for each value of z. Further guidance can be seen in the regions near the injection point. Asymmetry was also observed with respect to the thickness. Generally highly oriented zones are those regions in which the polymer matrix solidifies first, with slight variations due to the forces produced by front advance of the flow. Coincidence between the orientation state using the tensor orientation and the maximum eigenvalue which also defines the state was observed. The calculated error reaches the 0.82% which provides reliable results with the application of both methods. Table 1 summarizes the values obtained for the zone 1.

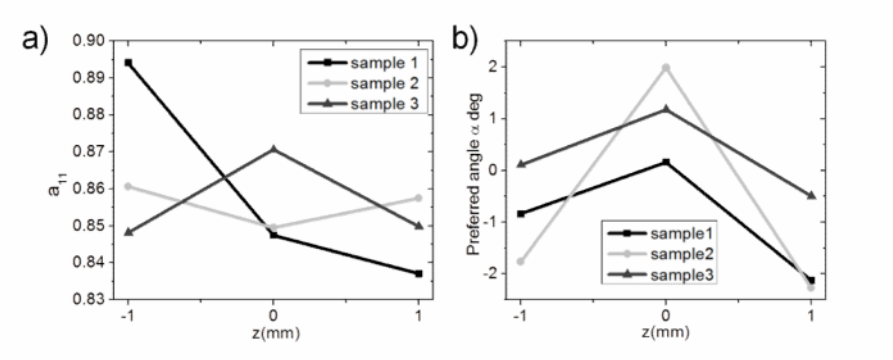

Figure 5. Distributions of the orientation state by means of a_{11} and the preferred angle in the main direction flux respect to z depth are shown

Figure 5b shows the distribution of the preferred angle of orientation and its evolution with the thickness of the specimens. Similar results were found for other preparations (40% wt/wt). The preferred angle of the fiber measured with respect to the direction of flow is very narrow and is in the range of ±2 grades. It shows a fairly symmetrical distribution of preferential angle and high deviations in the center of the flow. This effect is due to the insulating nature of the polymers having the specimens which take more time to solidify in this region, so the fibers have more mobility and are more dispersed.

Table 1. Comparison between calculated values a_{11} and the order parameter S of the planar orientation state in the zone 1 of the specimen (30% GAK) are shown

	A1_1mm_30%	A1_2mm_30%	A1_3mm_30%
a_{11}	0.8232	0.8237	0.8705
S (order parameter)	0.8234	0.8241	0.8777
Error	0.024%	0.049%	0.827%

CONCLUSIONS

The orientation tensor method has been used to evaluate the orientation state of short fibers in polymeric composites without dependence of the aspect ratio of fibers. Both orientation tensor and imaging processing technique were employed to calculate the orientation state of fibers in composites. It was possible to compare results obtained using the first element of the orientation tensor a_{11} and the higher eigenvalue of each orientation state. Unlike Yasuda's results depicted in figure 2, the orientation state in this report was calculated by taking into account the a_{11} element of the equation (8) for the two-dimensional case.

The central portion of the samples loaded with 30 and 40% of GAK showed greater dispersion angles and lesser preferred orientation state due to the time taken for this region to solidify. However it offers the possibility of experiencing the fiber orientation through the application of shear that will control the orientation state in that area and to infer on their future mechanical properties. The difference in results between the analysis through the main element of the tensor orientation and the largest eigenvalue is minimal and reaches the 0.82% allowing for the estimation of orientation state by any of the methods described with complete reliability.

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A NEW NATURAL FIBER: TOQUILLLA STRAW A POTENTIAL REINFORCEMENT IN THERMOPLASTIC POLYMER COMPOSITES

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Toquilla straw (*Carludovica palmata*) obtained from the Manabí province in Ecuador has been used traditionally for weaving the Panama hat. Due to its importance and high disposability as a renewable source and the methodological characterization which is required to understand its physical and chemical properties. To this end, sections of treated and untreated specimens were studied to analyze the mechanical, thermal, spectral and morphological properties. From thermal and mechanical analysis's that were carried out on the Toquilla straw fibers revealed a temperature onset of about 230°C before the degradation and Young's modulus as well as stress in the range of natural fibers used as reinforcement in thermoplastic polymer composites. Possible changes in their composition and structure were monitored using Fourier Transform Infrared spectroscopy as well as the morphology by means of a scanning electron microscope.

Keywords: natural fibers, reinforcement polymer composites, mechanical properties

INTRODUCTION

Nowadays, agriculture is playing an important role in the bio-based economy, providing feedstocks for the production of liquid fuels, chemicals and advanced materials (FAO 2013), such as natural fiber composites for industry. The mechanical properties of several natural fibers (Delgado *et al.*, 2012; Gupta *et al.*, 2012) or hybrid materials (Noorunnisa Khanam *et al.*, 2010) are attracting much attention to produce composite materials in the automotive industry (Bledzki *et al.*, 2006), aeronautical and civil buildings (Nguyen *et al.*, 2009). Natural fibers have the advantages of low cost, low density, biodegrability, abundance and cause less environmental impacts than man-made fibers. Vegetable fibers are classified according to their origin as well as the part of the plant (i.e., leaf, seed, bast, fruit, grass and stalk) and their properties which can be affected by factors such as climate, maturity, harvesting or physical or chemical treatments (Kalia *et al.*, 2009).

Carludovica palmata grows in the rainforest ranging from Guatemala to Bolivia and is considered a member of the *Cyclanthaceae* family and is a close relative to the Palmae (*Arecaceae*) family (Lopez *et al.*, 2008). This plant is sometimes referred to as Jipijapa or Montecristi when made into Panama hats to remember the names of villages in Ecuador where the finest hats are made. Other names used to refer to this plant are iraca, lucaina, lucua, palmiche, cestillo, nacuma, rabihorcado, murrapo, alagua and rampira to refer to this plant (Solano, 1997).

In this study, we report the mechanical, thermal, spectral and morphological characterization of the *Carludovica palmata* or Toquilla straw fibers. These fibers are the fundamental element of the most traditional Ecuadorian product recently inscribed in the Representative List of the Intangible Cultural Heritage of Humanity and widely known as the Panama Hat (UNESCO, 2012). For a better understanding and use of the treated natural fibers, in particular toquilla straw, information on the different properties

is also required. An attempt to describe the changes occurring in the structure of toquilla straw fiber due to this process is presented. Treated and untreated fibers were characterized by using mechanical testing, differential scanning calorimeter, thermogravimetric analysis, infrared spectroscopy and scanning electron microscopy.

METHODS

Bundles of Toquilla straw were obtained from Manabí province in Ecuador with and without any pre-sizing, chemical and physical treatment for this study. Two kinds of specimens were studied. The first one, treated Toquilla straw following the traditional process in which the strands are boiled in a water bath for 10 h and then dried under the sun. In addition, the bleaching process with sulfur vapors to whiten the plant was carried out. Toquilla straw was arranged in fiber bundles as reported in another cases (i.e., hemp, jute, arundo) (Elkhaoulani *et al.*, 2013; Fiore *et al.*, 2014) the main chemical components being cellulose, hemicelluloses, lignin and pectin which make the morphological structure of the plant fibers. In spite of the different diameters of the Toquilla straw for three different sections, an estimation of area and diameters of about 0.61 mm^2 and 400μm was a possible measure. The apparent density of the fiber (in bulk) of 1.06 g/cm^3 was estimated using hexane (ρ=0.760 g/cm^3) as a solvent under the Archimedes method.

Weights of a bundle of strands and the apparent density (in bulk) were measured in air and solvent by a procedure detailed below (Mwaikambo and Ansell, 2001).

$$\rho_A = \frac{\rho_s \cdot Wf_a}{Wf_a - Wf_s} \quad (1)$$

where ρ_s is the density of hexane; Wf_a and Wf_s are the weights of the strands in air and hexane, respectively.

EXPERIMENTAL

The universal machine (Instron-5582) at a constant speed of 3mm/s was employed for tensile strength measurements, according to ASTM C (1557-03) standard test. All samples for tensile strength testing were selected from the same stem.

Toquilla straw specimens were obtained by sectioning in three different parts of the leaf for mechanical characterization, as shown in figure 1a. The first section is named as the basis, the second one the central section and lastly a final section of the leaf. Afterwards, ten samples from each section of the raw single straw each having the length (100mm) and the treated straw were selected for testing of their elongation at break, Young's modulus, stress and thermal properties. Morphology of the Toquilla straw is shown in figure1b which revealed a spiral design. The fiber-cells with a polygonal shape were observed with a light microscope and SEM experiments showing variability in diameter in the cross section area images. It is shown along the specimen the morphology is like other natural fibers.

The thermal stability of raw and treated Toquilla straw was investigated within a dynamic range from 25°C to 900°C and rate of 10°C/min with a purging N_2 gas stream of 200mL/min. The thermo gravimetric analyzer (TGA/SDTA 851 Toledo Mettler, Columbus, OH) and the controlled environment with an inert gas (Ar, 50mL) were

employed. The thermograms were recorded using 7.03mg and 4.41mg of treated and untreated specimens, respectively.

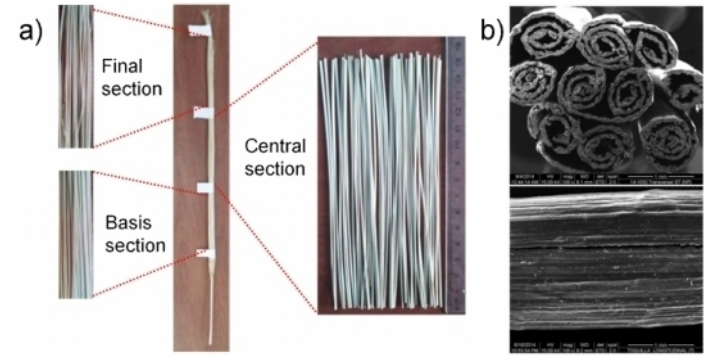

Figure 1. Toquilla straw a) corresponding to basis, central and final sections and several strands from the central section of experiments are shown. (b) Morphology of the Toquilla straw for cross section and longitudinal was recorded by SEM

Infrared spectroscopy experiments were performed using FTIR spectrometer (Perkin Elmer PC 1600). A series of 20 scans were collected for each measurement over the 4000 to 400 cm^{-1} spectral range and 4cm^{-1} of resolution.

The morphology of the central section of Toquilla straw was investigated by a scanning electron microscope (SEM, FEI-Quanta Inspect). Before SEM analysis, samples were coated with a thin layer of metallic platinum using a sputter coater (SC7620 sputter, Emitech). Moreover, cross-section areas of the Toquilla straw and thickness less than 5 μm and longitudinal sectioning were prepared by means of a Micron HM 360 Microtome for viewing with a light microscope (Micros Austria, Crocus 2).

RESULTS AND DISCUSION

Table 1. Mechanical properties of the Toquilla straw for three different sections using the Weibull distribution

Sections	Parameters	Treated fiber	Standard error	Untreated fiber	Standard error
Final	Tensile stress [MPa]	20.69	2.31	73.5	5.28
	Young Mod. [GPa]	3.43	0.40	3.09	0.08
Central	Tensile stress [MPa]	134.4	10.1	127.9	2.55
	Young Mod. [GPa]	4.79	0.11	4.06	0.07
Basis	Tensile stress [MPa]	47.13	4.65	131.8	9.28
	Young Mod. [GPa]	3.95	0.21	4.30	0.01

Young's modulus of about 4.1±0.7GPa and 3.8±0.6GPa were estimated for sixty samples both treated and untreated fibers, respectively. By comparing the tensile modulus and tensile strength values of raw fibers with treated ones, it revealed that the

sulfur treatment reduced the mechanical properties of sectioned zones named basis and final by almost 30% (see Table 1). Untreated and treated specimens of the central section revealed a tensile strength of about 130±10 MPa. The different sections of the Toquilla revealed a similar mechanical response as reported for other lignocellulosic fibers (De Rosa *et al.*, 2010). Due to the dispersion in dimensions of fibers, a statistical treatment using a two parameter Weibull distribution to estimate the tensile strength and the Young's modulus was used, as shown in figure 2.

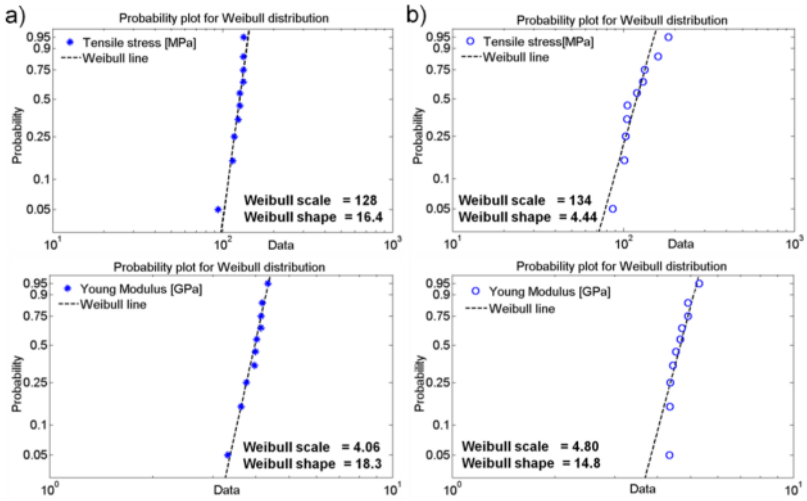

Figure 2. Statistical treatment using the Weibull distribution to mechanical properties for a) raw Toquilla straw and b) treated fibers of the central section

These mechanical properties were associated to scale parameter after fitting experimental data using the Weibull distribution method widely used to analyze natural fibers (Andersons *et al.*, 2005; Peponi *et al.*, 2008). Tensile stress calculated in Toquilla straw reveals its potential uses as reinforcement in polymeric composites which is higher than 40MPa reported for some polymers.

Thermogravimetric analysis (TGA) for the treated natural fiber reveals that thermal decomposition occurred in four stages (see figure 3a). The first range characterized with a rapid weight loss of about 12% up to 90°C of temperature due to the moisture caption and water evaporation. In this same range, untreated fibers show a low percentage of weight loss, less than 5% also observed in DTG curves in (b). *C. Palmata* samples which were not previously dried for this study. This fact suggests that volatile contents after treatments with sulphur vapors degrade below 90°C. For a range from 200°C to 350°C, both thermograms show similar behavior, with a weight loss of 40% and 50% for treated and untreated specimens, respectively.

A closer inspection in DTG curve shows a light peak of about 250°C prior to major decomposition occurring from 270 to 400°C, with a maximum temperature of decomposition around 330 and 340°C for treated and untreated fibers, respectively. Inset in Figure3b, revealed a displacement of temperature onset of about 10°C for treated samples. In this range of temperature, several natural fibers such as jute, curua, ramie and kenaf have shown an increase thermal stability after drying at 105°C for 1 h

(Ornaghi *et al.*, 2014). Above 350°C up to 475°C, the percentage weight losses are around 25%. Degradation of cellulose and lignin contents is associated for this range of temperature. In higher temperatures (800°C), the weight loss is less than 10% and associated to ash. The use of the Toquilla straw as reinforcement is assumed as retardant effect to thermal degradation within the composite material.

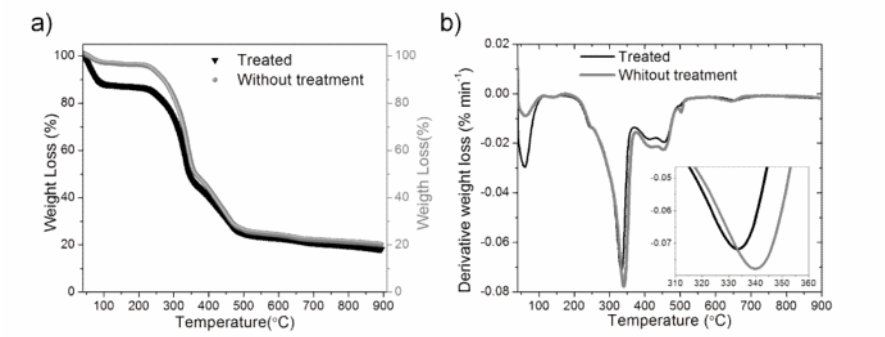

Figure 3. a) Thermogravimetric (TGA) and b) differentiate thermogravimetric (DTG) analysis curves of treated and untreated Toquilla straw specimens

From spectral analysis shown in figure 4, the spectral region from 3500 cm^{-1} to 2500 cm^{-1} is related to the stretching vibration of O-H, C-H and CH_2 group of lignin/cellulosic and hemicellulosic component of natural fibers.

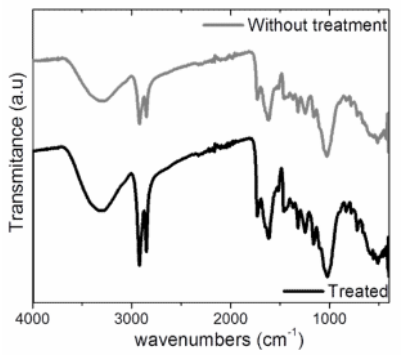

Figure 4. Fourier Transform Infrared spectra measured for untreated and treated Toquilla straw fibers

The stretching vibration of the hydroxyl group in lignin and cellulose correspond to the broad absortion band around 3300cm^{-1}. The band around 1729 cm^{-1} region may be attributed to C=0 stretching vibration of the acetyl group in hemicellulose. The peaks at 1650 cm^{-1} and 1616 cm^{-1} are attributed to non-esterified pectins. A closer inspection, the light shoulder around 1650 cm^{-1} in the treated specimen spectra is not present for untreated specimen spectra. The stretching vibration at 1515 cm^{-1} is assigned to aromatic skeleton vibration in lignin. By comparing both treated and untreated samples

few changes were observed. This fact can suggest that the different mechanical response of both specimens is due to structural changes or crystalline effects.

CONCLUSIONS

The Toquilla straw revealed degradation temperature of about 350°C showing its potential uses as thermoplastic reinforcement in composite materials. The tensile strength measured on the central section showed higher values than most common polymeric compounds. Spectral analysis did not show relevant changes of treated and untreated samples that allowed us to conclude the origin of variability of the mechanical responses calculated in the sections.

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HYBRID PE/PA/NANOPARTICLE COMPOSITES RESISTANT TO HIGH TEMPERATURES

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In the field of polymeric materials, leading companies turned to the development of modified polymers with superior physical and mechanical properties compared to those of the basic constituents, individually considered, which offers a degree of versatility that was not obtained with any other material. Composite materials are produced with anisotropic properties both at the nano, micro- and macroscopic scale, formed by the assembly of several components whose organization and design allow use of the maximum specific properties of each component in order to obtain higher overall final properties of each component. The most common composites are polymeric, in which the combination of plastomers with batches, nanoparticles and other ingredients lead to a significant variety of materials with much improved properties compared to the original and even directed towards predetermined properties according to the market requirements. This paper shows resistant polymeric nanocomposites (TEO) based on compatibilized polyethylene (PE) and polyamide (PA), with advanced characteristics based on reinforcement materials in the form of powders with nano structure and chemically activated surface, that provides qualitative performance and meeting the current requirements of quality and aesthetics for consumer goods.

Keywords: polymers, hybrid composites, thermal resistance

INTRODUCTION

In the field of polymeric materials, leading companies have been oriented towards the development of modified polymer structure elastomer/plastomer/ nanoparticles and superior physical and mechanical properties compared to those of the base constituents taken individually. These properties offers high use value in fields which require materials with performance characteristics: dimensional stability, resistance to weathering, UV radiation, ozone, microorganisms, solvents, aggressive chemical agents, waterproof and disperse systems, solid-gas, gas-liquid, electric resistance, lack of toxicity. In addition to the above, other important property is thermal resistance at a wide range of temperatures, etc. Properties are the result of a complex of original characteristics which arise from physical, mechanical and chemical interactions that occur during manufacturing processes that result in a polymer nanostructure.

Polymer nanocomposites (PNCs) are the class of composite materials comprising at least one of their components so called fillers bearing nanometer size scale (< 1000 nm) dispersed into a polymer matrix.

The physical, chemical and biological properties of the PNCs are largely related to physical characteristics of polymer matrix, their composition, dimensions of fillers, compatibility of fillers with polymer matrix, interfacial interactions between polymer matrix and nanofiller phase (Hussain *et al.*, 2006; Bitinis *et al.*, 2011; Faghihi *et al.*, 2013). Over past decades, significant progress is made on development of clay containing PNCs using various thermosetting and thermoplastic polymer

matrix. The development of PNCs for particular class of application is best exercised through appropriate combination of filler and polymer substrates (Das *et al.*, 2011; Zuzana *et al.*, 2012).

Polymer nanotechnology (Schaefer and Justice, 2007; Yoo and Paul, 2008) is a new domain in the nanosciences. Recently, polymer nanocomposites draw attention mainly due to their spectacular hybrids properties, which are synergistically derived from the two or more components of the composite. Due to the large contact area created by nanofillers is possible to obtain polymer nanocomposites with new properties. The advantages of nanoparticles (Alexandrescu, 2014) include reinforcing efficiency with minimal loss of ductility and impact strength, thermal stability, flame retardant, improved abrasion resistance, vapor and gas permeability, low shrinkage, minimum waste. In the last two decades has been developed a large variety of new multicomponent polymeric materials. Multiphase polymer nanocomposites have been identified as the most versatile economical method to produce new resistant polymers that are able to meet the complex requirements of performance.

MATERIAL AND METHODS

Materials

In order to achieve the thermal resistance hybrid composites, the following materials were used: (1) HDPE injection ERACLENE MP 64, HD 60-70 UA (Basplast), (2) polyethylene-*graft*-maleic anhydride viscosity 500 cP (140°C)(lit.); PE-g-MA (Sigma-Aldrich Chemie, USA), (3) Standard PA for injection - Sebamid 6 s3c (Basplast), (4) Montmorillonite – mmt, nanoclay, surface modified i.31.ps, contains 0.5-5wt% aminopropyltriethoxysilan, 15-35wt% octadecylamine (Sigma-Aldrich Chemie, USA).

Method

The PE/PA hybrid composites, compatibilized with maleic anhydride grafted polyethylene – PE-g-MA (Scaffaro *et al.*, 2008), and reinforced with chemically modified layered mineral clay of the montmorillonite type argile were carried out on a Counter-rotating twin screw extruder (Machado *et al.*, 1999) granulator, TSE 35 type. Finally being processed into finished products (boards) by molding method using a Electrically heated press, considering the optimal technological parameters of processing. After stabilization for 24 hours at room temperature, the plates are submitted to physico-mechanical determinations.

The method (Figure 1) for achieving multiphase polymer nanocomposites based on PE / PE-g-MA / PA / MMT on the extruder-granulator, is as follow: polyethylene is added at 150°C and a speed of twin screws 150-200 rpm, is mixed until it becomes easy to process (softening plastics) then increase the temperature to 215°C, add PA, MMT and PP-g-MA and continue mixing at speed of 250-280 rpm until ingredients are embedded and the mixture is uniform, obtaining cylindrical granules in the end.

Figure 1. Counter-rotating twin screw extruder granulator, TSE 35 type

The obtained polymer nanocomposite granules are added in the molds, to process them according to test specimens used for physical-mechanical characterization for finished products, using the electrically heated press, TP 600, by means of compression method, between its platters at temperature of 220°C and 300KN pressure for 2 minutes preheating, 5 minutes actual forming in the press and 10 minutes cooling.

Table 1. PE, PE/PA, PE/PE-g-MA/PA samples

Samples	MU	M0	M1	M2	M3	M4	M5
Polyethylene	%	100	90	70	89	85	65
Polyamide	%	-	10	30	10	10	30
PE-g-maleic anhydride	%	-	-	-	1	5	5
Total	%	100	100	100	100	100	100

Table 2. PE/PE-g-MA/PA/MMT polymeric nanocomposite samples

Samples	MU	M6	M7	M8	M9
Polyethylene	%	88	82	84	78
Polyamide	%	10	10	10	10
PE-g-maleic anhydride	%	1	1	5	5
Montmorillonite	%	1	7	1	7
Total	%	100	100	100	100

RESULTS AND DISCUSSIONS

The structural determinations were carried out on an IR molecular absorption spectrometer with double beam, in the range of 4000-600 cm^{-1}, using a 4200 FT-IR equipped with ATR crystal diamond and sapphire head. Samples analyzed are divided into two categories:

- PE;
- Polymeric architectures (M1 – M9).

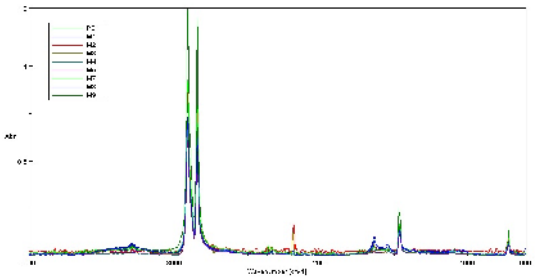

Figure 2. Overlapping IR spectra of PP / PP-g-MA / PA / MMT polymer architectures

From overlapping spectra is noted the presence of PE and PA in compounds. The presence of PA in variable percentages by the intensity of characteristic peaks is observed. Note that PE is in excess of the other components, being the main ingredient. PE-g-MA and MMT cannot distinguish so well, primarily due to the small amount (PE-g-MA max.5% and 4% -MMT).

The results of the physico-mechanical properties of the samples obtained for the polymer mixture based on polyethylene and polyamide compatibilized with PE-g-MA and montmorillonite reinforced, compared with the reference samples M0 - M5 are shown in Figures 3, 4, 5.

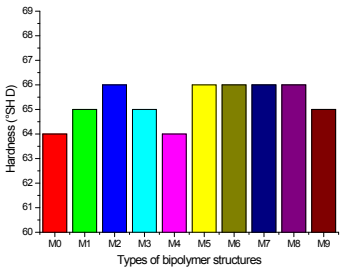

Figure 3. Variation of hardness of mixtures M0-M9

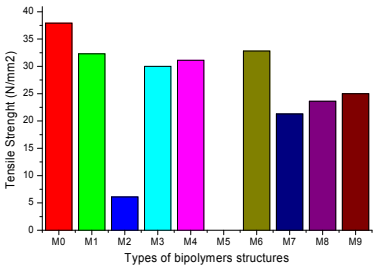

Figure 4. Variation of tensile strength of mixtures M0-M9

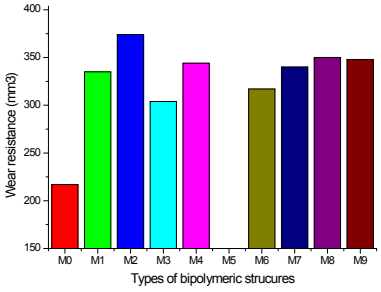

Figure 5. Variation of abrasion resistance of mixtures M0-M9

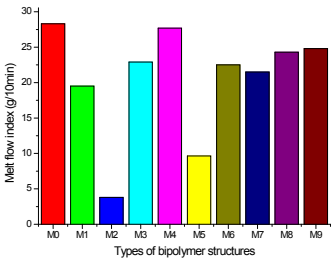

Figure 6. Variation of Melt flow index of mixtures M0-M9

Hardness (Figure 3) of polymer nanocomposites increases slightly compared to polyethylene as such – 64°Sh at 65°Sh and 66°Sh when increasing concentrations of polyamide (30%). MMT does not significantly affect the hardness values instead adding compatibilizer PE-g-MA decreases with 1°Sh hardness values, in composites this decrease is offset by increased percentage of polyamide and MMT.

Tensile strength (Figure 4) - decreases compared to the reference simple. Increase with the addition of compatibilizer PE-g-MA and MMT, but decreases with increasing the percentage of PA added in compositions. Neither the presence of MMT nor higher percentage (4%) affects the tensile strength values.

Wear resistance (Figure 5) - is within the standard, increasing with the amount of polyamide and compatibilizing agent. The presence of montmorillonite has no influence on the wear resistance.

Density - increases in proportion to the amount of polyamide added to the mixture, and the MMT has a weak influence over density values.

The more viscous materials are so requires a greater force to be extruded through MFI's die. According to the graph shown above, it is seen that the flow index polymer composites tested are highly influence by temperature, amount of reinforcing agent and the amount of coupling agent. Values of flow indexes significantly decrease proportional to the amount of PA due to increased melt viscosity, MMT compatibilizer positively influencing the flow.

CONCUSIONS

Samples of PE / PA without compatibilizer are difficult to obtain due to high melting temperature difference of the two elastomers; PE-150°C and PA-210°C, which makes the dispersion of the matrix PA on PE matrix to be achieved at high speed (300 rpm) and to be non-uniform (PA grainules can be observed in the mixture), the mixture is rigid and becomes brittle after cooling.

Introducing compatibilizer (PE-g-MA) significantly improves the mixture processing, dispersion of discontinuous phase - PA is uniform, the mixture is homogeneous, flexible and non-brittle after cooling.

Increasing the percentage of compatibilizer does not visibly improve processability.

Montmorillonite increases hardness mixture, it disperses evenly, it is not necessary to increase the rate of mixing and the samples are not brittle after cooling.

Quantities of more than 4% MMT technical do not change mixing process, proven by physical and mechanical tests.

Hardness of thermo resistance polymer nanocomposites increases slightly compared to polyethylene as such – 64°Sh at 65°Sh and 66°Sh when increasing concentrations of polyamide (30%). MMT does not significantly affect the hardness values, instead adding compatibilizer PE-g-MA decreases with 1°Sh hardness values, in composites this decrease is offset by increased percentage of polyamide and MMT.

Similar to hardness, tensile strength decreases compared to the reference simple. Increase with the addition of compatibilizer PE-g-MA and MMT, but decreases with increasing the percentage of PA added in compositions. Neither the presence of MMT nor higher percentage (4%) affects the tensile strength values.

Wear resistance increase, is within the standard, with the amount of polyamide and compatibilizing agent. The presence of montmorillonite has no influence on the wear resistance.

Density increases proportional with the amount of polyamide added to the mixture,

and the MMT has a weak influence over density values.

Values of flow indexes significantly decrease proportional to the amount of PA, but MMT compatibilizer positively influencing the flow.

Acknowledgements

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DETERMINATION OF SKIN PROPERTIES IN DIFFERENT TYPE, GENDER AND AGE USED FOR RIPENING OF CHEESE

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In traditional Tulum cheese production, sheep and goat skin sacks are commonly used. The realization of ripening process in Tulum cheese affects significantly the quality of the cheese. Considering the differences in casing materials, the ripening process, consequently the properties of the Tulum cheese are affected. In the present study, raw skin properties in different type, age and gender were investigated. Physical, chemical and structural characteristics of goat and sheep skins, which have different age (6 month, 1 and 2 years old) and gender (male and female), were determined according to thickness, tensile strength and elongation at break, tear strength, water vapour permeability, air permeability tests and, analyses such as pH, matters soluble in dichloromethane, shrinkage temperature and, Total KjeldahlNitrogen. For this purpose, 5 dry salted skins from each type, totally 60 skins were used. It was revealed that a big difference between the skin characteristics was determined depending on type of skins, ages, and genders used for ripening process. It is believed that the findings will lead to new technological advancements to create new casing production materials.

Keywords: Tulum cheese, goat skin, sheep skin

INTRODUCTION

Cheese is a food derived from cow, sheep, and goats' etc. milk and has a high content of proteins, calcium, fat, and phosphorus. Hundreds types of cheese from various countries are produced. Their production styles, textures and flavours can bevaried depending on the various factors (Kocak, 1996). Traditional cheese production is popular in Turkeydue to the eating habits of local population. One of the most produced traditional cheeses in Turkey is the "Tulum" cheese. "Tulum" means in Turkish skin sack and in traditional Tulum cheese production, sheep and goat skin sacks are commonly used. The realization of ripening process in Tulum cheese affects the quality of the cheesesignificantly. Casing material is one of the important effects on the ripening process. In literature, although there are various researches about the effects of ripening (Oluk, 2014) and casing materials on the quality of Tulum cheese, only a few studyis present regarding the skin used for casing material for the cheese production (Hayaloglu*et al.*, 2007; Gun, 2012).

The aims of this study are: to determine the casing properties of raw skins of different type, age and gender and to compare the properties of Tulum cheese ripened in these casings; to determine the properties of used and waste casings and to investigate changes in the packing material and to investigate the evaluation possibilities for another usage area. After considering the properties of raw and used casings on the properties of Tulum cheese, advantages and disadvantages of natural casings will be determined. Additionally determining the optimal properties of natural casings could lead the design and production of alternative industrial casings. Accordingly, in the first

part of the study, the characterization of the sheep and goat skins prior to Tulum cheese production was determined and their properties were compared according to their different type, age and genders.

MATERIAL

In the study, sixtydry salted raw skins, selected from different races (domestic goat and sheep skins), ages (6 months, 1 year and 2 years) and genders (male and female), were used. Each group had five repetitions. Analytical grade chemicals were used for the chemical analysis.

METHOD

The skins were cut along the backbones into halves. One part of the skins was used for the casing material. Pairing halves were tested and analyzed for determination of the skin characteristics. The skins were shaved before the tests and analysis.

Sampling was performed according to TS EN ISO 2418 standard (Anon. 2006c). The test samples were conditioned according to TS EN ISO 2419, at 23 ± 2°Ctemperature and 50% ± 5 relative humidity (Anon. 2006d).

For determination of the chemical and physical properties of the skins, TS 4117 EN ISO 2589 determination of thickness (Anon. 2006a), TS 4119 EN ISO 3376: tensile strength and percentage extension (Anon. 2006b), TS 4118-2 EN ISO 3377-2: tear load - Double edge tear (Anon. 2005a), TS EN ISO 4048: determination of matter soluble in dichloromethane (%) (Anon. 2009), TS 4120 EN ISO 3380:determination of shrinkage temperature (Anon. 2005b) and TS EN ISO 14268:determination of water vapor permeability (Anon. 2014) methods were used.

Air permeability properties were determined with air permeability test equipment (DEVOTRANS). The measurements were done at 200 Pa for 5 minutes for determining the amount of air passing through the skins from one side to other side as m^3.

Determination of Nitrogen Content and "Hide Substance" were carried out by using Kjeldahl principle.

For displaying cross sections of the samples, images were taken by Tabletop SEM (Toshiba, TM1000).

RESULTS AND DISCUSSION

Physical test results of thickness, tensile strength, percentage extension and tear load of the skin samples are given in Table 1.

The mean of thickness values for sheep skins was found 1.98 mm within a range of min. 0.95mm and max.4.84mm. The mean of thickness values for goat skins was found 1.57 mm within a range of min. 0.77mm and max. 2.55mm. The mean values of skins belonging to different age and gender were found similar for both sheep and goat skins. But, sheep skins were found thicker than the goat skins.

The mean of tensile strength and percentage extension values were found 8.78 N/mm^2, 32% and 14.3 N/mm^2, 27% for sheep skins and goat skins respectively. The tensile strength properties of goat skins were significantly higher than the sheep skins; although sheep skins had higher extension values.

The mean tear load values of sheep skins and goat skins was found 31.69 N/mm and 46.58 N/mmfor respectively. Goat skins performed better tearing strength than the sheep skins, and that might be one of the reasons that they are preferred more than the sheep skins for Tulum cheese production.

While lots of data exist in literature on the strength properties of semi-processed skins (pelts) and processed skins (leathers), no data has been found on raw materials.It could be because of the material type that is not in the form of consumer goods in that stage. But, these sheep and goat skins, used as main raw materials for leather industry, are also used in the same form for casing material of Tulum cheese production and these data may provide useful information in the search of alternative casing materials.

Table 1.Physical Test Results

	Thickness (mm)	Tensile strength (N/mm^2)	Percentage Extension (%)	Tear Load (N/mm)
Sheep, 6m, Female.	2.0	8.25	28.8	29.36
Sheep, 6m, Male	2.0	7.58	31.0	27.64
Sheep, 1y, Female	2.1	9.16	32.92	31.18
Sheep, 1y, Male	2.0	7.72	37.75	32.04
Sheep, 2y, Female	1.9	7.75	41.75	29.77
Sheep, 2y, Male	1.8	12.18	19.68	40.15
Goat, 6m, Female	1.4	12.13	19.08	34.75
Goat, 6m, Male	1.5	14.81	31.88	51.05
Goat, 1y, Female	1.7	12.38	27.82	41.67
Goat, 1y, Male	1.6	12.71	20.03	40.12
Goat, 2y, Female	1.7	15.34	32.96	59.29
Goat, 2y, Male	1.4	18.00	30.48	55.57

Water vapor and air permeability tests are related to the porosity of the material and have importance on the usage area of leather. Water vapor permeability of leathers can be changed depending on the characteristics of the structure, isolation of fiber bundles, thickness and the processes (Adiguzel and Sari, 2004). It wasrevealed that, vapor and air permeability of goat skins were higher than the sheep skins (Table 2). These results could be related to skin, fiber and hair characteristics, conservation status and fat content of the goat and sheep skins.

Table 2.Water vapor and Air Permeability Results

	Vapor Permeability ($mg/cm^2.h$)	Air Permeability From wool side (cm^3/cm^2 sec)	Air Permeability From flesh side (cm^3/cm^2 sec)
Sheep, 6m, Female.	0.56	43.97	6.07
Sheep, 6m, Male	0.54	162.90	56.27
Sheep, 1y, Female	0.31	52.93	5.70
Sheep, 1y, Male	0.65	372.27	29.97
Sheep, 2y, Female	0.41	134.60	4.93
Sheep, 2y, Male	0.41	93.40	12.73
Goat, 6m, Female	0.82	277.90	33.53
Goat, 6m, Male	0.63	196.17	14.30
Goat, 1y, Female	0.8	155.87	10.17
Goat, 1y, Male	0.46	138.00	38.90
Goat, 2y, Female	0.67	63.60	9.27
Goat, 2y, Male	0.77	169.07	13.93

Chemical characteristics of skin samples were determined in accordance with the analysis of pH, matter soluble in dichloromethane, shrinkage temperature, nitrogen content and hide substance (%)and the results were given in Table 3. The pH values of samples were found similar within the range of pH 6.3-7 without effected from type, gender and age. However, it wasdetermined that sheep skins contained more fatty substances than the goat skins and that changes were depending on the gender which female skins had more fat than the male skins.

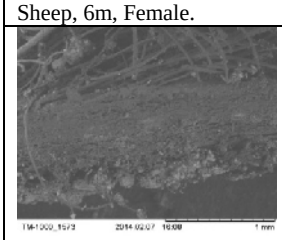
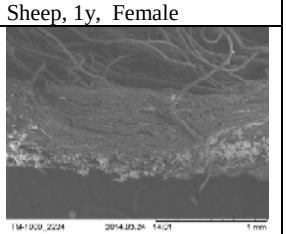
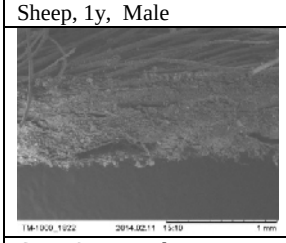
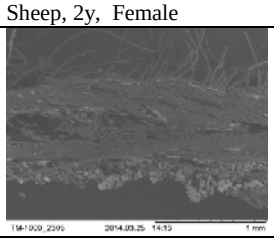
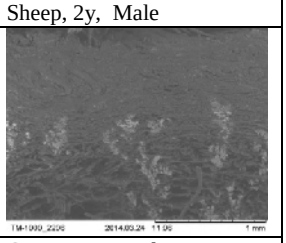
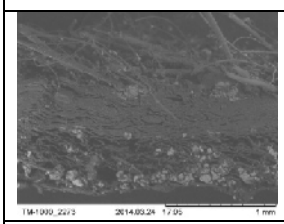
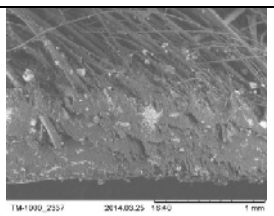
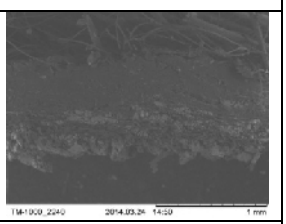
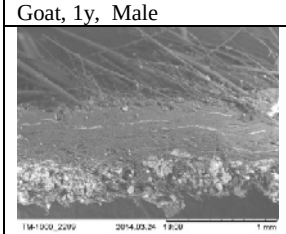
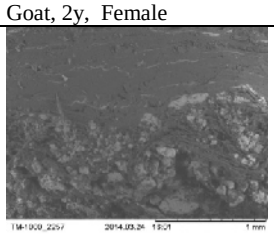
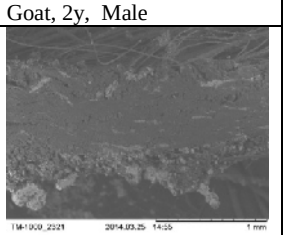
Determination of shrinkage temperature is a measure of hydrothermal stability of hide/skin and leathers which is related with fibre stabilization as a result of tanning. In practice, it is used to measure the success of the tanning (Mutlu*et al.*, 2014). In this research, shrinkage temperature was determined in order to find out if the status of the skins and also to compare the differences if any occurs after the cheese production. The measurements showed that the shrinkage temperatures of conserved raw sheep and goat skins varied between 65-70°C with an average of 68°C for sheep skins and 67°C for goat skins. Gustavson (1956) has stated that shrinkage temperatures of sheep and goat skins were between 64-66°C and 63-65°C respectively. O'flaherty*et al.* (1962) has stated that shrinkage temperatures of raw skins were 67°C in average.

When Nitrogen content and hide substance content percentages of the skins were examined, it was seen that sheep skins had lower values than goat skins. The average Nitrogen content and hide substance content percentages were found 8.7%-50.9% and 10.28%-59.24% for sheep and goat skins respectively.

Table 3.Chemical Analysis Results

	pH	Matter soluble in dichloromethane (%)	Determination of shrinkage temperature (^{0}C)	Nitrogen Content (%)	Hide Substance (%)
Sheep, 6m, Female.	6.9	15.11	67	7.33	42.89
Sheep, 6m, Male	6.8	7.0	67	8.93	52.27
Sheep, 1y, Female	6.7	8.58	70	8.75	51.21
Sheep, 1y, Male	6.6	8.99	69	8.94	52.28
Sheep, 2y, Female	6.6	13.88	67	8.16	47.71
Sheep, 2y, Male	7.0	8.29	68	10.09	59.04
Goat, 6m, Female	6.3	4.78	66	10.94	62.89
Goat, 6m, Male	6.6	5.98	70	8.90	51.81
Goat, 1y, Female	6.3	5.4	66	10.66	61.33
Goat, 1y, Male	6.6	2.77	65	10.82	62.20
Goat, 2y, Female	6.3	8.08	69	9.64	55.44
Goat, 2y, Male	6.3	4.31	70	10.74	61.77

Table 4. Displays of SEM images

Sheep, 6m, Female.	Sheep, 6m, Male.	Sheep, 1y, Female
		
Sheep, 1y, Male	Sheep, 2y, Female	Sheep, 2y, Male
		
Goat, 6m, Female.	Goat, 6m, Male.	Goat, 1y, Female
		
Goat, 1y, Male	Goat, 2y, Female	Goat, 2y, Male
		

The cross section images of sheep and goat skins can be seen in Table 4. The structural weakness and looseness and even layer separation in sheep skins can be seen from the displays. Contrarily, goat skin images show denser packed fibers. Accordingly, it is expected that these structural differences could have an effect on the properties of the casing material.

CONCLUSION

In this research, physical, chemical and structural properties of sheep and goat skins, that have different gender and age and will be used in Tulum cheese production, are determined.

Results of the present study indicate that:

- Sheep skins are thicker than the goat skins.
- The tensile strength properties of goat skins have been significantly better than sheep skins; but sheep skins have higher extension values.
- Goat skins perform better tearing strength than the sheep skins
- Water vapour and air permeability of goat skins are higher than the sheep skins.
- Sheep skins contain more fatty substances than the goat skins
- Shrinkage temperatures have an average of 68°C for sheep skins and 67°C for goat skins.
- The average Nitrogen content and hide substance content percentages are 8.7%-50.9% and 10.28%-59.24% for sheep and goat skins respectively.

The above findings will be used for the comparison of the skin properties after the Tulum cheese production and these results will be revealed the most suitable casing material for cheese production in food industry.Additionally, this will also lead alternative casing researches with the similar properties of skin samples.

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NANOMATERIALS FOR LEATHER SURFACE FUNCTIONALISATION

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Nanomaterials are an innovative research direction for the leather and footwear industry due to their high potential of replacing potentially toxic volatile organic materials and their ability to develop smart properties. The paper presents three directions for preparing nanomaterials with advanced properties compared to existing commercial products based on nano titanium dioxide (TiO_2) and nano zinc oxide (ZnO): electrochemical, hydrothermal and thermal synthesis. Nanomaterial performance was improved by doping and co-doping nano titanium dioxide in order to broaden the photoactivity range or increase the share of nanomaterials known for their thermal resistance properties. Characterization of these materials by specific techniques that highlight the crystalline structure (X-ray diffraction), particle size (DLS), absorption ability in UV-Vis (DRS), photodegradation ability of organic pollutant models (Vis spectroscopy) and thermal analysis (DSC) proves the performance of the new nanomaterials. By applying innovative technologies for leather finishing with composite polymers containing photocatalysts based on TiO_2/ZnO nanoparticles doped with metals/non-metals on can obtain leather products with advanced surface properties. The properties that these nanomaterials can transfer to leather surface are: self-cleaning, thermal resistance, biocide resistance, degradation of volatile substances, odorous substances etc.

Keywords: nanomaterials, photocatalysts, self-cleaning leather

INTRODUCTION

In the last years, studies indicated that great potential of nanoparticles (NPs) can be efficiently utilized for the manufacturing of high-added value products, including leather (Fujishima and Zhang, 2006). Semiconductor oxides TiO_2 and ZnO are investigated and used in photocatalytic oxidation of harmful organic compounds, including volatile organic compounds, as well as inorganic compounds due to the oxidative active species produced on their surface under UV irradiation, namely: free or attracted gaps, OH, O_2^- and O_2^{2-} radicals (Fujishima *et al.*, 2008). At present, research focuses on achieving a highly efficient photocatalytic action of these materials in visible spectrum light as well, in order to use the vast potential of solar photocatalysis (Rehman *et al.*, 2009). To achieve this, various functionalization techniques to make them absorb photons at a lower energy, including surface modification, doping and co-doping with metals/non-metals, were used (Rehman *et al.*, 2009). TiO_2/ZnO based photocatalysts can be used like photocatalytic coatings to decompose surface contamination, creating self-cleaning surfaces. Moreover, TiO_2 have antibacterial and antifungal properties, conferring a self-sterilizing effect to finished surfaces. To induce selfprotection properties of nanooxides on leather surface, composite materials based on acrylic polymers for film-forming on leather surface were used (Bitlisli and Yumurtas, 2008).

The present paper deals with some experimental results regarding synthesis, characterization and applications of the TiO_2/ZnO nanomaterials.

RESULTS AND DISCUSSION

Electrochemically Obtained TiO_2NPs

Electrochemical TiO_2NPs Synthesis from Choline Chloride Based Ionic Liquids

Electrochemical method consists in anodic dissolution of a Ti electrode in choline chloride based ionic liquids. System types are presented in Table 1.

Table 1. Ionic liquids systems used in TiO_2 electrochemical synthesis

No.	System type	Electrolyte composition	Working parameters
1	ILEG-IzOH	2:1 (volumes ratio) of ILEG(choline chloride:ethylene glycol 1:2 molar ratio): IzOH+ of tetrabutyl-ammonium bromide (Bu4N-/Br)	Temperature: 30-60°C Current density: 2-7 A/dm^2;
2	IL - EtOH	1:1 (volumes ratio) of IL (choline chloride:urea 1:2 molar ratio): EtOH + Bu4N-/Br	

Characterization of TiO_2 Nanopowder

- XRD investigations indicate anatase crystalline phase of TiO_2, a very high purity of powders and very fine particles.
- BET surface areas for TiO_2NPs have values of about 70 m^2/g, higher than commercial anatase TiO2. The adsorption/desorption isotherm is characteristic to mesoporous materials and the high surface area is mainly due to its nanometric size and then to the mesoporous structure.
- TEM micrographs of TiO_2NPs show nanometric dimensions of 10-20 nm, in accordance with XRD data.
- UV-Vis diffuse reflectance spectra indicates the shifting of the absorption toward the visible range of the solar spectrum, for TiO_2NPs obtained from ionic liquids, compared with commercial one, especially for TiO_2 from IL-EtOH system (Fig. 1). This behavior suggests a better photocatalytic activity under visible light illumination.

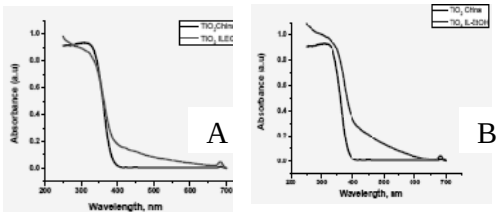

Figure 1. UV-Vis diffuse reflectance spectra of TiO_2 obtained from (A) ILEG-IZOH, (B) IL-EtOH electrolytes compared with commercial TiO_2 China

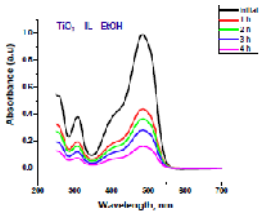

Figure 2. Absorbance spectra for a 20 ppm ORANGE II solution, in the presence of 1g/L TiO_2

Photocatalytic degradation for an azo dye, namely Orange II, with 20 ppm concentration, in the presence of 0.1% TiO_2, under visible halogen lamp (150W) illumination, indicates a very good activity (Fig. 2). Also, TiO_2NPs exhibited a significant increase in OII discoloring efficiency as

compared with commercial TiO_2, under UV irradiation. Moreover, ionic liquids are potentially recyclable, biodegradable and with no harm on human health and environment.

ZnO NPs Obtained from Ionic Liquids Media

ZnO NPs Synthesis Using Chemical Precipitation from Liquids Ionic

The electrochemical synthesis were performed in ionic liquid media based on choline chloride ($HOC_2H_4N(CH_3)_3Cl$), ethylene glycol and urea.

Table 2. Ionic liquids systems used in ZnO electrochemical synthesis

No.	System type	Electrolyte composition	Molar ratio Zinc acetate/NaoH	Zinc acetate concentration in reaction media
1	ZnO ILEG	ChCl:Ethylen-glycol-H_2O (3:1)	1:4	0.25M
2	ZnO IL	ChCl;urea-H_2O (3:1)	1:4	0.25M
3	ZnO standard	H_2O	1:4	0.25M

Characterization of ZnO Nanopowder

Composition and structure of the nanosized ZnO was analyzed by X-ray diffraction (XRD) and the visible light behaviour by UV-VIS absorption spectra recordings.

Table 3. Composition and crystallite sizes

System type	Composition, % (weight)	Crystallite size/ system	Network parameters a	c
ZnO-ILEG	ZnO – 45% NaCl – 35% Na_2CO_3 -15% Oxyclozanide ($C_{13}H_6Cl_5NO_3$) 1 – 5%	16.3nm Hexagonal	3.253Å	5.213Å
ZnO-IL	ZnO – 43% NaCl – 53% Oxyclozanide ($C_{13}H_6Cl_5NO_3$) 1 – 5%	16.1nm Hexagonal	3.253Å	5.213Å
ZnO standard	ZnO – 100%	31.6nm Hexagonal	3.253Å	5.213Å

All the obtained nanopowders present high crystallinity, in hexagonal system and have smaller size in ionic liquid media, of 16.1-16.3 nm, compared with 31.6 nm in the case of synthesis in aqueous media.

X-ray diffraction evidenced the presence of other chemical compounds, which were removed by washing.

UV-Vis diffuse reflectance spectra, evidenced the shifting of the absorption in visible area of spectrum greater for the ZnO obtained from ionic liquids than for ZnO from aqueous media, and higher for ZnO obtained from ChCl-urea than for the one from ChCl-Ethylenglycol electrolyte.

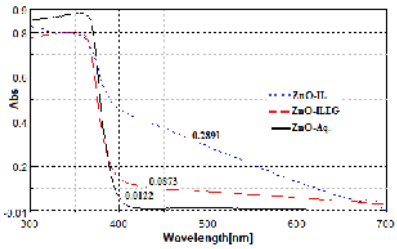

Figure 3. UV-Vis diffuse refelectance spectra for ZnO nanopowders

TiO_2 and Si Doped TiO_2 Obtained by Hydrothermal Method

Hydrothermal Synthesis of TiO_2 and Si Doped TiO_2

TiO_2Cl_2 and Na_2SiO_3 precursors were mixed, stirred and then ammoniacal solution was added to achieve the alkaline pH required for TiO_2 and Si doped TiO_2 precipitation. Suspension was transferred to an autoclave, in which, under precisely temperature and pressure working parameters, crystalline nanostructured materials were obtained. Thus, TiO_2NPs, 2% and 5%Si doped TiO_2NPs were obtained.

Characterization of TiO_2 and Si Doped TiO_2 Nanopowders

• XRD investigations indicate the main crystalline phases identified in TiO_2 and Si doped TiO_2 nanopowders and crystallite sizes (Table 3).

Table 4. Crystalline phases and crystallite sizes for TiO_2 and Si doped

System type	Crystalline phases (DRX)	Crystallite sizes (Scherrer), nm
TiO_2	91.5 % anatas; 8.5% brookit	15 nm
TiO_2-2%Si	90.6 % anatas; 7.1% brookit	16.2 nm
TiO_2-5%Si	90.1% anatas; 7.5% brookit	16.7 nm

• Differential scanning calorimetry evidenced the presence of two endothermic peaks, corresponding to the elimination of the water adsorbed on the surface (peak 1), and of the OH groups from constitution water (peak 2). In addition, an exothermic peak was observed (peak 3), which could be attributed, to the growth of grains (Table 4).

Table 5. Results obtained from thermal analysis (DSC method)

System type	Peak 1 (endotherm)		Peak 2 (endotherm)		Peak 3 (exotherm)	
	T, °C	ΔH, J/g	T, °C	ΔH, J/g	T, °C	ΔH, J/g
TiO_2-1	74.7	54.89	-	-	523.9	-8.374
TiO_2-2%Si	77.7	120.5	308.8	1.577	557.5	-1.932
TiO_2-5%Si	60.2	59.71	232.2 / 323.7	1.129/ 1.169	564.2	-0.605

These results indicate that 2% Si doped TiO_2 could induce heat and fire protection for leather treated with them.

Leather Surface with Self-Cleaning Properties Induced by NPs

The new synthesized nanoparticles were used for leather surface finishing. The procedure consisted in ultrasound mixing of solid nanoparticles in polymer finishing solutions and then spraying it on the leather surface. The surface properties of the treated leather stained with 0.05 mL of 200 ppm MB and pen ball ink lines were analyzed over time.

Photo-Degradation of a MB Spot on Leather Surface

The degradation of the MB dye on leather surface, under UV and visible light irradiation was done with Datacolor Check II Instrument based on CIELab color coordinates and recording of parameters: L* (lightness), a*(red-green color), b*(yellow-blue), C* (chroma) and h* (hue angle). Comparative representation of the L* parameter variation, which measures the lightness of a color, from completely opaque (0) to completely transparent (100) is represented in Fig. 4 and Fig. 5.

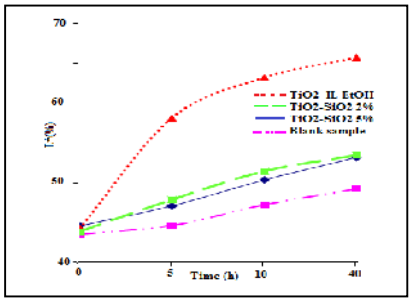

Figure 4. L* variation for treated leather in UV light

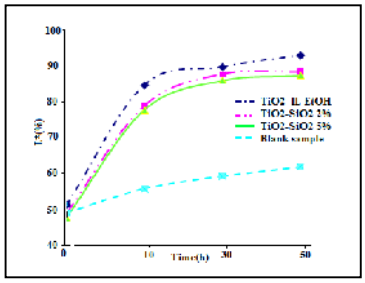

Figure 5. L* variation for treated leather in visible light

While, in UV illumination TiO_2-IL has a better photocatalytic activity than hydrothermally obtained Si-TiO_2, in visible light, all samples degrade MB dye spots.

Photographic Images of Photodegraded Leather Samples

Figures 6 and 7 present photographic images of the leather treated with polymeric composite based on TiO_2-5% SiO_2 (Fig. 6), TiO_2 obtained from IL-EtOH system (Fig. 7) and untreated leather (Fig. 8).

The experiments confirmed the photocatalytic activity increasing under Vis light exposure in the case of doped nanoparticles on leather surface.

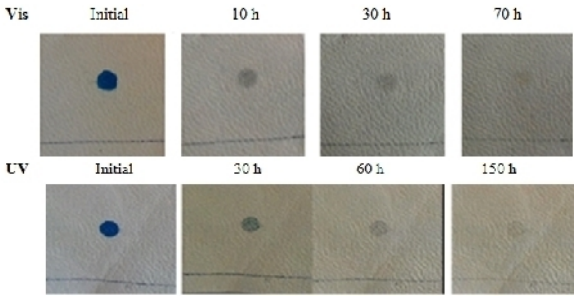

Figure 6. MB degradation onto a TiO_2-5% SiO_2 treated leather surface

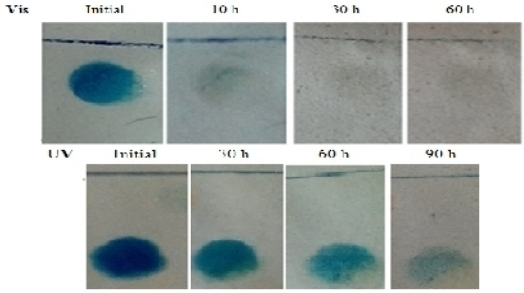

Figure 7. MB degradation onto a treated leather surface using TiO_2 from IL-EtOH

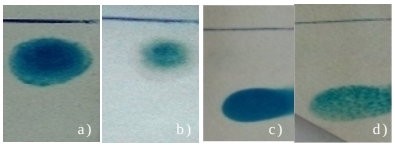

Figure 8. Dye degradation onto untreated leather: Vis light: a) initially, b) after 120h of exposure; UV light: c) initially, d) after 120h of exposure

CONCLUSIONS

The experimental results evidenced a new technique for TiO_2 and ZnO NPs synthesis from ionic liquid media with high photocatalytic activity, both in UV and visible light. TiO_2 doped with Si obtained by the hydrothermal method can provide heat and fire protection for leather treated with them. The self-cleaning properties were confirmed by colorimetric measurements for the MB spots applied to the treated leather surface exposed to UVA (λ=365nm) and visible light irradiation.

The self-cleaning leather surface using nanomaterials as photocatalysts represents a step forward in innovative upholstery material development with improved durability.

Acknowledgments

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PREPARATION AND APPLICATION OF SODIUM SILICATE COMPOUND SWELLING AGENT

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Low modulus sodium silicate was prepared with a polysilicon byproduct-silicon tetrachloride, and used as a swelling agent. Calcium chloride, hydrazine and protease were selected successfully as auxiliaries. Then dosages of the auxiliaries were optimized through the relative weight increase, relative thickness increase, proteoglycan removing and histology study. Based on the auxiliary optimization experiment, a compound swelling agent was prepared and the swelling ability was investigated with a scanning electron microscope and proteoglycan removing test. Finally, the environmental impact of the swelling process was evaluated by total solid of the swelling effluent. The results showed that, when the swelling agent was consisted of 4.0% sodium silicate, 1.0% hydrazine and 0.2% protease, the relative weight increase, relative thickness increase and proteoglycan removing ability reached the highest value, meanwhile fibers were also fully opened. Compared with the traditional liming process, a less total solid value was presented in the swelling effluents. The results could provide a new way for silicon tetrachloride recycling, also provide valuable references for cleaner swelling process.

Keywords: swelling, sodium compound swelling agent, polysilicon

INTRODUCTION

The fiber opening process determines the penetration of chemicals in leather making, also affect the sensory and physical properties of leather. However, some disadvantages of traditional liming are always mentioned by leather chemists, including lower solubility and sludge in liming effluent.

To overcome disadvantages of traditional liming process, many kind of lime-free fiber opening agents was discovered. One of the most famous lime-free swelling agents was sodium silicate. It had been proved that the fiber opening ability of sodium silicate was comparable to that of conventional process. Meanwhile, sodium silicate will not have any negative impact for leather. Compared with the traditional liming process, lower COD, BOD_5 and total solid (TS) data was presented in swelling effluent (Subramani *et al.*, 2008).

In this study, a polysilicon byproduct-silicon tetrachloride was used as a raw material for sodium silicate preparation. Then calcium chloride, hydrazine and protease were selected and optimized successfully as component of silicate compound swelling agent. Fiber opening ability and environmental impact of this compound swelling agent was also studied. The results could provide valuable references for both polysilicon and leather industry.

EXPERIMENTAL

Materials

Wet salted goatskins were selected as raw material. All the chemicals used in leather making process were commercial grade. Silicon tetrachloride was a polysilicon

byproduct collected from Yongxiang polysilicon LLC. The 2709 protease was purchased from Long Kete LLC. Chemicals used for the analysis were all research grade.

Preparation of Sodium Silicate

100g silicon tetrachloride was added into 300g ice water (T≤4°C), and then heated at 120°C to remove the hydrochloric acid. A 10mL water and equal weight sodium hydroxide were added then sodium silicate solution was finally prepared. The properties of this sodium silicate solution were analyzed according to GB/T 4209-2008, the results showed the content of Na_2O and SiO_2 were 12.92% and 15.87% respectively, modulus was 1.27, total dissolved solid was 28.76% and pH was 13.50.

Application of Sodium Silicate and Auxiliaries

Goat skins were soaked conventionally, and soaked skins were unhaired with 1398 neutral protease following a conventional dip and pile method. The unhaired pelts were cut into pieces along the backbone, and then used for the following trials. 4% sodium silicate, X% auxiliary, and 300% water (based on the weight of unhaired pelt) were added into the drum for swelling. The drums were run for 5min per hour in 7 hours then left overnight. The control was prepared following a conventional liming process.

Measurement of Swelling Pelt Weight and Thickness Increase

The relative weight increase was calculated as follows:

$$\frac{w_2 - w_1}{w_1} \times 100\% \tag{1}$$

where w_1 - weight of unhaired pelt, w_2 - weight of swelled pelt.

The relative thickness increase was calculated as follows:

$$\frac{d_2 - d_1}{d_1} \times 100\% \tag{2}$$

where d_1 - thickness of unhaired pelt, d_2 - thickness of swelled pelt.

Scanning Electron Microscope Analysis

The unhaired pelt was cut into two sides along the backbone. One side was prepared with silicate compound swelling agent (4% sodium, 1.0% hydrazine, 0.20% 2709 protease) and then takes as sample. The other side was prepared with a traditional liming process as a control. The samples were then dehydrated with ethyl alcohol and observed with a JSM-5900 scanning electron microscope (Philips Company).

Analysis of Proteoglycans in Waste Liquors

100mL of swelling effluent was filtrated and then analyzed for proteoglycan content with a standard method (Mantle and Allen, 1978).

Analysis of Total Solid Content in Waste Liquors

100mL of swelling effluent was collected and analyzed for total solid (TS) content following the standard procedure (Clesceri *et al.*, 1989).

RESULTS AND DISCUSSION

Optimization of the Kind of Auxiliaries

Table 1. Effect of auxiliaries on the proteoglycan removal

Sample	Sodium silicate-$CaCl_2$	Sodium silicate-hydrazine	Sodium silicate-2709 protease	Sodium silicate	Lime
Proteoglycan removal / (mg/L)	41.67	52.59	55.17	39.12	32.68

The effect of auxiliaries on the proteoglycan removal was shown in Table 1. It indicated that compared with the traditional liming process, sodium silicate swelling agent presented a better proteoglycan removal ability. Furthermore, a significant increase of proteoglycan removal was found while hydrazine and 2709 protease were used as auxiliaries. Furthermore, except the Sodium silicate-$CaCl_2$ group, proteoglycan removal ability was all raised with the addition of auxiliaries. Therefore, hydrazine and protease were selected as auxiliaries of sodium silicate compound swelling agent.

Optimization of the Dosage of Hydrazine

Table 2. Effect of hydrazine dosage on the swelling ability

Dosage of hydrazine / (%)	Relative weight increase / (%)	Relative thickness increase / (%)	Proteoglycan removal / (mg/L)
0.5	58.90	50.44	42.34
1.0	70.26	76.4	56.66
1.5	60.11	60.75	52.39
2.0	64.34	50.58	51.62

Table 2 indicated that the relative weight increase, relative thickness increase and the proteoglycan removal ability were correlated with the dosage of hydrazine. Swelling ability was raised with the dosage increasing until 1.0%. Therefore, 1.0% of hydrazine was selected as auxiliary of sodium silicate compound swelling agent.

Optimization of the Dosage of 2709 Protease

Table 3. Removal of proteoglycan and pelt property of sodium silicate with different dosage of 2709 protease

Dosage of 2709 protease / (%)	Relative weight increase / (%)	Relative thickness increase / (%)	Proteoglycan removal / (mg/L)
0.10	56.33	80.74	49.85
0.15	67.86	78.11	31.59
0.20	70.42	93.33	60.33
0.25	46.15	69.2	49.23

Table 3 indicated the swelling ability of the compound swelling agent was affected by the content of protease. Meanwhile, the highest relative weight increase, relative thickness increase and proteoglycan removal ability was obtained while the content of protease was 0.20%. Therefore, 0.20% of protease was selected as auxiliary of sodium silicate compound swelling agent.

Evaluation of Fiber Opening

Table 4. Proteoglycan removal of pelt

Sample	Lime	Sodium silicate compound swelling agent
Proteoglycan removal/(mg/L)	33.29	57.44

Proteoglycan removal ability of compound swelling agent was shown in table 4. It was evident that the proteoglycan removal ability of compound swelling agent was better than that of control. A better fiber opening ability was obtained with the compound swelling agent (Figure 1).

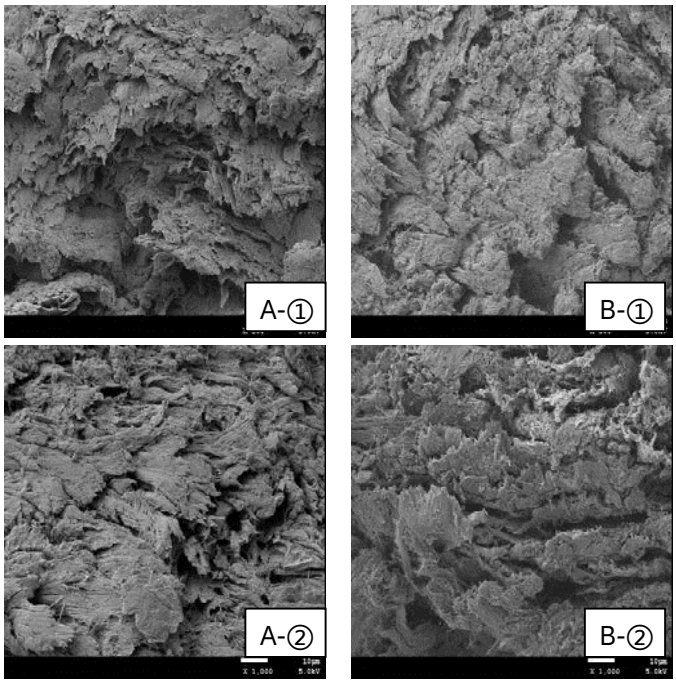

Figure 1. Scanning electron micrographs of swelled pelts (A: sample, B: control; 1:×500, 2:×1000)

Environmental Impact

Table 5. TS in waste fiber opening liquor

Sample	Lime	Sodium silicate compound swelling agent
TS/(g/100mL)	1.5763	1.0547

As shown in Table 5, the TS value of experimental fiber opening process was lower than that of control. The TS loading in experimental fiber opening liquor was decreased by 33% compared with the control process. Lime was the major contributor for TS in the waste liquor in conventional alkali swelling process due to the low solubility of lime. For the experimental fiber opening process, the reduction in TS is mainly due to the elimination of lime. An equivalent spent liquor after fiber opening for experimental and control process was collected and placed for a week. It could be seen clearly that there was a lot of lime residual in control liquor while there was only hair and broken little skin pieces in the experimental liquor. Hence, there was a significant reduction in the TS loading for experimental process.

CONCLUSIONS

A polysilicon byproduct-silicon tetrachloride was used as a raw material for sodium silicate preparation. Meanwhile Calcium chloride, hydrazine and protease were selected as auxiliaries and the content of each auxiliary was optimized. The results showed that, when the swelling agent was consisted of 4.0% sodium silicate, 1.0% hydrazine and 0.2% protease, the relative weight increase, relative thickness increase and proteoglycan removing ability reached the highest value, meanwhile fibers were also fully opened. Compared with the traditional liming process, a less total solid value was presented in the swelling effluents. The results could provide a new way for silicon tetrachloride recycling, also provide valuable reference for cleaner swelling process.

Acknowledgement

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OFF-AXIS MECHANICAL PROPERTIES OF FRP COMPOSITES

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Composite structures made of fibre reinforced polymer (FRP) composites are usually built-up of several individual unidirectional laminas which may have their natural material axes at different orientations with respect to the loading direction. Off-axis mechanical properties of the unidirectional FRP lamina can be determined either experimentally or predicted theoretically. One way to theoretically predict the off-axis stiffness and strength properties of a unidirectional orthotropic lamina is by applying the macromechanical concepts. This paper presents the available macromechanical approaches utilized to calculate the off-axis stiffness and strength properties of a unidirectional orthotropic lamina for which the loading directions are different from the principal material axes. In addition, a case study is presented, in order to apply the macromechanical tools to a FRP lamina made of glass fibres and epoxy matrix.

Keywords: FRP composite lamina, off-axis strength properties, off-axis stiffness properties.

INTRODUCTION

From macromechanical point of view, the off-axis mechanical properties of the unidirectional FRP composites are anisotropic, due to their variation with respect to the orientation of the reference plane. The aim of the macromechanical approach is to correlate the stiffness and strength properties along an arbitrary direction with the basic properties of the unidirectional FRP composite referred to its principal material directions (Daniel and Ishai, 2006). FRP composite laminates consist of two or more laminas, bonded together so that they can act as integral structural elements (Agarwal *et al.*, 2006). For this reason the understanding of the individual lamina characteristics should precede the analysis of the laminated structures theory.

Orthotropic Laminas

A lamina or a ply consists of a flat or curved arrangement of unidirectional fibers embedded in a support matrix and it represents the basic element of a composite material. For the orthotropic lamina, the material axes are perpendicular and stand as symmetry planes.

Generally Orthotropic Lamina

The generally orthotropic lamina is that for which the material axes (1, 2) do not coincide with the global coordinates axes (x,y), that may be the axes of the loading directions (Barbero, 2011). The material axes are rotated with respect to the reference system by angle Θ, as presented in Figure 1.

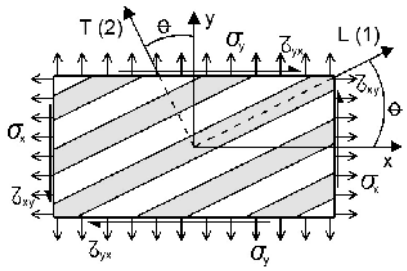

Figure 1. Generally orthotropic lamina

The constitutive equations for the generally orthotropic lamina are presented in Equations 1 and 2.

$$\{\sigma_i\} = \left[\overline{Q}_{ij}\right]\{\varepsilon_i\} \tag{1}$$

$$\{\varepsilon_i\} = \left[\overline{S}_{ij}\right]\{\sigma_i\} \tag{2}$$

where, $\{\sigma_i\}$ and $\{\varepsilon_i\}$ are the components of the stress and strain matrices, respectively. The matrices $\left[\overline{Q}_{ij}\right]$ and $\left[\overline{S}_{ij}\right]$ are the reduced transformed stiffness and compliance matrices, respectively. The elements $\overline{Q}_{ij}$ and $\overline{S}_{ij}$ are functions of the elastic properties of the lamina along its principle axes (1,2) and of the fibre orientation angle, Θ.

Stiffness Properties

Axial Modulus of Elasticity, E_x

Assuming that the only nonzero stress component acting on the lamina is σ_x, the axial modulus of elasticity (E_x) can be expressed in terms of the engineering constants in the principal material coordinates and of the fibre orientation Θ.

$$E_x = \frac{1}{\frac{1}{E_1}c^4 + \left(\frac{1}{G_{12}} - 2\frac{\nu_{12}}{E_1}\right)s^2 c^2 + \frac{1}{E_2}s^4} \tag{3}$$

where, E_1, E_2 and G_{12} are the axial and shear moduli of elasticity in the principal material axes, ν_{12} is the first Poisson's coefficient and the trigonometric functions *sinΘ* and *cosΘ* are denoted with *s* and *c*, respectively.

The variation of the axial modulus of elasticity is presented in Figure 2. It can be seen that the values of E_x decrease as the angle between the material axes and the global coordinates axes increases, between E_1 and E_2.

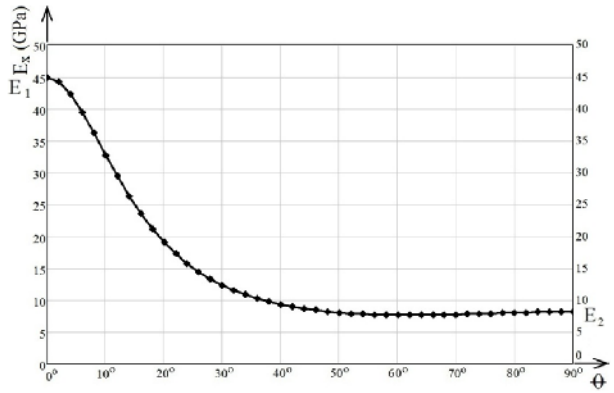

Figure 2. Variation of E_x with respect to Θ

Axial Modulus of Elasticity, E_y

Imposing that the only stress component different from zero is σ_y, the axial modulus of elasticity E_y can be also expressed with respect to the fibre inclination angle Θ and to the elastic properties of the lamina along its principal axis.

$$E_y = \frac{1}{\frac{1}{E_1}s^4 + \left(\frac{1}{G_{12}} - 2\frac{\nu_{12}}{E_1}\right)s^2 c^2 + \frac{1}{E_2}c^4} \tag{4}$$

Figure 3 presents the variation of E_y with respect to angle Θ. Unlike the case of the longitudinal modulus of elasticity in x direction, the interval between 0° and 60° is characterized by a smaller rate of increase while in the 60°-90° interval, E_y has the highest rate of increase. Appling Equation 4 for Θ = 0° and Θ = 90°, E_y equals E_2 and E_1, respectively.

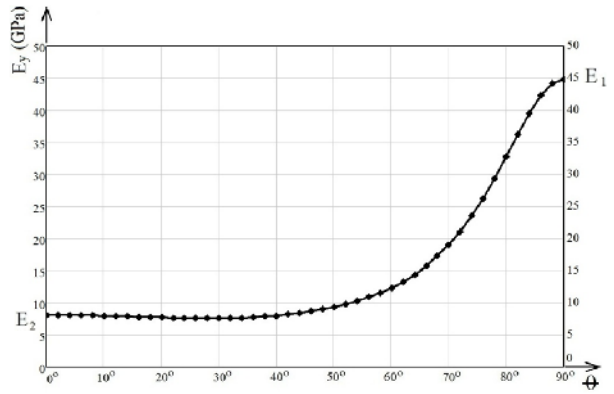

Figure 3. Variation of E_y with respect to Θ

Shear Modulus of Elasticity, G_{xy}

The shear modulus of elasticity can be calculated under the assumption of pure shear state of stress. In this case, the only non-zero stress component is τ_{xy}; the shear modulus of elasticity, G_{xy} can be also expressed as a function and of the elastic properties of the lamina in its principal directions and of the fibres inclination angle.

$$G_{xy} = \frac{1}{2\left(\frac{2}{E_1}+\frac{2}{E_2}+\frac{4\nu_{12}}{E_1}-\frac{1}{G_{12}}\right)s^2c^2+\frac{1}{G_{12}}(s^4+c^4)} \tag{5}$$

The variation of the shear modulus of elasticity is presented in Figure 4. It can be seen that G_{xy} has the highest values when Θ is 45° while G_{xy} equals G_{12} when Θ is 0° or 90°.

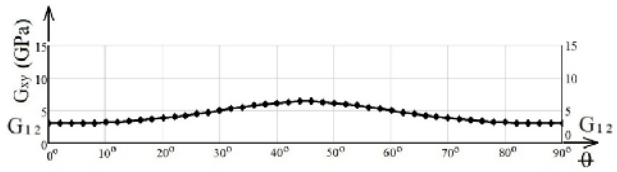

Figure 4. Variation of G_{xy} with respect to Θ

Poisson's Ratios, ν_{xy}, ν_{yx}

The first Poisson's ratio ν_{xy}, can be calculated considering that the only nonzero stress component is σ_x (Equation 6), while the second Poisson's ratio ν_{yx}, can be obtained when σ_y is different from zero, (Herakovich, 1998), (Equation 7).

$$\nu_{xy} = \frac{\left[c^2s^2\left(1+\frac{E_1}{E_2}-\frac{E_1}{G_{12}}\right)-(c^4+s^4)\nu_{12}\right]}{\left[c^4+c^2s^2\left(-2\nu_{12}+\frac{E_1}{G_{12}}\right)+s^4\frac{E_1}{E_2}\right]} \tag{6}$$

$$\nu_{yx} = \frac{\left[c^2s^2\left(1+\frac{E_1}{E_2}-\frac{E_1}{G_{12}}\right)-(c^4+s^4)\nu_{12}\right]}{\left[s^4+c^2s^2\left(-2\nu_{12}+\frac{E_1}{G_{12}}\right)+c^4\frac{E_1}{E_2}\right]} \tag{7}$$

Figure 5 presents the variation of ν_{xy} and ν_{yx} with respect to the inclination angle of the fibres, Θ. The first Poisson's ratio has the highest values when Θ is 29° and equals ν_{12} or ν_{21} when Θ is 0° or 90°, respectively. Similarly, the second Poisson's ratio has the same values as ν_{12} or ν_{21} when the inclination angle of the fibres is 0° or 90° but ν_{yx} reaches its maximum value when Θ is 61°.

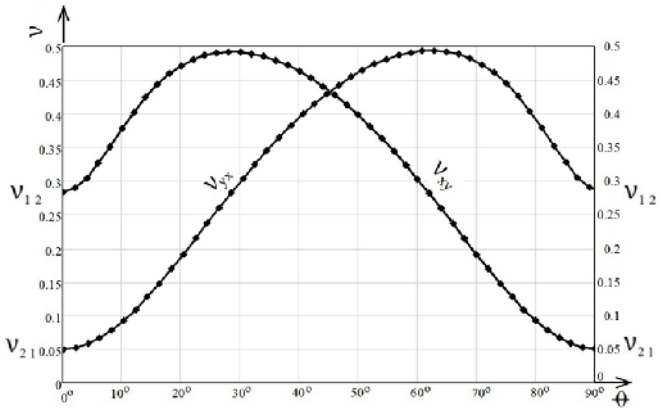

Figure 5. Variation of ν_{xy} and ν_{yx} with respect to Θ

Strength Properties

Off-axis Tensile Strength

The maximum tensile strength along any direction can be calculated with Equation 8 which is derived from the Tsai-Hill failure criterion (Kaw, 2005).

$$f_{x(\Theta)t} = \frac{1}{\sqrt{\frac{c^4}{f_{Lt}^2} + \frac{s^4}{f_{Tt}^2} + c^2 s^2 \left(\frac{1}{f_{Lts}^2} - \frac{1}{f_{Lt}^2} \right)}} \tag{8}$$

where, f_{Lt} and f_{Tt} are the longitudinal and transverse tensile strength of the lamina along its principle directions and f_{LTs} is the in-plane shear strength of the lamina.

CASE STUDY

Determine the mechanical properties in the global coordinates system (x,y) of the following 45° angle unidirectional E glass / Epoxy composite (Taranu et. al., 2013). The properties of the lamina along its principal axes are: f_{Lt} = 900 MPa, f_{Tt} = 19.5 MPa, f_{LTs} = 25.9 MPa, E_1 = 44.30 GPa, E_2 = 6.77 GPa, G_{12} = 2.95 GPa, ν_{12} = 0.278 and ν_{21} = 0.053.

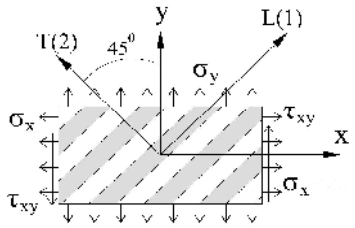

Figure 6. 45° angled unidirectional E glass / Epoxy composite lamina

Because Θ = 45° (s = c) it results that $E_x = E_y$ and $\nu_{xy} = \nu_{yx}$.

$$E_x = \frac{1}{\frac{1}{E_1}c^4 + \left(\frac{1}{G_{12}} - 2\frac{\nu_{12}}{E_1}\right)s^2 c^2 + \frac{1}{E_2}s^4} = 8052.9MPa = E_y \quad (3)$$

$$G_{xy} = \frac{1}{2\left(\frac{2}{E_1} + \frac{2}{E_2} + \frac{4\nu_{12}}{E_1} - \frac{1}{G_{12}}\right)s^2c^2 + \frac{1}{G_{12}}(s^4 + c^4)} = 5469.4MPa \quad (5)$$

$$\nu_{xy} = \frac{\left[c^2s^2\left(1 + \frac{E_1}{E_2} - \frac{E_1}{G_{12}}\right) - (c^4 + s^4)\nu_{12}\right]}{\left[c^4 + c^2s^2\left(-2\nu_{12} + \frac{E_1}{G_{12}}\right) + s^4\frac{E_1}{E_2}\right]} = 0.365 = \nu_{yx} \quad (6)$$

$$f_{x(\Theta)t} = \frac{1}{\sqrt{\frac{c^4}{f_{Lt}^2} + \frac{s^4}{f_{Tt}^2} + c^2s^2\left(\frac{1}{f_{Lts}^2} - \frac{1}{f_{Lt}^2}\right)}} = 31.15MPa \quad (8)$$

CONCLUSION

This paper presents the macromechanical approach that can be applied to determine the off-axis stiffness and strength properties of FRP composite laminas. These theoretical methods of predicting the properties of an FRP product subjected to a certain state of stress having reference directions different from the materials principal ones, can turn to be effective not only from the economical point of view but also from the time consuming one.

Experimental determinations for various inclination angles (Θ) of the fibers orientation are prohibitive and difficult to be carried out. Moreover, the off-axis properties of an FRP composite lamina should be previously determined by theoretical approaches followed by selective experimental tests aiming to validate these results.

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SYNTHESIS AND SWELLING PROPERTIES OF POLY[ACRYLAMIDE-*co*-ACRYLIC ACID] SUPERABSORBENTS OBTAINED BY ELECTRON BEAM IRRADIATION

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The aim of this study is to investigate the gel fraction, sol fraction, water absorbency and crosslink density of superabsorbents based on polyacrylamide/acrylic acid. Superabsorbents were prepared by free radical co-polymerization in aqueous solution of acrylamide with acrylic acid and different concentration of initiator at room temperature (25°C). Samples were subjected to electron beam treatment with doses ranging between 3 and 4.5 kGy.

Keywords: copolymerization, acrylamide, acrylic acid, superabsorbent, electron beam.

INTRODUCTION

Superabsorbent polymers were defined as three-dimensional networks of hydrophilic polymers that can absorb and retain a significant amount of water. In agriculture, superabsorbent polymers are especially used for soil conditioning and to increase the efficiency of fertilization. Thus, this class of materials has been developed to improve the physical properties of soils by: increasing water holding capacity and efficiency of its use, increasing soil permeability and stopping their erosion, decreasing the frequency of irrigation, reducing the tendency to form crust, increasing agricultural performance especially on unstructured soils, reducing fertilizer losses and fostering their uptake by plants (Baker *et al.*, 1994; Mihailescu *et al.*, 2004; Mihailescu *et al.*, 2007; Seybold, 1994;). Three main types of hydrogels (synthetic soil conditioners) have so far been developed as agricultural polymers: (1) starch-graft copolymers obtained by graft polymerization of polyacrylonitrile onto starch followed by saponification of the acrylonitrile units (2) cross-linked polyacrylates (3) cross-linked polyacrylamides and cross-linked acrylamide-acrylate copolymers (Ekebafe *et al.*, 2011). Most of the hydrogels marketed for agriculture come from the latter group as they are claimed to remain active for a much longer time (Ekebafe *et al.*, 2011). Cross-linked polyacrylamides hold up to 400 times their weight in water and release 95% of the water retained within the granule to growing plants (Ekebafe *et al.*, 2011). Radiation initiation of chemical reactions has been increasingly used for creation of novel hydrogels. A radiation technique is more preferable than a chemical one, because of the advantage to control gently the level of crosslinking by variation of the absorbed dose. This method offers unique advantages for the synthesis of new and modification of existing materials: it is a simple, additive-free process at all temperatures, reactions such as polymerization, crosslinking and grafting can easily be controlled, and the treatment can be limited to a specific area (Karadag *et al.*, 2004). Recent articles reports a series of methods for obtaining these superabsorbent copolymers with a view to enhance their absorbency, gel strength, and absorption rate. Hekmat *et al.* have synthesized a

hydrogels by using acrylamide free radical, potassium acrylate, and linear polyvinyl alcohol (Hekmat *et al.*, 2009). Ammonium nitrate was used (loaded) in the hydrogel as the fertiliser. Sayeda M. Ibrahim et all. (Sayeda *et al.*, 2007) obtained a superabsorbent hydrogels based on crosslinked carboxymethyl cellulose polymer and acrylamide monomer by electron-beam irradiation.

The aim of this study is to investigate the swelling properties of polyacrylamide/ acrylic acid hydrogels. Hydrogels have been prepared by electron beam irradiation at room temperature (25°C). The influence of absorbed dose on gel fraction and swelling behavior was investigated.

EXPERIMENTAL

Materials

In order to obtain the hydrogels, the following materials (Table 1) have been used: acrylamide (molar mass 71.08 g mol^{-1}; density 1.13 g/cm^3); acrylic acid (molar mass 72.06 g mol^{-1}; density 1.051 g/mL) and potassium persulfate (molar mass 270.322 g mol^{-1}; density 2.477 g/cm^3) - serves as initiator in the copolymerization process. All materials were procured from E-Merck, Germany.

Table 1. Used monomers in preparation of hydrogels

	Formula	Abbreviations
Acrylamide	$H_2C=CH-CONH_2$	AMD
Acrylic acid	$H_2C=CH-COOH$	AA
Potassium persulfate	$K_2S_2O_8$	PP

Preparation and Irradiation of the Samples

Series of hydrogels having different compositions of AMD and AA were synthesized as given in Table 2.

Table 2. Synthesis details of poly(acrylamide-co-acrylic acid) hydrogels

Sample code	AMD (mol/l)	AA (mol/l)	$K_2S_2O_8$ (mol/l)	Dose (kGy)
H_{1-1}	5	0.5	0.7×10^{-3}	3
H_{1-2}				3.5
H_{1-3}				4
H_{1-4}				4.5
H_{2-1}	5	0.5	3.5×10^{-3}	3
H_{2-2}				3.5
H_{2-3}				4
H_{2-4}				4.5
H_{3-1}	5	0.5	7×10^{-3}	3
H_{3-2}				3.5
H_{3-3}				4
H_{3-4}				4.5

Experimental Installation and Sample Irradiation

Experiments were carried out with an experimental installation consisting mainly of the following units: an electron linear accelerator (ALIN-10) of 6.23 MeV energy and

75 mA peak current of the electron beam and an irradiation chamber containing the samples of monomer solution. The ALIN 10 is a travelling-wave type, operating at a wavelength of 10 cm and having 164 W maximum output power. The optimum values of the EB peak current I_{EB} and EB energy E_{EB} to produce maximum output power P_{EB} for a fixed pulse duration τ_{EB} and repetition frequency f_{EB} are as follows: E_{EB} = 6.23 MeV, I_{EB} =75 mA, P_{EB} = 164 W (f_{EB} = 100 Hz, τ_{EB} = 3.5 μs). The EB effects are related to the absorbed dose (D) expressed in Gray or J kg^{-1} and absorbed dose rate (D*) expressed in Gy s^{-1} or J kg^{-1} s^{-1}. Electron beam dose rate was fixed at 2.4 kGy/min in order to accumulate doses between 3-4.5 kGy and samples were irradiated in atmospheric conditions and at room temperature of 25°C.

RESULTS AND DISCUSSION

In the present study, the hydrogels have been obtained by maintaining a fixed concentration of AMD and AA in the reaction mixture and varying only the concentrations of $K_2S_2O_8$ (as crosslinker) and the absorbed dose. The following parameters were determined: the soluble fraction, the gel fraction, the water absorbency and the crosslinking density.

Gel Fraction and Sol Fraction

Samples of the prepared hydrogels were accurately weighed (W_0), extracted with distilled water and then dried in a vacuum oven at 80°C to a constant weight (W_1). The soluble fraction was calculated according to the following equations (Nizam *et al.*, 2007):

$$Sol\_fraction(\%) = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

$$Gel\_fraction(\%) = 100 - Sol\_fraction \quad (2)$$

where W_0 is the initial weight of dried sample and W_1 is the weight of sample after extraction with water and dried.

The results presented in Figures 1 and 2 show that when both EB dose and initiator concentration increase, there is a decrease of soluble fraction and an increase of gel fraction (crosslinked polymer content).

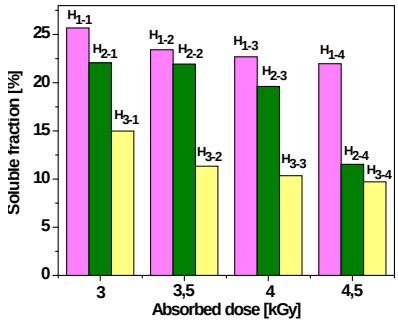

Figure 1. Soluble fraction versus absorbed dose and PP concentration

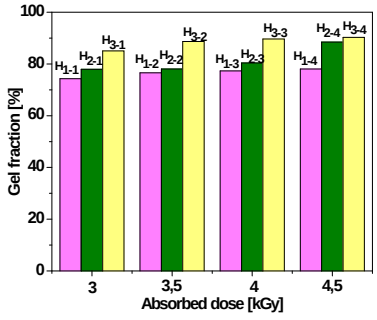

Figure 2. Gel fraction versus absorbed dose and PP concentration

The highest values for soluble fraction and gel fraction were obtained for the blends with the concentration of 7 x 10^{-3} PP and irradiated at 4.5 kGy. The addition of initiator significantly increases gel fraction. Thus, in an irradiation cure system, the gel content and soluble fraction of samples increases with increase in irradiation dose. This is due to the formation of a three-dimensional network structure.

Water Absorbency

The swelling measurements of the copolymer were carried out in water at room temperature. Two hundred grams of distilled water was added to 0.1 g of the dry copolymer in a 400 cm^3 glass beaker covered with a glass lid. The polymer was allowed to swell for 24 h. The fully swollen gel was then separated from the unabsorbed water by filtering it through a 100-mesh sieve aluminum screen for 2 h at room temperature and the swollen copolymer gel was then weighed (Yiamsawas *et al.*, 2007). The water absorbency was calculated as shown below:

$$Water\_absorbency(Q) = \frac{W_2 - W_0}{W_0} \quad (3)$$

where W_0 is the weight of the dry polymer (g) and W_2 is the weight of the water swollen gel (g).

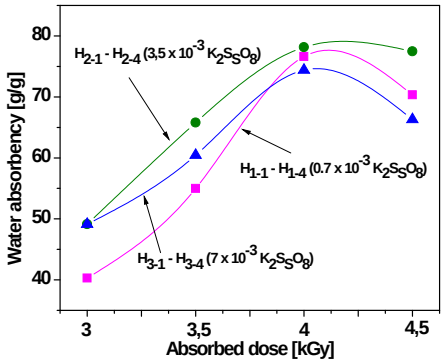

Figure 3. Water absorbency versus absorbed dose and PP concentration

The results on water absorbency of the copolymers synthesized by electron beam irradiation with various concentration of PP are shown in Figure 3. At high initiator concentrations, more crosslinks can be formed to give rigid chains that reduce the swelling of the gel. More than that, the absorption of water depends on the irradiation dose at which the samples have been obtained. It was found that maximum water absorption occurs for the samples obtained with 4 kGy. For irradiation with upper doses the absorption of water decreases, because in addition to the crosslinking reactions degradation reactions occur.

Crosslinking Density

For a polymer network, crosslinking densities of the copolymer were determined using the Flory–Rehner theory (Yiamsawas *et al.*, 2007) as follows:

$$M_C = -V_1 d_P \frac{\nu_S^{1/3} - \nu_S/2}{\ln(1-\nu_S) + \nu_S + \chi \nu_S^2} \quad (4)$$

where: M_C is the number-average molar mass of the chain between crosslinks; V_1 is the molar volume of the solvent, in this case water = 18 cm^3 mol^{-1}; d_P is the polymer density (g cm^{-3}); υ_S is the volume fraction of the polymer in the swollen gel (cm^3) and is equal to 1/S; χ is the Flory–Huggins interaction parameter between the solvent and the polymer. The value χ was taken from the literature (Yiamsawas *et al.*, 2007; Ding *et al.*, 1991; Karadag *et al.*, 1997) as follows:

$$\chi = 0.431 - 0.311\nu_S - 0.036\nu_S^2 \quad (5)$$

To determine the equilibrium volume swelling (S), it is necessary to place a sample of known density into water until mass measurements indicate the cessation of the uptaken liquid by the polymer (Yiamsawas *et al.*, 2007). If no extractable is present and all the imbibed solvent causes swelling, the volume swelling, S, is given by:

$$S = \frac{(W_2 - W_0)/d_S}{W_0/d_P} \quad (6)$$

where W_0 and W_2 are the same parameters defined earlier, d_S and d_P are the densities of water and polymer, respectively.

Here, the crosslink density, *q*, is defined as a mole fraction of the crosslink units.

$$q = \frac{M_0}{M_C} \quad (7)$$

where Mo is the molecular weight of the polymer repeating unit and is calculated using the relation (Karadag *et al.*, 1997).

$$M_0 = \frac{(m_1 \times M_1) + (m_2 \times M_2) + (m_3 \times M_3)}{m_1 + m_2 + m_3} \quad (8)$$

where m_{AMD}, m_{AA} and m_{PP} are the mass in g of acrylamide, acrylic acid and the initiator, and M_{AMD}, M_{AA} and M_{PP} are the molar mass in g mol^{-1} of acrylamide, acrylic acid and the initiator, respectively.

Table 3. Variation of the volume swelling (S), the volume fraction of the polymer in the swollen gel (υ_S), the Flory–Huggins interaction parameter (χ), the number-average molar mass of the chain between crosslinks (Mc) and the crosslink density (q) with PP content and irradiation dose

Samples	S	$\upsilon_{S\ (cm^3)}$	χ	M_c (g mol^{-1})	M_0 (g mol^{-1})	qx10^4
H_{1-1}	96,38	0,0104	0,4278	470.211	136,10	2,89
H_{1-2}	94,03	0,0106	0,4277	450.094	136,10	3,02
H_{1-3}	76,63	0,0131	0,4269	312.353	136,10	4,36
H_{1-4}	70,35	0,0142	0,4266	267.786	136,10	5,08
H_{2-1}	120,35	0,0083	0,4284	695.402	136,37	1,96
H_{2-2}	109,62	0,0091	0,4282	590.203	136,37	2,31
H_{2-3}	74,60	0,0134	0,4268	297.647	136,37	4,58
H_{2-4}	77,48	0,0129	0,4270	318.638	136,37	4,28
H_{3-1}	74,61	0,0134	0,4268	297.743	136,70	4,59
H_{3-2}	70,19	0,0142	0,4266	266.729	136,70	5,13
H_{3-3}	74,40	0,0134	0,4268	296.211	136,70	4,61
H_{3-4}	78,67	0,0127	0,4270	327.434	136,70	4,17

Table 3 shows that the number-average molar mass between the crosslinks of hydrogels increases with PP content and irradiation dose. Since AAm and AA in hydrogels includes many hydrophilic moieties (nonionizable and ionizable), AMD/AA hydrogels can swell significantly. Crosslink density is reverse due to the value of the number-average molar mass between crosslinks.

CONCLUSIONS

This study was carried out to illustrate the synthesis of poly[acrylamide-co-acrylic acid] superabsorbents in the presence of different concentration of initiator under the effect of electron beam irradiation. The characteristics of the superabsorbents are influenced by the chemical composition and the electron beam absorbed dose. In an irradiation cure system, the gel content and the soluble fraction of samples increases with increase of irradiation dose. This is due to the formation of a three-dimensional network structure. The water absorbency of the crosslinked copolymer was measured by swelling in distilled water at room temperature. At high initiator concentrations ($K_2S_2O_8$), more crosslinks could be formed to give rigid chains that reduce the swelling of the gel. The number-average molar mass between crosslinks of hydrogels increases with $K_2S_2O_8$ content and irradiation dose. Crosslink density is reverse due to the value of the number-average molar mass between crosslinks.

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NANOPARTICLES AND DEPOSITION METHOD FOR PHOTOCATALYTIC TEXTILES AND DURABLE WOOD

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This paper presents the research results obtained in ERA NET MANUCOAT project, coordinated by INCDTP in collaboration with the following partners: INCDMNR-IMNR, SC MGM STAR CONSTRUCT SRL –Romania and IRIS-Spain. Requirements for the textiles with multi-functional properties and durable wood are increasingly higher. New photocatalytic textiles with sensitivity in the visible spectrum, antibacterial and antifungal properties and wood with increased durability to environmental conditions were developed. To this aim, the obtaining of nanostructured undoped and Ag-doped TiO2 powders with characteristics that enable their deposition by physical methods and with an extended absorption in the visible region and establishment of the technological parameters for physical deposition technique represents the main issue. The innovative elements presented consist in the development of hydrothermal technology to obtain doped TiO2 NPs Anatase with extended absorption in the visible region and increase of the photo degradation rate and the manufacturing of new flexible, smooth nanostructured layers on textile and wood materials through improved physical method (plasma electro-spay, RF sputtering). The influence of different nanoparticles composition and coating methods of textile and wood is discussed through the main analyses and their results: contact angle, SEM/EDX, surface and volume resistivity, degradation rate of the methylene blue/methyl orange, antifungal and antibacterial effect, washing fastness.

Keywords: TiO_2 nanoparticles, sputtering method, photocatalytic textiles

INTRODUCTION

During the past several years the demand of different textiles with multifunctional properties such as self-cleaning and antibacterial was intense. Also TiO_2 NPs was selected for it photocatalytic activity, non-toxicity, high availability, biocompatibility, and low price. In order to obtain textile materials with antimicrobial performances, many procedures are used: impregnation of the fibrous material with a solution, suspension or emulsion of the bactericidal (fungicidal) product; padding of an antimicrobial product, from its soluble state into an insoluble one on the fibrous material; binding of an antimicrobial product on the fiber through chemical bonds (ionic, coordinative, covalent); immersion of a bactericidal product either in the spinning solution or melt, during preparation of the chemical fibers (Coman *et al.*, 2010).

The increasing environmental concerns and demands for an environmentally friendly processing of textiles leads to the development of new technologies like

physical methods for deposited of NPs on textiles and wood substrate: plasma electrospray and RF sputtering.

In the same respect we are develop the synthesis of TiO_2 nanoparticles is an economical alternative to relatively expensive and highly polluting multi-steps wet chemical processes. Based on the relationships between the physical and chemical characteristics of doped titanium and their photocatalytic and antibacterial performance revealed by the literature data, the powders selected to be deposited on textile and wood substrates and their expected properties are: titanium Anatase phase amount > 95%, crystallite size at the nanoscale, silver dopant type, dopant concentration 0,5-2,0 mol%, photocatalytic activity in the visible range and antibacterial properties (Gupta *et al.*, 2013).

EXPERIMENTAL PART

Nanoparticles

The Anatase TiO_2 nanoparticles or TiO_2 doped with Ag were synthesized through an innovative hydrothermal technology in aqueous media, at low temperatures and high pressures in one step without any further thermal treatment.

The characteristics of NPs synthesized and used in our experiments are:

- TiO_2 nanoparticles: Anatase = 26.16 nm, Brookite = 8.19 nm in percentage of 98% Anatase and 2% Brookite;
- TiO_2 doped with silver nanoparticles: Anatase = 23.13 nm, Brookite = 25.73 nm and Silver = 59.90 nm in percentage of 93.5% Anatase, 6.0% Brookite and 0.5% Silver.

Textiles and Wood

Perla fabric designed for curtains, covers and other uses in public spaces. Diferent Wood samples

Physical Deposition Methods

For the electrospray deposition the nanoparticles was dispersed into a liquid that is forced with a controllable solvent pump to go through an electrified capillary, typically made of steel. Before nanoparticles deposition the fabric surface was activated in DBD atmospheric plasma, non-thermal, in order to improve nanoparticles adhesion on surface, decontaminate fabrics prior to deposition, removing or decreasing the microorganism load, clean the surface from dirt or fats. The speed of fabric was between 1-10 m/minute. For this deposition method, IRIS Spanish partner up-scaling the equipment at pilot scales.

The sputtering deposition was made in the vacuum equipment VU-2M that were pilot up-scaled, by sputtering circular source TORUS 2'' HV, radio frequency power supply R301 MKII, automatic matching network EJAT3 and matching network controller EJMC2.

The nanoparticle of titanium dioxide and silver doped titanium dioxide were sintered in targets that were bonding with an electrically conductive silver-filled epoxy paste on copper backing plates. The Bonding Sputtering Targets has many benefits for the sputtering process: faster transfer heat and the possibility to be used even after the target crack occurs. A glow discharge process, plasma treating is necessary for cleaning and

activating the surfaces prior to deposition. The sputtering process occurs in vacuum and argon atmosphere.

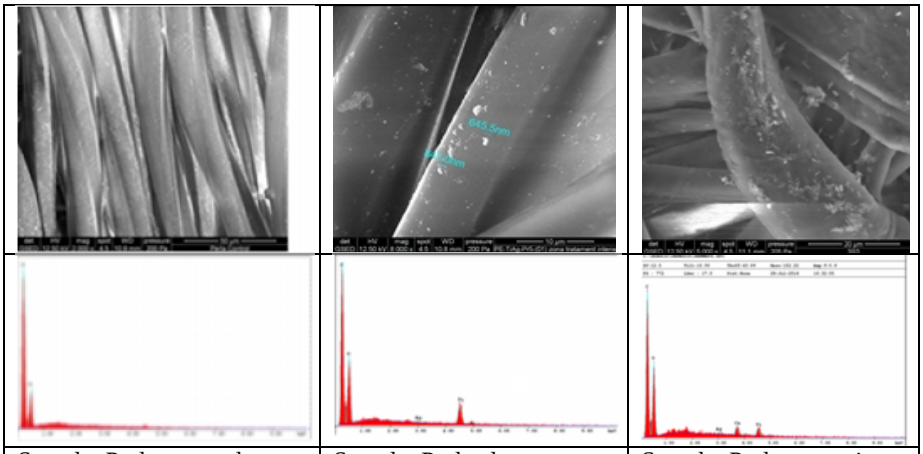
The assessment of characteristics of samples treated with TiO_2 Ag1% by electrospray and sputtering methods was made in terms of SEM images, EDX pattern, phisical-mecanical properties, bactericidal action and self-cleaning effect.

The methods used for tests are scanning electron microscopy (SEM) and EDX spectra, photocatalytic activity after stained with Methyl Orange (MO) and Methylene Blue (MB), Antibacterial effect was assessment according SR EN ISO 20743:2013-Textiles. Antibacterial activity of textile products, SR EN ISO 20645:2005-Textile fabrics. Antibacterial activity control. Agar diffusion plate test and ASTM E2149-10 Standard test method for determining the antimicrobial activity of immobilized agents under dynamic contact conditions.

RESULTS AND DISCUSSION

SEM/EDX Analyses

The SEM/EDX images of Perla sample treated with TiO_2Ag1% by electro-spray and sputtering are shown in figure 1.



Sample: Perla -control	Sample: Perla electro spray TiO_2Ag1% (5m/minute)	Sample: Perla sputtering TiO_2Ag1% (10 minute)

Figure 1. SEM and EDX images for sample Perla

- SEM images for electro- spray deposition at fabric speed 5m/minute, show a non uniform deposition of nanoparticles in the form of clusters; the size of nanoparticles agglomerations are 640÷840nm; EDX identified Ti in smaller amounts and Ag characteristic peaks are not visible;

- SEM images for 10 minute Sputtering deposition, show a non uniform deposition of nanoparticles; on the surface of samples the amount of Ti is 7.65% by weight and the amount of Ag i is 1.74% by weight;

Regarding to samples wood treated by sputtering, these was stained very hard.

Physical-Mechanical Characteristics

The physical-mechanical characteristics, tensile strength, elongation, tear strength and abrasion resistance, were determined only on electro-spray NPs deposition sample. The tensile strength and elongation aren't influenced by plasma treatment. A small influence is on tear strength and abrasion resistance is greatly enhanced. Also surface and volume resistivity is not significantly influenced by plasma treatment and electrospray deposition, table 1.

Table 1. Physical-mechanical characteristics of Perla electro spray TiO_2Ag1%

Sample / Characteristics		Perla Control	Perla electrospray TiO_2Ag1% (5m/minute)
Tensile properties, N	Warp	1687	1659
	Weft	854	850
Elongation, %	Warp	52.3	51.7
	Weft	39.4	42.2
Tear force, N	Warp	71.2	61.0
	Weft	55.4	51.4
Abrasion resistance by the Martindale method, cycles		38931	49441
Surface resistivity, Ω		2.98×10^{13}	1.84×10^{13}
Volume resistivity, Ω		1.08×10^{13}	1.84×10^{13}

Antibacterial Effect

Perla samples treated with TiO_2/Ag1% by sputtering presents inhibitory effect after 1h and 24 h for Gram-negative strains (Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumani, Klebsiella pneumoniae) and Gram-positive strains (Enterococcus faecalis, Staphylococcus aureus) and also for Candida albicans.

Photocatalytic Effect

The photocatalitic effect was evaluated by the color difference between treated samples and control sample according to standard EN ISO 105-B02:2013 Textiles - Tests for color fastness - Part B02: Color fastness to artificial light: Xenon arc fading lamp test (ISO 105-B02:2013). The half covered samples are exposed to light in Xenon Arc lamp light fastness tester equipped with a xenon lamp, suitable filter systems to simulate visible light. The light fastness of the dyes, measuring the degree to which a dye resist fading due to the light exposure is evaluated on grey scale (note from 1 to 5; 5 is best, meaning the dye is not destroyed by the light and 1 is worst, the dye is destroyed by light) or blue scale (1-8; 8 best). TiO_2, being a photocatalytic compound, accelerate the degradation. Consequently, if the dyes (methyl orange or methylene blue) show a high degradation noted by a small grade (e.g. 1 or 2) the textile material coated with TiO_2 Ag1% has a high ability to absorb visible light and to destroy the dye being a very good photocatalyst.

In parallel to the above mentioned Standard photocatalytic activity can be measurement with Hunterlab equipment (more objective).

The grade of photocatalytic activity to methylene blue and methyl orange of Perla sample treated by electrospray with TiO_2/Ag1% at a fabric speed of 5m/minute is presented in figure 2.

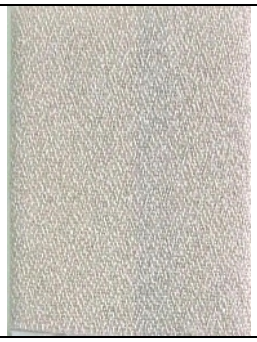
Color grade difference	Perla sample-Control	Perla sample stained by dipping 5 minutes in methylene blue (c=100 mg/L)
		
ISO 105 B02- grade	3-4	3
Color difference on Hunterlab equipment	4	3
Color grade difference	Perla sample-Control	Perla sample stained by dipping 5 minutes in methyl orange (c=100 mg/L)
		
ISO 105 B02- grade	4	2-3
Color difference on Hunterlab equipment	4	1.5

Figure 2. Images after 7h Xenon lamp exposure an grade of photocatalytic activity to of Perla sample treated by electrospray with TiO_2/Ag1%

Taking into account both measurements, it could be concluded that Perla samples treated by electro-spray with stable solution of TiO_2/Ag1% have a good an efficient photocatalytic activity.

CONCLUSION

The intermediaries' tests allow to conclude:

Perla samples treated with TiO_2/Ag1% by sputtering presents total inhibition of grows for all strains: Gram-negative strains (Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumani, Klebsiella pneumoniae) and Gram-positive strains (Enterococcus faecalis, Staphylococcus aureus) and also for Candida albicans;

Perla samples treated by electro-spray with stable solution of TiO_2/Ag1% have a good an efficient photocatalytic activity;

Samples wood treated by sputtering with TiO_2/Ag1% was stained very hard;

Physical-mechanical characteristics of sample are not affected by the plasma treatment applied before NPs deposition both by electrospray and sputtering.

As a general conclusion the result of treatment with TiO_2 and TiO_2 doped with Ag depends on substrate characteristics and the treatment technique should be selected according the application field of textile and wood.

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SR EN ISO 20645:2005-Textile fabrics. Antibacterial activity control. Agar diffusion plate test;

SR EN ISO 20743:2013-Textiles. Antibacterial activity of textile products;

NEW PIGMENT PASTE FOR LEATHER FINISHING

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Environmental problems that the leather industry faces today regarding leather finishing include restrictions on the use of heavy metals in pigment pastes, ethoxylated alkylphenols, formaldehyde and other toxic crosslinking agents. Environmental and toxicity related concerns have led to new alternatives for leather finishing auxiliaries. The quality of pigment pastes used, playing a major role in obtaining leather finishing film, influences some physical-mechanical, technological, aesthetic and ecological properties, which, cumulated, confer value of use and commercial appearance to various leather items: footwear, garments, bags and upholstery. This paper presents a study on the physical-chemical characterization and ATR-FTIR spectroscopy of a new pigment paste and leather finishing composition made using the new pigment paste, acrylic binder and biodegradable non-ionogenic emulsifier (which replaces nonylphenol ethoxylate). The finishing film obtained using the new finishing composition shows higher thermal stability compared to those currently used, as evidenced by differential thermal analysis (DTA).

Keywords: leather, finishing, pigment paste

INTRODUCTION

Leather finishing is done using disperse systems which contain the following auxiliaries: pigments, binders, dyes, natural and synthetic waxes, preservatives, plasticizers, thickening agents, fillers, odoriferous substances, penetrating agents, solvents (Lange, 1982; Heideman, 1994).

The quality of pigment pastes used, playing a major role in obtaining leather finishing film, influences some physical-mechanical, technological, aesthetic and ecological properties, which, cumulated, confer value of use and commercial appearance to various leather items: footwear, garments, bags and upholstery (Chirita and Chirita, 1999; Urban, 2002). Pigments are organic or inorganic chemical compounds which constitute the dye base for coatings.

Pigments used in leather finishing must have certain characteristics, among which the most important are: fastness to light, resistance to weathering and high temperatures, bright and vivid color, high coating power, high dispersion degree, compatibility with the other components of coating dyes.

In leather finishing operations there are restrictions regarding the use of heavy metals in pigment pastes, ethoxylated alkylphenols, formaldehyde and other toxic crosslinking agents (OSPAR, 2004; Triderma, 2010; Veco, 2010).

Environmental and toxicity related concerns have led to new alternatives for leather finishing auxiliaries (Niculescu and Leca, 2007).

The paper presents the characterization of a new pigment paste by physical-chemical analyses and ATR-FTIR spectroscopy, compared to pigment paste used in industrial production. The paper also presents the development of a finishing composition made using the new pigment paste and characterization by differential thermal analysis (DTA) of films obtained on glass from the finishing composition, compared to coating films

obtained according to current leather finishing processes. For the new finishing composition the following chemicals were used: non-ionic tensioactive agent – lauryl alcohol ethoxylated with seven moles of ethylene oxide, which replaces nonylphenol ethoxylated with 9 moles of ethylene oxide, which is used usually in leather finishing. Its utilization in industrial production was forbidden by the Directive 76/769/EL/2003, as a consequence of assessment of its ecotoxicity which shows a 30% biodegradability only.

EXPERIMENTAL

Materials

Black iron oxide (Pebeo, France), content of Fe_2O_3 – 94%, bulk density – 0.8-1.2 g/cm^3, water absorption – 32% g/g, particle size – 0.6±0.1µm.

Acrylic binder Bindex Brillant (Pebeo, France), homogenous emulsion, dry substance – 30.24 %, density – 1.965 g/cm^3, pH – 6.5, Hoppler viscosity – 4.000 cP.

Castor oil (S.C. Happynatura SRL, Bucharest), total fatty matters – 64%, viscosity Ford cup Φ 6 – 57 s, saponification index – 14 mg KOH/g, acidity index – 9 mg KOH/g, iodine index – 92g 100/g oil.

Nonionic emulsifier – lauryl alcohol ethoxylated with 7 moles of ethylene oxide (SC Elton Corporation SA, Bucharest), density at 40°C – 0.950 g/cm^3, pH (10%) solution – 7-8. Wax emulsion AGE 7 used as handle modifier (made from beeswax, lanolin and triethanolamine monostearate and stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide: dry substance – 12%, pH (10% solution) – 7.0 (Niculescu *et al.*, 2013).

Roda-cryl 87, marked AC87, (Triderma, 2010), acrylic binder for ground coat, dry substance – 38.92%, pH (10% solution) – 6.0, Ford cup viscosity Φ4 – 14.5, density – 1.036 g/cm^3.

Black pigment paste (Roda Casicolor Black), viscous and homogenous fluid, dry substance – 22.45%, pH (10% solution) – 6.5-8.0, ash – 12.24%.

Methods

Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR) measurements were run with a Jasco instrument 4200 model, in the following conditions: wave number range – 4000-600 cm^{-1}; data pitch – 0.964233 cm^{-1}; data points – 3610: aperture setting – 7.1 mm; scanning speed – 2 mm/s; number of scans – 30; resolution – 4 cm^{-1}; filter – 30 kHz; angle of incident radiation – 45°.

Simultaneous Thermal Analysis of TG with DTA mode (ΔT) and DSC (mW) were run with a Perkin-Elmer instrument STA 6000 model; temperature: 25-950°C, heating rate 10°C/min.

Obtaining the New Pigment Paste

The formulations and methodology for obtaining the pigment pastes are described in Niculescu *et al.* (2013) and the composition is presented in Table 1.

Materials used for obtaining of new black pigment paste are:

- black iron oxide pigment, which is not toxic;
- acrylic polymer, which replaces protein binders used in the compositions of pigment pastes, thus eliminating crosslinking with formaldehyde, which is toxic;

- non-ionic tensioactive agent was used – lauryl alcohol ethoxylated with seven moles of ethylene oxide; for blank pigment paste nonylphenol ethoxylated with 9 moles of ethylene oxide was used as tensioactive agent;
- wax emulsion made from beeswax, lanolin and triethanolamine monostearate and stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide;
- castor oil used as plasticizer.

Table 1. The composition of new pigment pastes

New pigment paste composition	Quantities
Black iron oxide, (%)	30
Polyacrylic binder, %	40
Ethoxylated lauric alcohol, %	9
Castor oil, %	9
Wax emulsion, %	3
Water, %	9

Obtaining the Finishing Film on Glass Plate

Finishing compositions were prepared containing: 100 g/L new pigment paste (for sample) / pigment paste Roda Casicolor Black (for blank); 30 g/L wax emulsion; 300 g/L acrylic binder; 570 g/L water. With these dispersions, finishing films were obtained by deposition on glass plate and dried on air.

RESULTS AND DISCUSSION

Characterization of Pigment Pastes

New pigment pastes were characterized by physical-chemical analyses and ATR-FTIR spectroscopy.

Physical-chemical characteristics are presented in the Table 2.

Table 2. Physical-chemical characteristics of pigment pastes

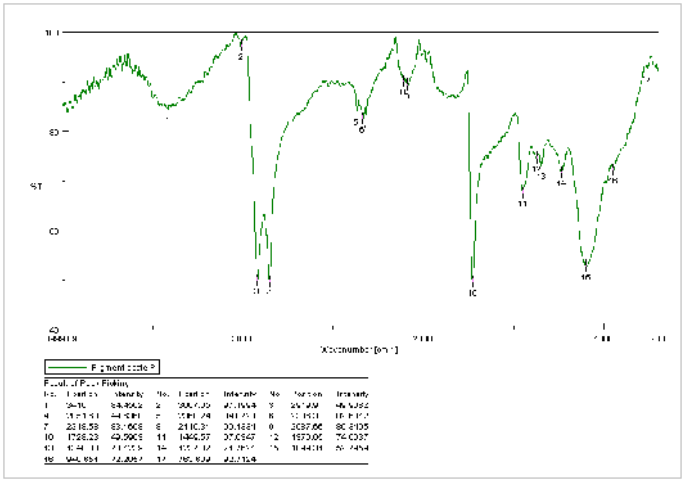
Characteristics / samples	New pigment paste	Blank Roda Casicolor Black
Dry substance, %	30.67	22.45
pH 10% solution	6.8	6.5-8
Ash, %	23.42	12.24

The new pigment pastes are viscous and homogeneous fluids and dry substance content indicates that they are more concentrated pastes. They are stable over time, without sediments of phase separation and have the characteristics of concentrated pastes.

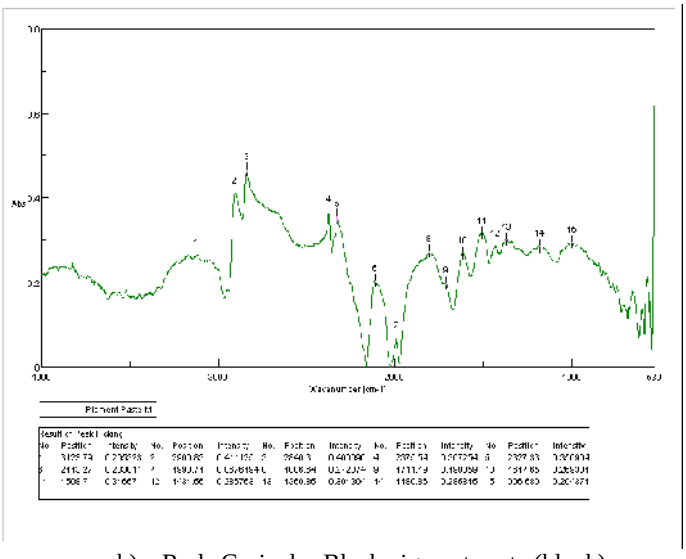
Rheological behavior of ecological pigment paste has been presented before (Niculescu *et al.*, 2014).

Characterization of Pigment Pastes by FT-IR

Both pigment pastes (new and blank), dried on the glass plate, were analyzed by ATR-FTIR and spectra are shown in Figure 1.



a) New pigment paste



b) Roda Casicolor Black pigment paste (blank)

Figure 1. ATR-FTIR spectrum for pigment pastes

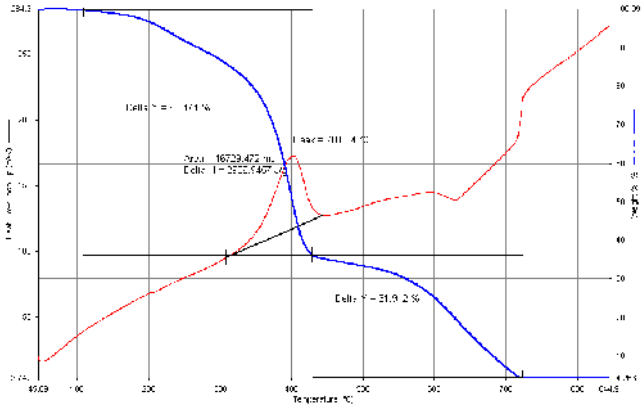
The ATR-FTIR spectrum from Figure 1 presents all the bands characteristic to acrylic polymers: in the range 3200-3500 cm^{-1}, a broad weak band assigned to carboxyl OH group, which usually overlaps the -NH band attributed to amide group. At 2919-

2851 cm^{-1}, 1449 cm^{-1} assigned to stretching and deformation vibrations of CH_3 and CH_2 groups. An intense band around 1728 cm^{-1} due to stretching of C=O groups from esters, and at 1099 given by ether groups. Both pigment pastes (new and blank) contain all the bands characteristic to acrylic polymers.

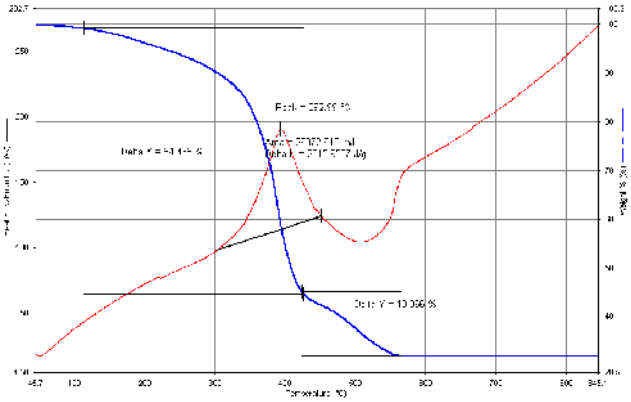
Characterization of Finishing Films

The finishing films obtained by deposition on glass plate of finishing compositions containing new pigment paste (Figure 2a) and Roda Casicolor Black pigment paste (Figure 2b) were characterized by DTA.

Figure 2 a and b presents TG and DTA curves for finishing films obtained by depositing on glass and drying, for the new pigment paste a), and b) for the finishing film used in industrial leather production.



a) Finishing film containing new pigment paste



b) Finishing film containing Roda Casicolor Black pigment paste (blank)

Figure 2. TG and DTA diagram for the finishing film obtained on the glass plate

Latent heat graphs show that the decomposition temperature for finishing film containing new pigment paste is 401.04°C and for finishing film with Roda Casicolor Black pigment paste (blank) is 394.79°C.

For finishing film containing new pigment paste, the temperature interval for mass loss is 110-725°C and mass loss is 63.474% in the temperature interval 110-430°C and 31.912% in the temperature interval 430-725°C. Total degradation of the finishing film occurs at the temperature of 725°C.

For finishing film containing Roda Casicolor Black pigment paste (blank) the temperature interval for mass loss is 118-565°C and mass loss is 54.435% in the temperature interval 118-425°C and 13.066% in the interval of 425-565°C. Total degradation of the finishing film occurs at the temperature of 565°C.

The specific thermal degradation parameters show that finishing film containing new pigment paste has an increased thermal stability compared with classic finishing film.

CONCLUSIONS

The new pigment paste has better characteristics than classic pigment paste used in leather finishing. Finishing film obtained with finishing composition containing new pigment paste is more stable at temperature as observed by DTA.

The research will continue with finishing tests on leather and test of biodegradability of the finishing composition /film in order to determine environmental impact.

Acknowledgements

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POLYMERIC NANOSTRUCTURES BASED ON POLYOLEFINS AND RUBBER FOR THE FOOTWEAR INDUSTRY

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Designing and constructing materials of technical and engineering interest with preset physical-chemical and mechanical properties have commanded the attention of researchers and engineers since the beginning of the technological era. Polymeric nanostructures based on rubber and plastics were identified as the best method to produce new polymeric materials able to satisfy complex performance requirements. Over the last few years, the global tendency to develop new advanced hybrid polymeric materials from a mixture of polymers (elastomers and plastomers: EPDM with thermoplastic polyolefins) and reinforcement agents with nano-sized particle has given new possibilities of extending their area of application. Nanostructures based on thermoplastic polymeric compounds – EPDM/polyolefins/nanoparticles – were selected because one polymer alone cannot meet all requirements regarding mechanical, physical and chemical properties. Thus, we combined characteristics of the two polymers, such as chemical resistance; low water permeability; resistance to high temperatures, ozone and radiation; flexibility at low temperatures; colour stability; processability properties adapted to the injection technology; green and waste-free technology; reduced working time; low energy consumption for processing into finished products; recirculation of material in approximately five cycles without changing its properties etc. Performance of polymeric nanostructures depends on the concentration and morphology of the elastomer and plastomer used, processing parameters, type and concentration of auxiliary materials used in compounding, the equipment and working parameters used in compounding, etc.

Keywords: polymeric nanostructures, EPDM, polyolefins, nanoparticles.

INTRODUCTION

Footwear manufacturers require advanced materials to process and usage, which resulted in the development of new polymeric structures with nanostructured reinforcing agents with optimized properties to conventional used materials in this area (Vilsan *et al.*, 2009). In this context, it has developed this theme of making a nanostructured material that combines the specific properties of each elastomer to obtain products with predetermined features, depending on the application. These polymeric structures obtained by combining in, different rubber proportions, polyolefin and nanoparticles, will be process to fit for use in the footwear industry and consumer goods (Sonmez *et al.*, 2014; Kurahatti *et al.*, 2010) .

In recent years, the worldwide trend of obtaining new advanced hybrid polymer consisting of a mixture of polymers (elastomers: EPDM thermoplastic polyolefin) and reinforcing agents, offers new possibilities to extend the aim of application (Stelescu *et al.*, 2013; Ionescu *et al.*, 2008; Manaila *et al.*, 2007). Polymeric structures based on thermoplastic polymers / EPDM ware selected as a single polymer cannot satisfy all the

requirements required for assembly of mechanical, physical and thermal features, needed in a number of specific applications. In this way, it will combine the characteristics of the materials used, such as chemical resistance, low water permeability of thermoplastic polymer with excellent resistance to heat, ozone and sunlight, very good flexibility at low temperatures, resistance to alkalis and acids, steam and water low permeability, excellent color stability of EPDM, etc. (Volintiru *et al.*, 1974; Anandhan *et al.*, 2011; Koo *et al.*, 2005; Manaila, 2013).

EXPERIMENTAL PROCEDURE

Materials

The following materials were used: (1) polypropylene (PP), impact copolymer Tipplen K 948, manufactured (by Tiszai Vegyi Kombinat RT (TVK), HUNGARY); (2) ethylene-propylene-diene (EPDM) terpolymer rubber, NORDEL IP 4760, specific gravity – 0.872, Mooney viscosity – 60 MU, ethylene content – 67.5 wt%, ethylidene norbornene (EBN) contents – 5.0 wt%, molecular weight distribution – medium, propylene content – 27.5 wt% (by – DuPont Dow elastomer, LLC, USA); (3) polypropylene-graft-maleic anhydride (PP-g-AM), average Mw~9.100 by GPC, average Mn~3,900 by GPC, maleic anhydride 8-10 Wt.%, manufactured (by Sigma - Aldrich Chemie USA); (4) montmorillonite (MMT), Nanoclay, surface modified I.31.PS, contains 0.5-5wt% aminopropyltriethoxysilan, 15-35wt% octadecylamine (Sigma-Aldrich Chemie, USA), (5) di(tert-butylperoxyisopropyl)benzen, powder 40% with calcium carbonate and silica (PD) - Perkadox 14-40B (1.65 g/cm^{3}density, 3.8% active oxygen content, pH 7, assay: 39.0-41.0%).

Procedure

The polymeric nanocomposites based on EPDM rubber and polyolefins, compatibilized with maleic anhydride grafted polypropylene – PP-g-MA, were carried out on a Counter-rotating twin screw extruder granulator, TSE 35 type (Tabel 1), then the obtained granules are prepared by means of blending technique, on a mixer-type Plastic-Corder Brabender Mixer (table 2 A and B, Table 3 A and B) and reinforced with chemically modified layered mineral clay of the montmorillonite type and crosslinking agent, and finally being processed into finished products (boards) by molding method using a Electrically heated press, considering the optimal technological parameters of processing. After stabilization for 24 hours at room temperature, the plates are submitted to physico-mechanical determinations.

Table 1. Sample for comparison (test specimen) PP, PP/EPDM, PP/EPDM/PP-g-MA

Symbol	MU	M_0	M_1	M_2	M_3	M_{11}	M_{21}	M_{31}
Polypropylene	%	100	90	70	50	90	70	50
EPDM	%	-	10	30	50	10	30	50
PP- g-AM	%	-	-	-	-	5	5	5

The method for achieving polymer nanocomposites based on PP/EPDM and PP/EPDM/PP-g-MA on a Counter-rotating twin screw extruder granulator, is as follow: polypropylene is added at 150°C and a speed of twin screws 150-200 rpm, is mixed until it becomes easy to process then increase the temperature to 175°C, add EPDM and

PP-g-MA and continue mixing at speed of 250-280 rpm until ingredients are embedded and the mixture is uniform, obtaining cylindrical granules in the end.

The obtained polymer nanocomposite granules based on thermoplastic polymers/ compatibilizer/nanoparticles/ crosslinking agents are prepared by means of blending technique, on a Plastic-Corder Brabender Mixer 350 E at mixing speed of 280 rpm, the temperatures in the three zones are 165/175/175°C, air cooled, 3-5 minutes mixing according to the added ingredients.

Table 2. Polymer nanocomposites formulation based on PP/EPDM/PP-g-MA/MMT (A and B)

A

Symbol	MU	$M_{11}M_1$	$M_{11}M_2$	$M_{11}M_3$	$M_{21}M_1$	$M_{21}M_2$	$M_{21}M_3$
PP	%	90	70	50	90	70	50
EPDM	%	10	30	50	10	30	50
PP- g-MA	%	5	5	5	5	5	5
MMT	%	1	3	7	1	3	7

B

Symbol	MU	$M_{31}M_1$	$M_{31}M_2$	$M_{31}M_3$
PP	%	90	70	50
EPDM	%	10	30	50
PP- g-MA	%	5	5	5
MMT	%	1	3	7

Table 3. Polymer nanocomposites formulation based on PP/EPDM/PP-g-MA/MMT/ PD (A and B)

A

Symbol	MU	$M_{11}M_1$	$M_{11}M_2$	$M_{11}M_3$	$M_{21}M_1$	$M_{21}M_2$	$M_{21}M_3$
PP	%	90	70	50	90	70	50
EPDM	%	10	30	50	10	30	50
PP- g-MA	%	5	5	5	5	5	5
MMT	%	1	3	7	1	3	7
PD	%	3	3	3	3	3	3

B

Symbol	MU	$M_{31}M_1$	$M_{31}M_2$	$M_{31}M_3$
PP	%	90	70	50
EPDM	%	10	30	50
PP- g-MA	%	5	5	5
MMT	%	1	3	7
PD	%	3	3	3

The obtained polymer nanocomposite granules are added in the molds, to process them according to test specimens used for physical-mechanical characterization for finished products, using the electrically heated press, TP 600, shown in figure 1, by means of compression method, between its platters at temperature of 165°C and 150 KN pressure for 2 minutes preheating, 10 minutes actual forming in the press and 10 minutes cooling (with water).

Figure 1. Electrically heated press, TP 600

RESULTS AND DISCUSSIONS

The obtained polymeric nanostructures have been tested in compliance with the physical-mechanical standards in effect and the results are presented in the tables below (Table 4, Table 5 A and B, Table 6 A and B).

After stabilization for 24 hours at room temperature, the plates are submitted to physico-mechanical determinations: density, g/cm^3; wear, mm^3; hardness, ^{0}Sh D; elasticity, %; tensile strength, N/mm^2.

Table 4. Physical-mechanical characterisation – Sample for comparison (test specimen) PP, PP/EPDM, PP/EPDM/PP-g-MA

Symbol	M_0	M_1	M_2	M_3	M_{11}	M_{21}	M_{31}
Wear, mm^3, SR ISO 4649/2010	192	310	147	171	219	181	243
Density g /cm^3 SR ISO 2781:2010	0.91	0.91	0.89	0.89	0.87	0.88	0.91
Hardness ^{0}Sh D SR ISO 7619-1:2011	70	65	54	42	62	53	43
Tensile strength, N /mm^2, SR ISO 37:2012	11.4	11.9	9.6	7.0	10.9	7.6	5.5
Elasticity, %, ISO 4662:2003	28	28	28	30	26	28	30

Density decreases with the amount of EPDM rubber and PP-g-AM compatibilizer added to the mixture; the presence of montmorillonite (MMT) should have a weak influence on density values. Wear increases, in the standard range, proportionally to the amount of copmatibilizing agent added to the mixture. The presence of montmorillonite should not influence the degree of wear. Tensile strength decreases proportionally with adding EPDM and compatibilizer – PP-g-MA, compared to the raw material – PP. It is noticed that by adding rubber and compatibilizer, elasticity is approximately constant.

Table 5. Physical-mechanical characterisation – Polymer nanostructure formulation based on PP/EPDM/PP-g-MA/MMT (A and B)

A

Symbol	$M_{11}M_1$	$M_{11}M_2$	$M_{11}M_3$	$M_{21}M_1$	$M_{21}M_2$	$M_{21}M_3$
Hardness ^{0}Sh D SR ISO 7619-1:2011	68	68	66	57	57	60
Tensile strength, N/mm^2, SR ISO 37:2012	15.5	12.2	10.8	19	16.4	11.7
Elasticity, %, ISO 4662:2003	32	30	28	30	30	30

B

Symbol	$M_{31}M_1$	$M_{31}M_2$	$M_{31}M_3$
Hardness ^{0}Sh D SR ISO 7619-1:2011	47	43	42
Tensile strength, N /mm^2, SR ISO 37:2012	8.5	7.8	5.6
Elasticity, %, ISO 4662:2003	32	30	28

Table 6. Physical-mechanical characterisation – Polymer nanostructure formulation based on PP/EPDM/PP-g-MA/MMT/PD (A and B)

A

Symbol	$M_{115}M_1$	$M_{115}M_2$	$M_{115}M_3$	$M_{215}M_1$	$M_{215}M_2$	$M_{215}M_3$
Hardness ^{0}Sh D SR ISO 7619-1:2011	63	63	64	57	58	58
Tensile strength, N/mm^2, SR ISO 37:2012	Could not be determined	5.6	10.2	8.2	9.8	10.7
Elasticity, %, ISO 4662:2003	26	26	26	26	26	24

B

Symbol	$M_{315}M_1$	$M_{315}M_2$	$M_{315}M_3$
Hardness ^{0}Sh D SR ISO 7619-1:2011	48	49	51
Tensile strength, N /mm^2, SR ISO 37:2012	8.1	8.5	10.6
Elasticity, %, ISO 4662:2003	24	24	28

- Hardness of polymeric structures by adding EPDM in varying proportions decreases below the raw material - PP, and by adding PP-g-AM compatibilizer, values are approximately similar to those mentioned above. In the case of polymeric nanostructures with 1% MMT hardness decreases compared to PP, and with 3% and 7% MMT it significantly decreases below polypropylene hardness, as well as by adding crosslinker (PD) hardness decreases compared to the value of raw material (PP).
- Due to the fact that by adding the crosslinker during the mixing process, EPDM rubber vulcanizes, tensile strength has values below those of polymeric nanostructures based on PP/EPDM/PP-g-AM, as well as below those of the control sample (PP).
- It can be seen that with the addition of MMT in different percentages, elasticity increases slightly versus polypropylene, with the addition of crosslinking agent - PD due process of EPDM rubber vulcanization, elasticity decreases.

CONCLUSIONS

Processing natural and synthetic elastomers involves the use of many auxiliaries with a well established role in influencing properties of finished products or cost price. To obtain products with preset physical-mechanical characteristics depending on their destination, it is necessary to use fillers and compatibilizers of various types and concentrations.

The polymeric nanocomposites based on EPDM rubber and polyolefins, compatibilized with maleic anhydride grafted polypropylene – PP-g-MA, were carried out on a Counter-rotating twin screw extruder granulator, TSE 35 type according to the added ingredients, and then the obtained polymer nanocomposite granules based on thermoplastic polymers/compatibilizers/nanoparticles/crosslinking agents were prepared by means of blending technique, on a Plastic-Corder Brabender Mixer 350 E at mixing speed of 280 rpm, the temperatures in the three zones are 165/175/175°C, air cooled, 3-5 minutes mixing according to the added ingredients.

Processing the obtained granules in boards form is possible by adding them in the molds, using the electrically heated press, TP 600, by means of compression method, between its platters at temperature of 165°C and 150 KN pressure for 2 minutes preheating, 10 minutes actual forming in the press and 10 minutes cooling, with water cooling.

Finally being processed into finished products by molding method using a Electrically heated press, considering the optimal technological parameters of processing and after stabilization for 24 hours at room temperature, the plates are submitted to physico-mechanical determinations.

Acknowledgements

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THE EFFECT OF FILLER ON CHARACTERISTICS OF SOME ETHYLENE VINYL ACETATE COPOLYMER COMPOSITES

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In this research work, the influence of the amount and type of filler on characteristics of some ethylene vinyl acetate copolymer (EVA) composites was studied. Materials used in the study were: ethylene vinyl acetate copolymers Elvax 260 (27.8% wt% vinyl acetate content), four types of filler (precipitated silica Ultrasil VN3, carbon black HAF, precipitated kaolin and precipitated chalk) and other ingredients (zinc oxide, acid stearic, polyethylene glycol, antioxidant, dibenzoyl peroxide Perkadox 14-40B, polyfunctional monomer). The blends were prepared by means of blending technique, on an electrically heated laboratory roller mill at 70±5°C, friction 1:1.1 and total blending time 6-14'. Samples were crosslinked using two methods: by classic method in the presence of peroxides and by electron beam irradiation. The minimum torque and maximum torque increased with increasing filler content, and the highest values were obtained for the mixtures containing precipitated silica. Increasing the filler content tended to increase the hardness, 100% modulus, tear strength and tensile strength of the composites. The best results were obtained by adding active fillers - precipitated silica and carbon black - to samples crosslinked with peroxides, as well as by electron beam irradiation.

Keywords: ethylene vinyl acetate copolymer, filler, accelerated electrons, physical-mechanical characteristics, curing characteristics

INTRODUCTION

The importance of fillers in the rubber compounds is well known. Fillers can be classified into black and non-black. Soon after carbon black was discovered to be an active filler in rubber, at the beginning of this century, it became one of the most important components in the manufacture of rubber products, with a consumption second only to rubber itself. Non-black fillers are classified as: fillers used mainly to reduce cost, semi-reinforcing fillers and reinforcing fillers used to achieve high, performance in non-black products (Evans, 2001).

Fillers are widely used to enhance the performance of rubbers and other polymeric materials. Filler characteristics such as size and shape of particles and aggregates, chemical nature and porosity of surface, dispersibility and tendency to agglomerate and form secondary filler networks determine its effect on rubber compounds. Surface activity relates to the compatibility of the filler with a specific elastomer and the ability of the elastomer to adhere to the filler. If the size of the filler particle greatly exceeds the polymer interchain distance, it introduces an area of localized stress. This can contribute to elastomer chain rupture on flexing or stretching. Fillers with particle size greater than 10,000 nm are therefore generally avoided because they can reduce performance rather than extend or reinforce it. Fillers with particle size between 1,000 and 10,000 nm are used primarily as diluents and usually have no significant effect, positive or negative, on rubber properties. Semi-reinforcing fillers range from 100 to 1000 nm. The truly

reinforcing fillers (active fillers), which range from 10 nm to 100 nm, can significantly improve rubber properties (Evans, 2001; Franta, 1989).

In this research work, the influence of the amount and type of filler on characteristics of some ethylene vinyl acetate copolymer (EVA) composites was studied. Four types of filler used in the study were: precipitated silica (average particle size: 10-100 nm, surface area 40-170 m^2/g), carbon black HAF (average particle size: 20- 36 nm, surface area 80 m^2/g), precipitated kaolin (average particle size: 200-500 nm, surface area 45-130 m^2/g) and precipitated calcium carbonate (average particle size: 40-700 nm, surface area 8-74 m^2/g). It is noticed that precipitated kaolin and precipitated calcium carbonate are semi-reinforcing fillers ranging from 100 to 1000 nm and precipitated silica and carbon black HAF are reinforcing fillers, which range from 10 nm to 100 nm and may significantly improve rubber properties (Evans, 2001; Franta, 1989).

Samples were crosslinked using two methods: by classic method in the presence of peroxides, and by electron beam irradiation. The radiation induced grafting and crosslinking of polymers are new techniques applied in modifying polymers. Use of radiations as power source is justified by the limited classic resources, on the one hand, and a number of specific benefits, on the other hand, such as: (1) removing the curing agents, (2) obtaining new high purity materials, (3) a fast process which enables an accurate monitoring, (4) an effective and uniform curing of the whole rubber body because of the high penetrating ability of radiation, (5) lack of wastes (Manaila *et al.*, 2008; Manaila *et al.*, 2007; Craciun, 2013).

Research papers have been published suggesting that appropriate polyfunctional monomers (PFMs), also called coagents, added in polymer matrix, could be used to obtain desired physical properties of the blend at lower irradiation doses. Coagents are multifunctional organic molecules which are highly reactive towards free radicals. Previous studies (Stelescu *et al.*, 2013; Stelescu *et al.*, 2012) show that the most efficient PFMs in the EVA case were polyfunctional monomer triallylcyanurate (TAC).

EXPERIMENTAL

Materials

Materials used in the study: (1) EVA copolymer Elvax 260 (27.8% wt% VA content, flow index (MFI) 5.5 g/10 min at 190°C and 2.16 kg load), (2) dibenzoyl peroxide Perkadox 14-40B (1.60 g/cm^3 density, 3.8% active oxygen content, 40% peroxide content, pH 7) and polyfunctional monomer triallylcyanurate Luvomaxx TAC DL 70 (TAC) (26% percentage of ash, density 1.34 g/cm^3, 30% active synthetic silica). as vulcanizing agents, (3) four types of filler: precipitated silica Ultrasil VN3, carbon black HAF, precipitated kaolin and precipitated chalk, (4) other ingredients (zinc oxide, stearic acid, polyethylene glycol, antioxidant Irganox 1010 - pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate).

Sample Preparation

EVA compounds which were filled with different dosage of precipitated silica Ultrasil VN3, carbon black HAF, precipitated kaolin and precipitated chalk were prepared by means of blending technique, on an electrically heated laboratory roller mill. For preparation of polymeric composites, the blend constituents were added in the following sequences and amounts: 100 parts EVA roll binding (2'), embedding 3 phr (parts to 100 parts rubber) PEG 4000, 1 phr Irganox 1010 antioxidant, 5 phr zinc oxide,

0.5 phr stearic acid (2'), adding 10, 30, and 50 phr fillers, respectively (2–4'), embedding 3 phr TAC and homogenization of blends and removing from the roll in the form of sheet (2-4'). Process variables: temperature 70±5°C, friction 1:1.1 and total blending time 6-12'. Plates required for physico-mechanical tests have been made by compression molded, using a electrically heated hydraulic press, at a temperature of 160°C, pressure of 150 MPa, to obtain sheets of dimension 150x150x2 mm^3. Samples were crosslinked using two methods: by classic method in the presence of peroxides and by electron beam irradiation. In samples crosslinked using the classic method, 8 phr dibenzoyl peroxide Perkadox 14-40B was added as vulcanizing agent and all cure times were adjusted to bring the respective cures to T_{90} for each sample—the vulcanization time was measured by means of Monsanto Rheometer (see Tables 1-2). For samples crosslinked by electron beam irradiation, plates have been made by compression molding for 5'. Then, the samples were packed in a polyethylene film and were irradiated at 100 kGy in the ILU-6M cavity electron accelerator.

Laboratory Tests

The cure characteristics of the compounds were determined by an oscillating disk rheometer (Monsanto), at 160°C and 30 min, according to the SR ISO 3417/1997. Delta torque or extent of crosslinking is the maximum torque (MH) minus the minimum torque (ML). Scorch time (t_{s2}) is taken as the time to reach 2% of the delta torque above minimum. Optimum cure time (t_{90}) is the time to reach 90% of the delta torque above minimum. The cure rate index (CRI) of the recipe was calculated according to the following formula:

$$\mathrm{CRI} = 100/(t_{90} - t_{s2}) \tag{2}$$

The cure rate index is a measure of the rate of vulcanization based on the difference between optimum vulcanization time, t_{90} and incipient scorch time, t_{s2}.

Mechanical properties of the vulcanizates were measured on a Schopper tensile tester with a nominal rate of the traverse of the moving grip of 460 mm/min. Modulus at 100% strain, tensile strength, and elongation at break tests were carried out according to the conditions described in ISO 37/2012, on dumb-bell shaped specimens of Type 2. Residual elongation is the elongation of a specimen measured 1 min after rupture in a tensile test. It was calculated using the formula:

$$\text{Residual elongation } (\%) = [(L - L_0)/L_0] \times 100 \tag{1}$$

where L_0 is the initial length between two marks and L is the length between the marks 1 min after the sample broke in a tensile test. Tearing strength tests were carried out using angular test pieces (type II) according to SR EN 12771/2003. Hardness of the vulcanized materials was measured using the Shore A scale with vulcanized samples of 6-mm thickness, by using a hardener tester according to ISO 7619-1/2011. Elasticity was evaluated with a Schoob test machine using 6-mm thick samples, according to ISO 4662/2009. All measurements were taken several times and the resulting values were averaged on three to five measurements.

RESULTS AND DISCUSSION

Cure Characteristics of the Blends

Tables 1 and 2 present the results of rheological measurements. For every measured sample, ML, MH, optimum curing time (T90), shorter time (t_{min}), scorch time (t_{s2}) and CRI were determined by reading the variables on the curing curves obtained with the

Monsanto rheometer. It is found that in blends filled with reinforced (active) fillers such as carbon black and precipitated silica (Table 1), upon increasing the amount of filler, an increase of MH, M90 and CRI and a decrease of curing time, t_{90}, occur. In blends where semi-reinforced fillers were added (Table 2), it is found that upon increasing the amount of filler, a slight decrease of t_{90} and a slight increase of CRI occur.

Table 1. Rheometric characteristics of samples filled reinforcing fillers: precipitated silica and carbon black

Rheometric characteristics/ filler type and amount	Control	Precipitated silica			Carbon black		
		10 phr	30 phr	50 phr	10 phr	30 phr	50 phr
The minimum torque ML (dNm)	1	0.2	13.0	21.2	0	11.8	13
The maximum torque MH (dNm)	35	40.5	54.1	70	38	41.8	48
Delta torque ΔM (dNm)	34	40.3	41.1	48.8	38	30	35
M90 (dNm)	31.6	36.5	50	65.9	34.2	38.8	44.5
Curing time, t_{90} (min)	21'45''	20'45''	19'30''	19'15''	21'30''	20'	19'30"
Shorter time, t_{min} (min)	45''	1'	40''	35''	1'	1'	40''
Scorch time, t_{s2} (min)	2'45''	2'30''	1'20''	1'15''	2'45''	2'30''	2'30''
Cure Rate Index, CRI (min^{-1})	5.26	5.48	5.50	5.55	5.48	5.71	5.88

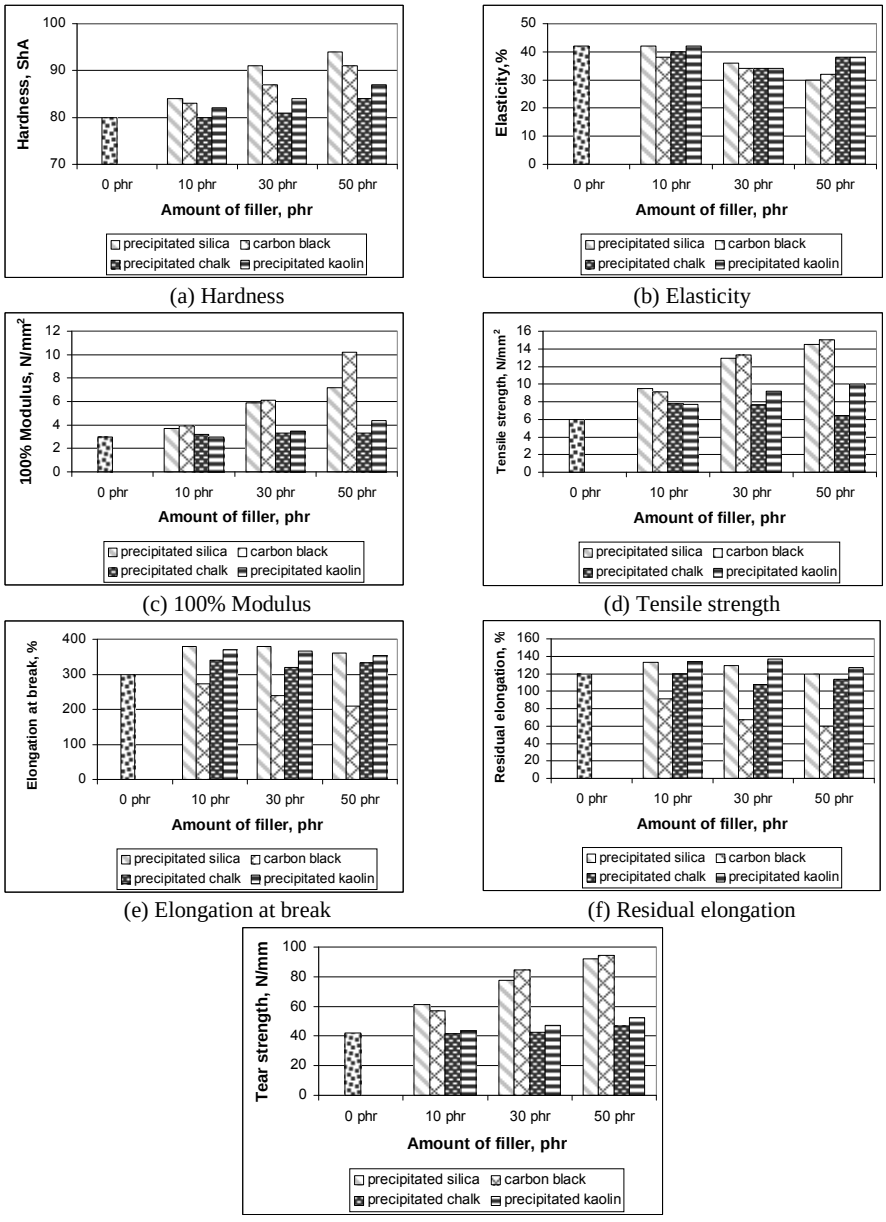
Table 2. Rheometric characteristics of samples filled with semi-reinforcing fillers: precipitated chalk and precipitated kaolin

Rheometric characteristics / filler type and amount	Precipitated chalk			Precipitated kaolin		
	10 phr	30 phr	50 phr	10 phr	30 phr	50 phr
The minimum torque ML (dNm)	27	28	21.8	9.5	10	10
The maximum torque MH (dNm)	47	49	45	38	42	46
M90 (dNm)	45	46.9	42.7	35.15	38.8	42.4
Delta torque ΔM (dNm)	20	21	23.2	28.5	32	36
Curing time, t_{90} (min)	21'15''	20'30''	20'30''	21'15''	20'45''	20'15''
Shorter time, t_{min} (min)	1'	40''	45''	45''	1'	1'
Scorch time, t_{s2} (min)	2'15''	2'	2'15''	2'15''	2'	2'
Cure Rate Index, CRI (min^{-1})	5.26	5.40	5.48	5.26	5.33	5.48

Physico-Mechanical Characteristics of the Blends

In Figure 1 are presented the results of the variations of physical-mechanical properties with the increase of the filler amount in composites and in Table 3 are presented physical-mechanical characteristics of samples filled with 50 phr precipitated silica and 50 phr carbon black respectively, crosslinked by irradiation with 100 kGy. From the obtained results, it is noticed that for blends filled with reinforced (active) fillers such as carbon black and precipitated silica and crosslinked using the classic method (Figure 1), upon increasing the amount of filler, an increase in hardness, 100% modulus, tensile strength and tear strength, and a decrease of elasticity occur. Comparing characteristics of the control blend with those of blends containing 50 phr active fillers, significant improvements of hardness, 100% modulus, tensile strength, and tear strength characteristics were found by adding carbon black and precipitated silica fillers, respectively, both by crosslinking using the classic method (Figure 1), and by crosslinking

by electron beam irradiation at a dose of 100 kGy (Table 3). In blends containing semi-reinforced fillers, upon increasing the amount of filler, only a slight improvement of hardness, 100% modulus, tensile strength, elongation at break and tear strength is noticed.



(a) Hardness (b) Elasticity

(c) 100% Modulus (d) Tensile strength

(e) Elongation at break (f) Residual elongation

(g) Tear strength

Figure 1. The variations of mechanical properties with the increase of the filler amount in composites

Table 3. Physical-mechanical characteristics of samples filled with reinforcing fillers: precipitated silica and carbon black, crosslinked by irradiation with 100 kGy

Physical-mechanic characteristics / filler type and EB dose	Control	Precipitated silica	Carbon black
Hardness, °ShA	86	96	91
Elongation, %	37	38	30
100 % Modulus, N/mm^2	4.1	10.6	9.5
Tensile strength, N/mm^2	14.6	15.8	14.6
Elongation at break, %	390	240	333
Residual elongation,%	175	79	127
Tear strength, N/mm	60	105	86

CONCLUSIONS

As a result of this study, it is found that in blends based on EVA filled with reinforced (active) fillers such as carbon black and precipitated silica, upon increasing the amount of filler, an increase in MH, M90, CRI, hardness, 100% modulus, tensile strength and tear strength as well as a decrease in curing time, t_{90}, and elasticity occur. In EVA blends where semi-reinforced fillers were added, it is found that upon increasing the amount of filler, a slight increase in t_{90} and a slight decrease of CRI, hardness, 100% modulus, tensile strength, elongation at break and tear strength occur. The best results were obtained by adding active fillers - precipitated silica and carbon black - to samples crosslinked with peroxides, as well as by electron beam irradiation.

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FLEXYBRICK – REVOLUTIONARY SOLUTION FOR POLYURETHANE APPLICATION

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Polyurethane has been used in thousands of application all around the globe in different fields. From boots to skateboards, furniture, thermoinsulation and even mobile phones, polyurethane is an indispensable material today. Even though in the construction field its applicability has been widely increased, the paper proposes an innovative application for this material. The considered material is an eco-friendly one with low self weight and reduced needed labor. Detailed analysis are done between curent used solutions – masonry, light weight concrete, wood and the innovative solution called FlexyBrick. FlexyBrick is a new product based on polyurethane. The following parameters are analyzed: thermal rezistance, self weight, compression strength, bending strength and price. FlexyBrick is reinforced with recycled or organic materials in order to reduce costs (crashed glass, sand, cigarettes filters, straw, chopped rubber) and to improve mechanical properties. Using this new infill material the heating costs will be reduced and the execution time will decrease. The polyurethane bricks are easily adapted to any climatic condition and allow the water vapor transfer from the inside to the exterior side of the masonry, preventing condensation.

Keywords: infill, eco-friendly material, polyurethane.

INTRODUCTION

Polyurethane is a mixture between two substances and was initially discovered in the 1940s. Since then it has been used in a wide range of items, from baby toys, skateboards, surfboards to beehive, snow blades for machines, vehicle suspension bushings, airplane wings, and it continues to be adapted for contemporary technology. Polyurethane can be manufactured in any colour, can take any shape, size or any geometrical complexity.

Polyurethane chemistry began in 1937 when H. Rinke first prepared 1,6-hexamethylene diisocyanate (HDI) and Otto Bayer developed the diisocyanate polyaddition process.

Widespread use of polyurethanes was first seen in Germany, during World War II, when it was utilised to replace rubber, which at the time was expensive and hard to obtain. During the war, other applications were developed, largely involving coatings of different types, from aeroplane finishes to resistant clothing (Olteanu and Toma, 2012).

By the end of the war, polyurethane coatings have started to be used on an industrial scale and could be custom formulated for specific applications. By the mid-50's, polyurethanes could be found in coatings and adhesives, elastomers and rigid foams. It was not until the late-50's that comfortable cushioning flexible foams were commercially available. With the development of a low-cost polyether polyol, flexible foams opened the door to automotive applications known today.

In the 1990s new two-component polyurethane and hybrid polyurethane-polyurea elastomers were used to spray-in-place load bed liners and military marine applications for the U.S. Navy.

While polyurethane is a product that most people are not overly familiar with, as it is generally 'hidden' behind covers or surfaces made of other materials, it would be hard to

imagine life without polyurethanes today (Olteanu *et al.*, 2011), (Pastia and Luca, 2013).

In Romania, the polyurethane was introduced in 1978 and it is manufactured by Oltchim SA. In 2007, consumption of polyurethane raw materials was more than 12 million metric tona, the annual average increase being of approximately 5%.

Polyurethane in Construction

Polyurethane is used in construction since 1950 and its main application is building insulation, thus achieving roof insulation panels, walls, ceilings and floors, Figure 1.

Metal-faced polyurethane sandwich panels are the system of choice today for large industrial buildings, refrigerated and other warehouses, office blocks, exhibition halls, fair pavilions, schools and sports halls. Prefabricated sandwich wall and lightweight roofing consist of metal facings bonded tightly together by a core of rigid polyurethane foam. The aluminium or steel facings themselves are surfaces coated and can be manufactured either flat or with profiles of various depths. Polyurethane sandwich panels come complete with specially formed tongue-and-groove joints ensuring a perfect fit and maximum integrity (Woods, 1982).

Polyurethane foam sandwich panel is fit for the projects which have serious requirements regarding constant temperature, or strict hygiene maintenance, because polyurethane core material is considered the best material in keeping warm and thermal insulation.

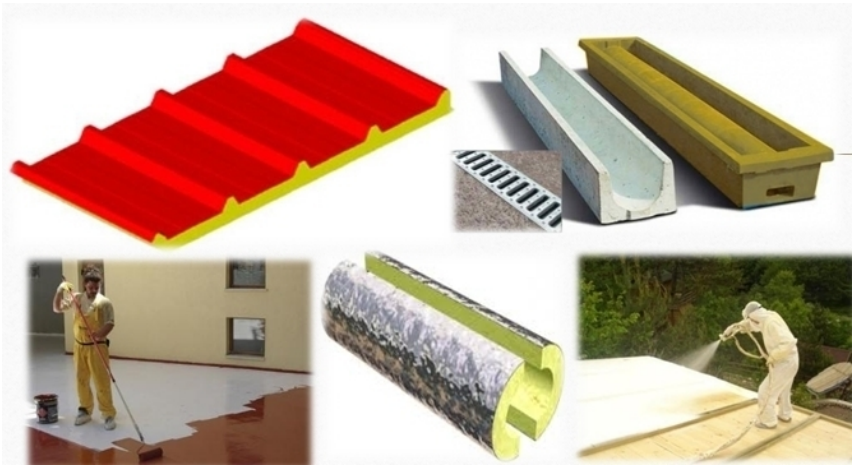

Figure 1. Polyurethane applications in construction

FLEXYBRICK CONCEPT

Base Constituent Material

Polyurethane properties depend on the type of isocyanate and polyols used. In some ways, a piece of polyurethane can be considered a giant molecule. As a consequence, typical polyurethanes do not soften or melt when heated. Isocyanates and polyols avaible options, compared to other additives and processing conditions, allow the

polyurethane to have a wide range of properties that make it such a widely used polymer. The polymerization reaction is a polymer containing urethane linkages, and it is catalyzed by tertiary amines such as diazabicyclo [2.2.2] octane, and metal compounds such as dibutyl tin dilaurate or bismuth octane (Hepburn, 1991).

If water is present in the reaction mixture, isocyanate reacts with water to form a urea linkages and carbon dioxide, and the resulting polymer contains both urethane and urea linkages. This reaction is reffered to as the expansion reaction and is catalyzed by tertiary amines such as bis (2 – dimethylaminoethy) ether. Another very important reaction in making rigid insulating foams is the trimerization reaction of isocyanate, which is catalyzed by potassium octoate. One of the desirable attributes of polyurethanes is their ability to be converted to foam. Making foam requires the formation of a gas, at the same time as the urethane polymerization. The gas may be carbon dioxide, either generated by the reaction of isocyanate with water, or added as a gas or liquid boiling volatile product. In the latter case, polymerization temperature leads to liquid vaporization. Thus, combining these two reactions, a foam expansion is being made, and because of the limited dimensions of the mold, physical properties of the polyurethane bricks are obtained.

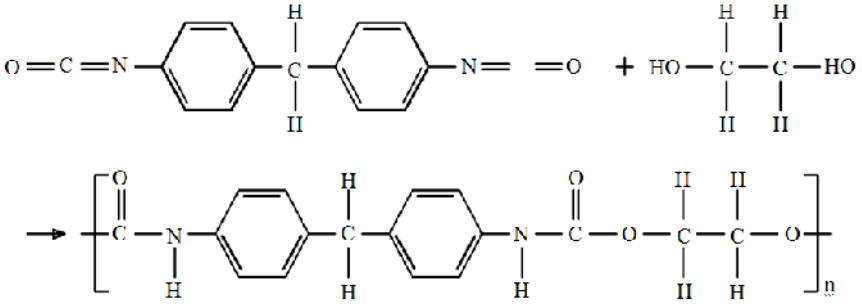

Figure 2. Chemic formula of polyurethane

FlexyBrick Masonry Block

FlexyBrick is the name given to the flexible masonry block the authors are proposing. This new polyurethane application comes as a response to an existing problem for the reinforced concrete frame structure with infill material – meaning poor interaction between the structural system and the infill material.

FlexyBrick, are made using a mold that has a special machine with a dosing device, in order to obtain the required density. During the manufacturing process it is desired the best quality of the material components, compliance with technological parameters and quality of finished products, in order to ensure proper operating behaviour.

Various sizes of polyurethane masonry block can be produced, depending on the construction site, the destination of the building, the size of the reinforced concrete frames structure. By creating appropriate mould, polyurethane can take even the shape of a round section. In this way polyurethane masonry block can replace the log required for the construction of a country-house, the environmental advantage being in this major issue.

Reinforcement

In order to prevent cracking and to provide higher mechanical properties it is required to use reinforcement for the masonry blocks.

Due to the progressive increase in the price of steel – concrete on the world market, and as a result to the technical and economic studies that were made, it was chosen polymer fibre as reinforcement. In order to find the most suitable reinforcement material, masonry blocks of various type of reinforcement have been tested in compression test. The used materials for this purpose were: fibre glass mesh, crashed rubber, geogrid mesh, metal mesh, broken glass. Another important advantage for using reinforcement is the reduction of polyurethane material, diminishing total cost also.

FlexyBrick Masonry

The FlexyBrick masonry is realized using polyurethane as adhesives in order to obtain a homogenous infill material. No specific training is required, only fast execution workers. The masonry is connected to the structural system with steel bars and has fibre glass mesh between the masonry block as longitudinal reinforcement.

Operations that must be strictly controlled:

- good adhesion between polyurethane masonry blocks and adhesive;
- horizontal and vertical joints shall be well filled with adhesive all over achieved;
- vertical joints will be weave as shown in Figure 3, with reinforcing mesh fiberglass, geogrid etc.;

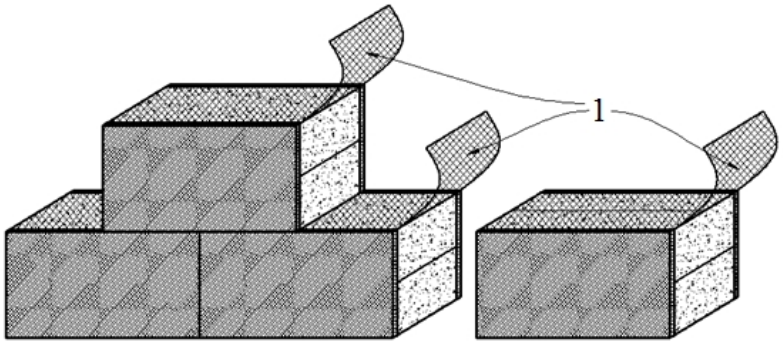

Figure 3. Masonry weaving using reinforcement mesh around the horizontal joints,
1. Reinforcement mesh

- vertical joints will be woven so that the superposition height of two succesive rows, both in the field and at the corners intersection, to make be minimum of ¼ from the brick lenght, and ½ of its thickness. Weaving is required in each row;
- the horizontality of the bricks rows will be watched;
- the interruption of the mansory work will be done in steps;
- the connections between walls, corners, junctions and branches will be made alternatively;
- the anchoring of the infill masonry is made with stell – concrete whiskers of Ø 8 = 50 cm or creating strep to achieve weaving ancient masonry;
- corrosion protection of the anchors will be provide;

- partition walls (bricks edge) are stiffen by weaving and anchoring steel – concrete bars Ø 6 OB37 every 3-4 rows in horizontal joints, according to the P2-85Standard.

OTHER USES OF POLYURETHANE

Lintels

Lintels are auxiliary construction elements that look like a beam, positioned above an opening in the masonry wall, which supports the gravitational load coming from adjacent mullions. Before fixing the lintel, the size of the hole in the masonry will be checked.

When building the part of the wall above the lintel, it is forbidden to introduce inside the lintel any kind of clamping elements by drilling, as these can affect its strength.

It should be taken into account that the extremities of the lintels need to lean on the walls with a length equal to the height of the lintel, which needs to be at least 25 cm, Figure 4.

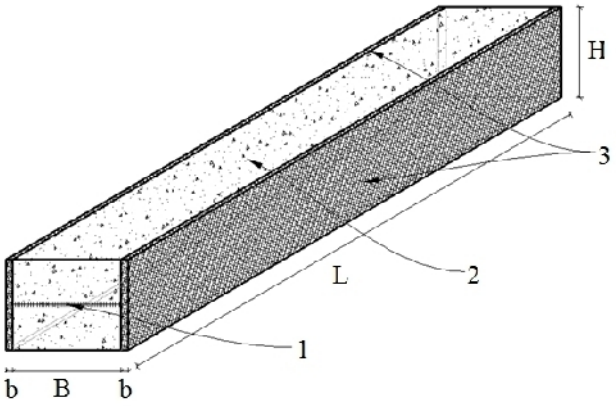

Figure 4. Lintels: 1. Reinforcement mesh; 2. Rigid polyurethane; 3. Fibrocement

Ventilated Facades

Ventilated facades are currently made of: tiles HPL (High Pressure Laminate), cement, ceramics, klinker, terracotta, artificial stones, recomposed materials (marble, granite, travertine), composite wood, aluminium, decorative glass, steel, natural stone. By its insulating qualities, for both low and high temperature, a ventilated facade has the economic advantage of reducing costs for cooling or heating. This is because the structure of this system is protecting the building against the environmental factors.

Using a ventilated facade, the building structure may represent the most modern and attractive line, its maintenance being simple, with no major problems. Getting the stereotomy provided by the arhitect means the individualization of the fixing system which is made through technological design.

The development of the technical documentation as stated above is customized for each application, depending on the complexity and specific characteristics.

CONCLUSION

Modern engineering takes architecture to a new level, making possible to build all sorts of structures, even the parametric ones. In order to change the perspective in the engineering field reserch is carried out all around the world to produce new materials or improve the characteristics of the existing ones.

The article presented an revolutionary application for a relative olt material, polyurethane. The possibility to have in the future a entire structure made of material, produced entirely mechanically and adapted to all the elements a structure has, becames more possible

Among the advantages there are: low price, big geometrical variaty, fast erection and increase thermal resistance properties. The research is still under development and the latest results will be presented in the near future.

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INFLUENCE OF COUPLING AGENTS ON THE POLYMERIC MATERIAL / DISPERSE MATERIAL INTERFACE

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This paper presents the method of obtaining and the characterization of polypropylene-based composites reinforced with glass fibers and treated with coupling agents based on polydimethylsiloxane (PDMS), in the presence of a coupling agent such as polypropylene grafted with maleic anhydride (PP-g-MA), processed using a twin screw extruder granulator. The influence of coupling agents plays an important role in adhesion to the interface and in determining the properties of the composite. A major disadvantage of polypropylene matrix is given by the lack of functional groups, while the introduction of treated glass fibers results in composites similar to those obtained in the presence of conventional reinforcing agents (talcum, calcium carbonate, etc.). In this regard, adding 3% PP-g-MA in the polypropylene matrix under the influence of temperature and shear forces developed within the processing machine (extruder) results in opening the maleic ring, and the resulting carboxylic groups react with functional groups present on the surface of reinforcing materials, improving dispersion and interfacial adhesion. The materials used, both glass fibers and the resulting composite, were characterized physico-chemically, morphologically by SEM, and structurally by XRD, FTIR, etc. The results demonstrate good compatibility between phases in the presence of a coupling agent and of treated glass fibers compared with samples obtained in the absence thereof.

Keywords: composite, coupling agent, glass fibres

INTRODUCTION

An important class of engineering materials is produced by compounding of short glass fibers and thermoplastics. These composites offer excellent mechanical properties, economy and are easily produced into different shapes by injection molding, compression molding, or extrusion (Karsli and Aytac, 2011).

In last few decades, intensive research efforts have been devoted, especially, to achieve a better understanding of the interphase between reinforcement and matrix since the mechanical properties of composite materials highly depend on the interphase properties (Fonseca *et al.*, 2014). The key factors to manufacture high mechanical performance lie on the equilibrium between the fibre content and their aspect ratio (length/diameter), and the adhesion level between the constituents (interphase) (Zhuang *et al.*, 2010). Interphases between reinforcement and matrix are formed by the inter-diffusion of sizing and matrix (Kano-Ibarretxe *et al.*, 2012), resulting in different compositions and properties compared to the bulk matrix.

Polypropylene is a commodity thermoplastic belonging to the polyolefin group of polymers, which are widely used due to their low cost and excellent chemical resistance. However an, inherent property of the polyolefin group is that they are nonpolar which means that they have a low chemical affinity with other materials,

therefore much of the interface strength is provided by mechanical interlocking and compressive residual stresses created during cooling (Yan *et al.*, 2013).

Many studies have been conducted on physical and chemical methods to improve the adhesion between the fiber and matrix through a modification of the fiber and/ or the polymer matrix (Feller and Grohens, 2004). A coupling agent is just like a molecular bridge in the interface of inorganic filler and organic polymer matrix. Because of the hydrophilic nature, the fiber does not wet or interact with hydrophobic polymer due to the difference in surface energies. It is necessary to treat the fiber with a coupling agent in order to improve the compatibility between filler and matrix. A widely used coupling agent is organic silanes, which formula can be simply written as $RSiX_3$, where R is a non-hydrolyzable organic group which can be combined with polymers, and X is a hydrolyzable group, for instance, an alkoxy group such as —OC2H5, —OCH3 etc (Liu *et al.*, 2008).

The polydimethylsiloxane coupling agent, with Si-O-Si backbone shows excellent heat and UV radiation stability, low-temperature flexibility, good hydrophobicity, and excellent moisture resistance (Bogoeva-Gaceva and Grozdanov, 2006).

According with the literature (Noranizan *et al.*, 2012), an efficient method to enhance the adhesion between different types of fibres and polypropylene matrices, and therefore improve the final overall mechanical characteristics of the composite, is the addition of polypropylene grafted maleic anhydride (PP-g-MA) to the system (Etcheverry and Barbosa, 2012; Biswas *et al.*, 2014).

MATERIALS AND METODS

Materials

The materials used in this study were the following: polypropylene homopolimer TIPPLEN H 949A, manufactured by Tiszai Vegyi Kombinat RT (TVK), HUNGARY; polypropylene-graft-maleic anhydride (PP-g-AM), average Mw~9.200 by GPC, average Mn~3,900 by GPC, maleic anhydride 8-10 Wt.%, manufactured by Sigma Aldrich USA; poly (dimethylsiloxane), grade: analytical standard, vapor pressure: 5 mmHg (20^0C), mol wt - average M_w ~95,000, average M_n ~50,000, manufactured by Sigma Aldrich USA, borosilicatic fiber type E, length=4.5 mm, diameter=13 µm, alkaline oxide content>1, manufactured by Polydis, RO.

Composites based on polypropylene reinforced with glass fibres are obtained in two phases:

- In the first stage – 100g of glass fibers were added in 1litre of solution of PDMS 0.5%, and allowed to react at room temperature for 8h. The fibers dried in advance at 80°C and thereafter are subjected to a heat treatment at 130°C for 20 min.

- The second stage consists in developing the composite on a counter-rotating twin screw extruder granulator: polypropylene powder is introduced in the extruder with the glass fibres untreated and treated with coupling agents poly (dimethylsiloxane) and polypropylene grafted with maleic anhydride at the temperature profile of 155°-160°-165°-170°-175°-180°C, residence time of 80s and rotation of 100 rpm. The composite is then granulated and dried in the pelletizer and subsequently subjected to physical-mechanical, structural and morphologic tests. We developed 7 variants of polymeric composites based on polypropylene reinforced with glass fibres untreated in an amount of 10, 20 and 30% and polypropylene/3%PP-g-AM reinforced with glass fibres treated with PDMS in an amount of 10, 20 and 30%.

The specimens for mechanical tests were prepared by electrically heated press (TP 600 Fontijne Grotnes, Nederland) at a temperature of 165°C, preheat = 10 min, pressing=15 min, cooling=12 min and pressure of 150kN.

Influence of PP-g-MAH compatibilizer on the morphology, structural and mechanical properties of short glass fiber reinforced polypropylene composites was examined.

Characterization: counter-rotating twin screw extruder granulator, TSE 35 type; Electrically heated press, TP 600 with the following characteristics: pump pressure max.300 bar, pressing surface 400 x 400 mm, two work spaces, work temperature 150-300°C ADJUSTABLE, manufactured by Fontijne Grotnes, Nederland; FTIR microscopy by using a Thermo iN10 MX FTIR microscope operated in reflection mode; X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature, Cu-Kα radiation from a Cu X-ray tube (ran at 15mA and 30 kV) was used; SEM images were recorded on a HITACHI S2600N electron microscope coupled with an EDS detector, on samples covered with a very thin silver layer.

RESULTS AND DISCUSSION

In Table 1 are presented the results of physical and mechanical determinations: hardness and tensile strength of polypropylene, PP / 3%PP-g-MA, of the polypropylene based composites reinforced with glass fiber percentage between 10-30% in the absence of the coupling agent and PP / 3% PP-g-MA / PDMS treated glass fibers in a percentage of 10-30% based composite.

- Hardness Values - varies considerable from 73°SH D for reference sample - PP (isotactic polypropylene) to 49°Sh D for PP / 3% PP-g-MA composite. The addition of relatively low amount of compatibilizer (PP-g-MA) influences the hardness by decreasing the degree of crystallinity and hence the viscosity of the polymer. For composites with 10, 20 and 30% treated and untreated fibers, an increase in hardness is observed with increasing amount of reinforcing agent.

- Tensile strength - for the reference mixture (PP) values are 6.21 N/mm^2 compared with the value of 5.6 N/mm^2 in the case of mixtures PP / 3% PP-g-MA which demonstrates that adding compatibilizer decreases tensile strength of the composite. 10-20% of untreated fiber composite shows improved value 25-28 N/mm^2 to 6.21 N/mm^2 reference value and 5.6 N/mm^2 obtained in the absence of polymer composite fiberglass. For composite with 10 and 20% treated fibers and in the presence of compatibilizing agent, the interactions (Van der Waals) that develops at the interface matrix polymer / compatibilizer / coupling agent on the surface of glass fibers induce an appreciable increase in tensile strength values 30-34.6 N/mm^2. This can be attributed to the fact that it deposits a significant amount of coupling agent, during treatment, on the surface of glass fibers. The composites with 30% treated fiber and PP-g-MA present, improves the tensile strength values to 22N/mm^2 of the composition with the same percentage of fibers, but untreated 11N/mm^2. These values demonstrate that the 30% levels of the untreated fiber composite lower the tensile strengths than those with 10% and 20% treated because: increase the melt viscosity and generate gaps in the material which are points for microcracks spreading in to large cracks.

Table 1. Tensile strength and hardness of PP, PP/3%PP-g-AM and developed composites

Symbol	Hardness ^{0}ShD	Tensile strength N/mm^2
PP	73	6.21
PP/3%PP-g-AM	49	5.6
PP/FG 10%	65	25
PP/FG 20%	68	28
PP/FG 30%	70	11
PP/3%PP-g-AM/ 10%FS treated with PDMS	63	30
PP/3%PP-g-AM/ 20%FS treated with PDMS	66	34.6
PP/3%PP-g-AM/ 30%FS treated with PDMS	67	22

Silanization was established on the basis of the comparative FTIR commercial glass fiber and treated the PDMS (Figure 1). It may easily see that the spectrum of the material change considerably due to silanization of fiber surface (CH_2 stretching bands intensifies the 2800-2900cm^{-1}) and the growth of physical bonded water (1740 and 3500cm^{-1}). Physical water appears because, after treatment applied, heat treated glass fibers PDMS at 130°C, they were washed with water to remove unreacted silane.

Composite materials based on glass fiber reinforced PP show the same range as that of PP not reinforced (Figure 2). The bandwith, Si-O-Si, characteristic for glass fibers can not be viewed due to the embbeding of fibers in the polymer matrix (Figure 3).

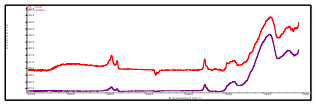


Figure 1. IR spectra of the treated and untreated glass fibers

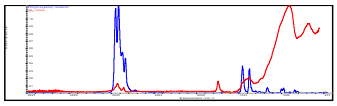


Figure 2. Standard IR spectra of PP and GF-treated PDMS

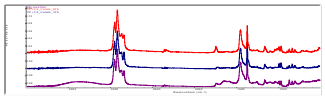


Figure 3. IR spectra of PP/3%PP-g-AM, PP/3%PP-g-AM/10 and 30%GF treated with PDMS

X Ray Diffraction (XRD)

Polypropylene has a high degree of crystallinity as evidenced by X-ray diffraction (Figure 4.). Using databases allowed identification of the bands characteristic of isotactic propylene and PP-g-MA used in a proportion of 3% compared to PP.

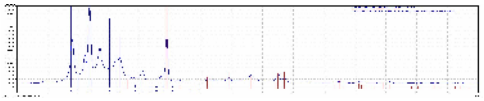


Figure 4. X-ray diffraction of the polypropylene plasticized with 3% PP-g-AM

If in the mass of plasticized polypropylene are embedded glass fibers, which may be identified, as the band intensity of 29.5 (Figure 5) is to note the large difference in intensity of this band where the analysis is performed on the surface or section resulting from mechanical tests. Much lower intensity for surface analysis is explained taking into account the embedding glass fibers in polymer matrix and thus shielding the signal, strong.

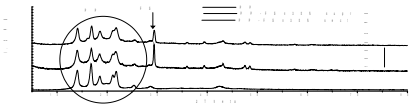
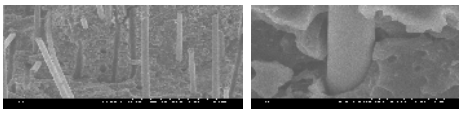


Figure 5. X-ray diffraction of the samples of PP, PP-treated GF 30%, registered in sectional respectively surface

Electron Microscopy

By scanning electron microscopy was possible to evaluate the efficiency of the dispersion of the treated and untreated glass fiber in the thermoplastic matrix and the capacity of wetting the surface fiber for thermoplastic matrix as a way to evaluate the efficiency of coupling agent.

Electron microscopy images recorded on samples PP / GF10% untreated (a) and PP/ 3%PP-g-AM / 10% GF treated with PDMS (b) were obtained in the section break and are presented in (Figure 6).



a) b)

Figure 6. Characteristic SEM images recorded on samples PP-FS10% untreated (a) respectively PP/3%PP-g-AM/GF treated with PDMS 10% (b)

Scanning electron microscopy (SEM) confirms micro morphological improvement of the connection strength: the compatibilizing agent PP-g-MA, PDMS-treated glass fibers and polymer matrix composites compared with using the untreated fiber. The compatibility of the thermodynamic characteristics of such compatibilized polymer matrix and the fibers treated with PDMS were found to be optimal because of strong adhesion between the fibers and the polymers matrix. This is evidenced also by SEM images (Figure 6b) of the fracture surfaces of obtained hybrid composites, seeing a significant deposition of coupling agent on the fiber surface. In addition, the film coating on the surface of the fibers (-Si-O-Si of polydimethylesilane) silane groups ionic and / or covalent can they bind very strongly to the existing functional groups on the surface of polypropylene -COO- groups, resulting from opening maleic cycle at high temperatures produced during extrusion.

Also presents some gaps in its structure, however the composite is more homogeneous, where the glass fibers are better attached to the polypropylene matrix, indicating that the PP-g-AM has acted positively on the interfacial adhesion of the polymeric matrix with reinforcement, which can be also indicated by the results of tensile strength the composites.

The images confirm the results obtained in mechanical tests where the compatibilized samples showed an increase of strength in relation to the non-compatibilized compositions.

CONCLUSIONS

Physical-mechanical and morphologic test results show good interfacial adhesion between polypropylene and glass fibers, due to both the surface coupling agent represented by PP-g-AM and to the treatment applied to glass fibers with poly (dimethylsiloxane) through reduction of thermal expansion coefficients which are completely different. SEM images show good compatibility between the polymer phase and disperse material and a good dispersion of glass fibers in the polypropylene mass. PP-g-AM has a double role, acting both as coupling agent and as plasticizer reducing the viscosity of the mixture. XRD provides information related to the existing crystalline phases, being able to identify peaks characteristic to polypropylene, glass fibers and a low amount of amorphous phase. Modifying the polymer matrix and the reinforcing agent improves adhesion, resistance to temperature and humidity.

Acknowledgements

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MECHANICAL BEHAVIOR OF A THIN LAYER GLASS FIBER STRENGTHENED OLD MASONRY

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Brick masonry structural system is commonly used in Romania and not only. This is due to its many advantages in terms of mechanical behavior based on increased capacity of stress redistribution. In case of seismic actions, many buildings have been partially damaged (in some cases more than 50%) leading to the investigation of appropriate strengthening solutions. In addition to traditional strengthening solutions, composite materials are an advantageous alternative due to the high strength versus weight ratio. This paper presents an experimental and numerical analysis on old brick masonry specimens made with weak lime mortar and limestone sand, subjected to uniaxial compression. The results were compared with finite element numerical analysis in order to show stress distribution between brickwork and strengthening layers applied. Several forms of strengthening application relative to masonry faces (interior and exterior) were considered in the numerical analysis. The beneficial effect on the mechanical behavior of strengthened masonry in terms of stress and strain distribution of both masonry and strengthening layers is shown to be dependent on the thickness of the strengthening layer.

Keywords: masonry strengthening, glass fiber mineral composites, mechanical behavior

INTRODUCTION

Masonry structures made with ceramic bricks were widely used in construction worldwide. Starting with Roman civilization these structural systems have progressed significantly due to the process of baking bricks in kilns. Mortar just as important as brick, is a key component of masonry and has also evolved. This technology has been taken up and used by many civilizations is spread all over the globe.

Depending on local resources, masonry have been adapted to the possibilities of the regions where they were applied. In Italy, lime, clay or volcanic ash are used as binder to make the mortar. Different combinations of mortar were identified in Romania as part of old masonry (Robertson, 1969; Mark and Hutchinson, 1986).

Research in the past years at the Faculty of Civil Engineering of Iasi focused on masonry systems made with local materials (Plesu *et al.*, 2012; Taranu *et al.*, 2013)

One of them is a sandy limestone called *pufar* mined near Iasi on Bucium hill (Pietraria village). Many churches and houses were made with masonry structure and mortar with *pufar* during the XVIIth - XVIIIth century. This mortar is characterized by reduced strength and large deformations capability. These masonry systems have a good behavior to multiple seismic actions that occurred during the life of the buildings analyzed. In this context the apparition and damage increasing to significant degradation require structurally efficient building solutions.

One of these solutions was identified by using a composite material with reinforced glass fiber mineral matrix (Taranu *et al.*, 2012; Baux, 2010; Thomas, 1972). The paper presents some experimental results and numerical analyses on unstrengthened masonry elements and strengthened with composite material.

MATERIALS

For the purpose of the experiments, 20 elements of masonry ceramic bricks with *pufar* mortar were made. The bricks were specially made by molding and baking clay, at 1:2 scale with dimensions of 117x60x36. The sand (*pufar*) was extracted from the hill near to Iasi and sieved with 2 mm sieve. Lime slurry was used as binder. Wet mortar has a good workability and adherence due to lime binder. Figure 1a presents a sample of simple masonry executed with same thickness of mortar joints.

In the second stage, for strengthening samples were used fiberglass mesh alkali resistant with 5x5mm network and mineral matrix. A strengthening layer of 1 cm thick, was applied by pouring fluid into a prismatic shape. The fluid mix is a combination of sand, Portland cement and calcium sulphate in β anhydride III' form manufactured from industrial wastes (Aranda, 2011; Toma, 2013). Figure 1 presents the stages of execution and pouring of masonry samples.

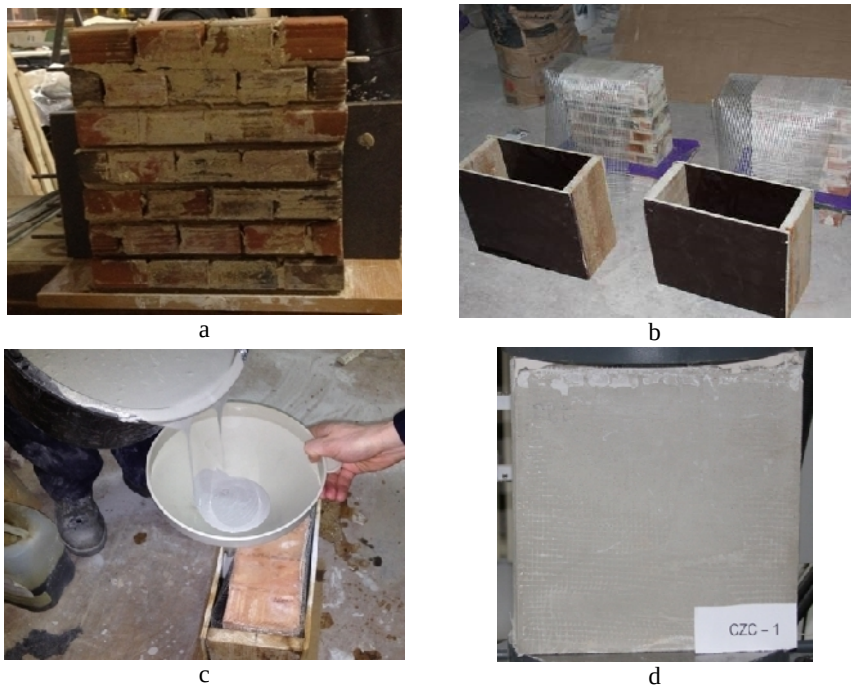
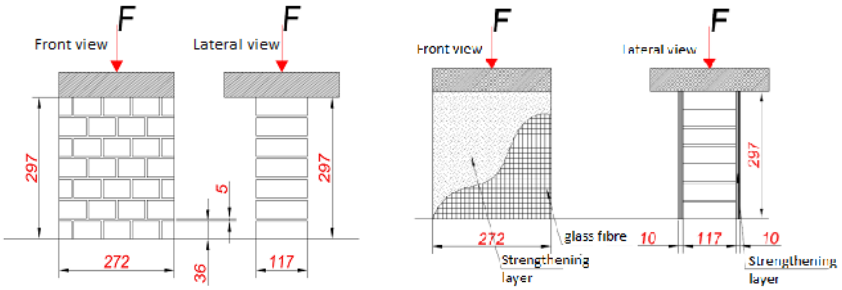


Figure 1. Preparation of masonry samples: a - simple unstrengthened masonry; b - glass fiber mesh reinforcement of masonry samples; c - pouring of the fluid mix; d - the final aspect of the strengthened masonry

All items were kept for 28 days under standardized conditions of temperature and humidity.

EXPERIMENTAL PROGRAM

Before testing the masonry samples, their component characteristics were determined. Masonry elements were tested in compression in a universal machine. Tests consisted of 5 samples of each type of masonry. The dimensions of the samples tested, as well as the loading schema are shown in Figure 2.



a b

Figure 2. Dimensions and loading schema: a - uniaxial compression on simple masonry, b - uniaxial compression on strengthened masonry

Testing was force controlled with a loading rate of 0.01 N/mm^2/s, until samples yielded completely. Displacements were measured with displacement transducer of the testing machine. Each of the 5 tests took about 25-30 min. The test was stopped when the failure of the samples occurred.

RESULTS AND DISCUSSIONS

The unstrengthened masonry samples tested in axial compression yielded by the appearance of vertical cracks parallel to the direction of force. They originally appeared in the layers of mortar after that in all the mass of bricks. The strengthened samples failure differs by taking in an early stage of deformation of thin strengthening layer. In these layers cracks were also propagated in the parallel direction to the loading of the sample. In Figures 3 and 4 the stress – strain curve and damaged samples are shown, respectively.

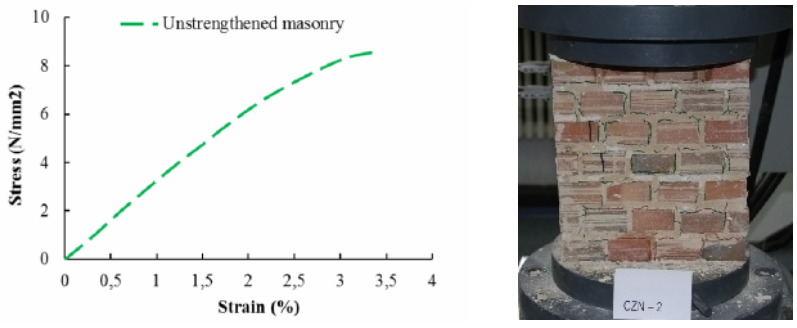


Figure 3. Stress strain curve for unstrengthened masonry

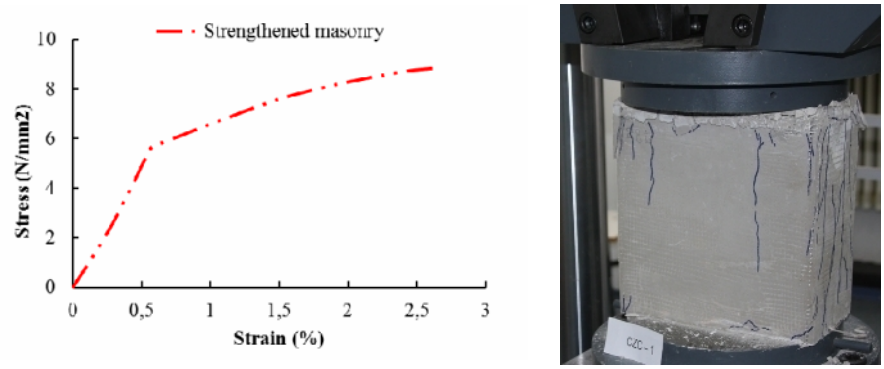


Figure 4. Stress strain curve for strengthened masonry

After completing the experimental tests values and mechanical characteristics were processed several numerical analyses were performed. FEM analyses assumptions were different depending on the composition of the samples. They were considered of distinct volumetric elements of strengthening layer, masonry, mortar or bricks. Figure 5 presents images of the models considered and their discretization.

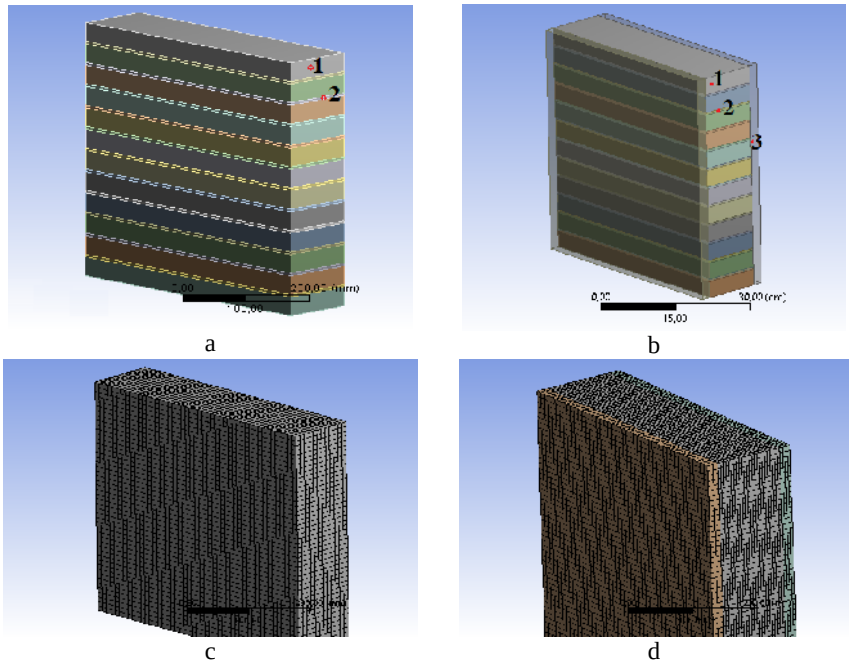


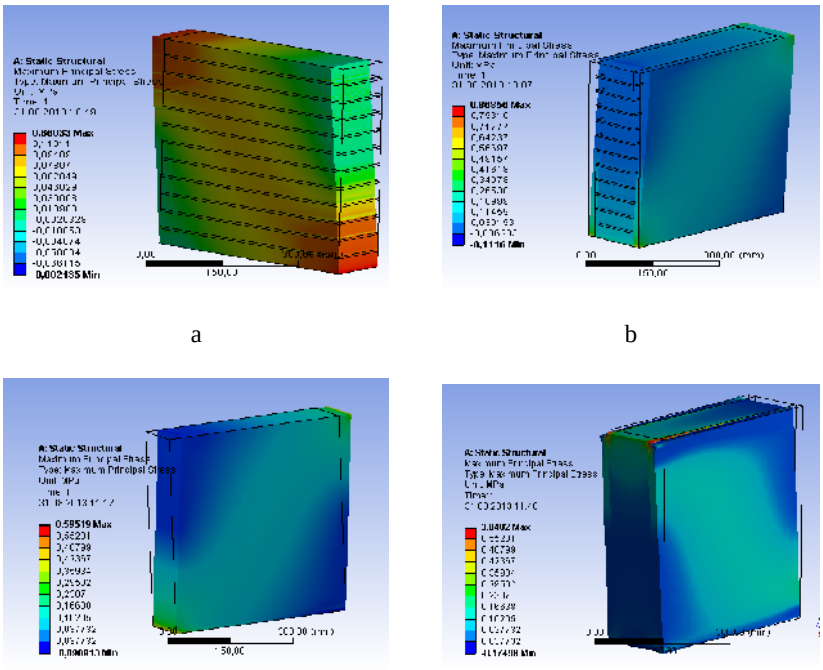
Figure 5. Numerical models analyzed: a - unstrengthened masonry (1 - brick, 2 - mortar); b - strengthened masonry (1 - brick, 2 - mortar, 3 - strengthening layer); c - homogenous simple masonry; d - strengthened masonry with 2 strengthening layers

For the numerical analyses performed on the samples the material characteristics presented in table 1 were used.

Table 1. Material characteristics

Material	Tensile strength (N/mm^2)	Compressive strength (N/mm^2)	Elastic modulus in compression (N/mm^2)
Mortar	0.29	2.30	208.95
Brick	2	14.72	426.23
Masonry	0.17	8.6	322.58
Mineral composite	11.78	26.84	9007

In the numerical model a horizontal load of 1000 N was applied in order to observe the distribution of the maximum and minimum principal stresses and strains. Figure 6 presents de image of map distribution of the maximum principal stresses in the case of the studied model.



a b c d

Figure 6. Maximum principal stress: a - unstrengthened masonry composed model; b - strengthened masonry composed model; c - unstrengthened homogenous model; d - strengthened homogenous model

Comparing the results of numerical analysis and the experimental test the influence of weak mortar on masonry strength is visible. When strengthening layer is applied an increase of overall stiffness and resistance is present.

CONCLUSIONS

Masonry structures are numerous and spread all over the world. The use of local materials led to different behavior of these structures. Due to the age of many of these structures and damages that occurred during their existence, the study of these systems and finding suitable and compatible building solutions is required. In this paper some experimental results of uniaxial compression tests on elements of masonry with local mortar made of calcareous sand, called *pufar* which was used at a large scale in Iasi region were presented. In addition to verifying the material properties some strengthening solution based on glass fiber reinforcement mineral matrix composite were proposed and tested. The mineral composite consists in 50% of fine sand, 25% ordinary Portland cement and 25% of calcium sulfate in β anhydride III' form, manufactured from industrial wastes and fiberglass mesh reinforcement.

The results show a considerable improvement of the behavior of masonry by applying thin strengthening layer on both faces of masonry. The strengthening layer increases capacity and helps to redistribution of efforts in a more uniform manner across the mass of element. This solution can be applied successfully if it is possible to pour on both sides of the walls. Also it was noticed a good compatibility between materials associated (masonry and strengthening mineral composite) both experimental tests and the numerical analyses.

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EXPERIMENTAL TESTS AND FINITE ELEMENT MODELLING OF GLASS FIBER REINFORCED MINERAL MATRIX COMPOSITES

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The actuality of composite materials and the mechanical potential provided to the structural systems for new construction and strengthening solutions have led to many research studies conducted with great interest. Polymer composite materials have been studied for over 50 years and have been successfully applied in many technologies. In case of building constructions mineral matrix composites have the advantage of high compatibility between strengthened structural element and composite material used. Also these solutions can be used to create new structural elements meeting the same exacting structural requirements as traditional construction systems. This paper presents the results of experimental tests performed on one story structural model laterally loaded after in order to obtain the failure mechanism and maximum strength capacity. The experimental results are compared with FEM numerical analysis. The conclusions show the influence of glass fiber reinforcement and stress distribution in these composite systems, experimentally observed and compared with the numerical analysis results.

Keywords: glass fiber mineral composites, tensile strengthening, experimental tests

INTRODUCTION

The concept of fiber reinforced composite materials was developed in past decades and brittle cement-based paste was reinforced with asbestos fibers when in about 1900 the so-called Hatschek technology was invented for production of plates for roofing, pipes, etc. Glass fiber reinforced concrete (GFRC) was developed in the 1940s, but it was not used until the 1970s (Brandt, 2008). Extensive research carried out since the early 1960 has shown that a suitable composite material can be produced by reinforcing special cements such as gypsum-aluminous slag cement and high alumina cement with low alkali borosilicate glass fiber, commercially available all over the world (e.g. E glass) (Majumdar, 1971).

The ordinary E-glass fibers are not resistant and durable in highly alkaline Portland cement paste and the alkali-resistant (AR) glass fibers without addition of zircon oxide ZrO2 which were introduced by Majumdar and Ryder (Feldman, 1993; Nakagawa and Akihama, 2000; Singh and Garg, 1994).

Recent research work carried out at the Faculty of Civil Engineering of Iasi has shown that an efficient combination between glass fiber and a fluid cement paste can be used in such a manner as to obtain precast low weight panels for modular buildings and also for strengthening solutions of masonry walls (Taranu *et al.*, 2013; 2011). The basic ingredient for obtaining this mineral matrix is the ordinary Portland cement which is also the key ingredient for concrete. If an amount of calcium sulphate in the β anhydride III' form is added, the result is almost spectacular. This mixture needs just 20 % water of entire quantity and it is transformed to a fluid mix which can be cast in small spaces like a 5 mm layer between polystyrene sheets. Calcium sulphate in the β anhydride III' form was developed by French researchers starting with 2008 (Aranda *et al.*, 2011; Baux, 2010).

This article presents some results of the research based on the structural behavior of a prototype building entirely made of glass fiber reinforced mineral composite

(GFRMC). The structural model was tested to a maximum lateral load carrying capacity and numerical analyzed with FEM.

MATERIALS

The fluid mix necessary for an appropriate matrix is the calcium sulphate in the β anhydride III' form which is an ecological binder. This is obtained from phosphogypsum, lactogypsum or citrogypsum and can be used as partial replacement of the Portland cement in a mineral matrix. Adding water and sand with pre-established mix proportions, one can obtain a fluid mineral matrix with fast setting time and good mechanical characteristics in a very short period of time. The fluid mix can be poured in narrow spaces, having a width of at least 5 mm, embedding the glass fibre reinforcement that may be present. However, before using this high workability mineral matrix in structural elements, one needs to follow a two-step procedure. The first step would be the setting of the formwork in the desired shape of the structural element. This can be done by joining polystyrene sheets to one another. In this way, the formwork also plays the role of thermal insulation as in Figure 1. The second step of the procedure is the positioning of the glass fiber mesh as reinforcement before pouring the fluid composite mix into the formwork. The mechanical characteristics were determined in laboratory and the average values are presented in Table 1.

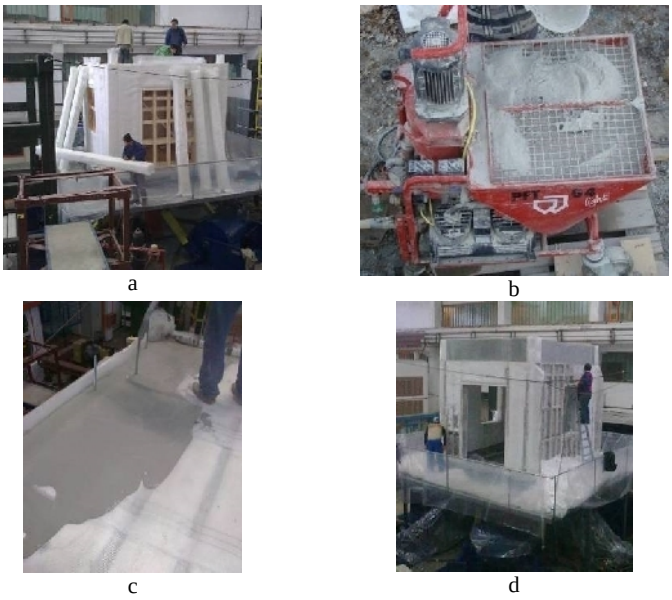
Table 1. Mechanical characteristics of the materials

Material	Compressive strength (N/mm^2)	Tensile strength (N/mm^2)	Compressive elastic modulus (N/mm^2)	Tensile elastic modulus (N/mm^2)
Glass fibre (alkali resistant without emollient obstructing yarn drifting - Mesh density (160 g/m^2)	-	2000	-	72413
Mineral matrix	31.29 [a] 25.72 [b]	7.28[c]	8840	9259
Composite (GFRMC)	28.78[a]	6.24[d]	9836	45595

[a] *Compressive strength on half-prisms;* [b] *Compressive strength on cylindrical samples;* [c] *Tensile strength from bending of the prismatic samples;* [d] *Uniaxial tensile strength on strips*

EXPERIMENTAL PROGRAM

The next step after performing the materials laboratory tests was the design and construction of a modular panel structure. Considering the laboratory facility and the testing machine capability, one story building model was designed with 3420 x 3420 mm in horizontal plan and a height of 3000 mm. Figure 1 shows the plan and the perspective view of the designed structure. The model was built directly on the shaking table platform. The base of the model made of a stiff steel frame was considered fixed and attached to the structure. After the mounting of the panels in their position the pouring and cast of the fluid mixture has started. This operation was done by an appropriate pump for mortars. The premixed binder in dry state form was mixed with the controlled amount of water in the pump. The setting time of the matrix was short and the entire structure was made in 4 hours.



a b c d

Figure 1. Stages of the building experimental model: a – mounting of the polystyrene panels; b – electro-mechanical pump; c – casting of the fluid mix; d – removing of the exterior polystyrene layer

The structural model was tested several times on the shaking table with different seismic actions. The performed tests had different levels of intensity from low with maximum acceleration of 0.1g to high with 0.4g. The maximum level showed that the model was stiff enough and that no damage was produced. After these tests, the model was positioned laterally to the shaking table and hinge connected to the shaking table. In this new position the structure was fixed at the base and laterally loaded by the shaking table with low frequency increasing load. The model was equipped at the same level of action with 2 LVDTs for the displacements measurement and a 200 kN force transducer. Figure 2 presents the equipped structural model before the experimental test.

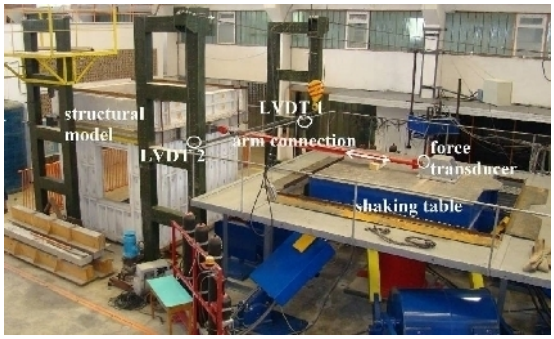


Figure 2. Structural model laterally connected to shaking table

In parallel with experimental test few FEM numerical analyses were performed. Figure 3 presents the loading schema of the side-wall of the structure which respect to the real environment of the test and was FEM analyzed.

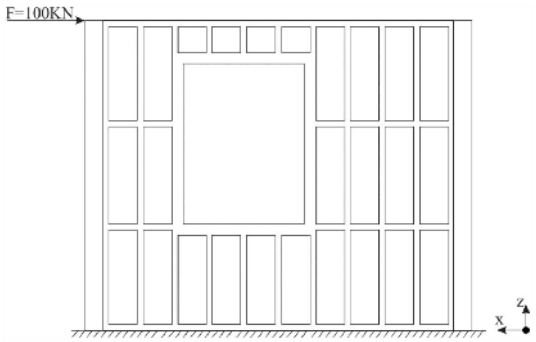


Figure 3. Loading schema of the GFRMC structural wall

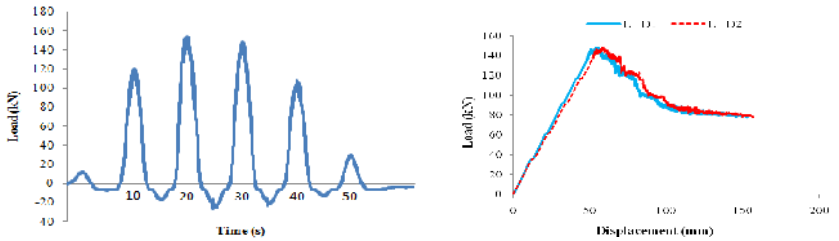
RESULTS AND DISCUSSION

The first step of the experimental program was the selection of the loading vs. time action. Few cycle loading of the shaking table with a low frequency starting from 0.1 Hz and variable amplitude were imposed. The first test reveals that strength of the model at the base connection was very low and walls were detached of the base (Figure 4a). The entire model was still stable and stiff. This fact required additional connection of the structure to the base for an improved fitting (Figure 4b). Two additional tie rods were mounted around the window parapets. After this preparation the loading test was resumed.

The additional connections have proved to be effective meaning that the model was fully loaded with the laterally applied force. The last test consists in a push movement of the structure until this was totally damaged. The failure mechanism occurs in the window railings areas and in the corners of the windows. Figure 5 presents the load vs. time curve and the load vs. displacement curve recorded also.

a b

Figure 4. a) Failure of the base connection; b) additional connection of the structural model



a b

Figure 5. a) Load vs. time applied on the model; b) load vs. displacements (left D1; right D2)

As was expected in the corners of the windows, the maximum tensile stresses appeared. The strength of the material was exceeded and local cracks developed. Some details with the failure mechanism are presented in Figure 6 and numerical results of the FEM analysis also.

a b

Figure 6. a) Structural model damaged after the laterally loading test; b) maximum principal stress in FEM analysis

From the load displacement curve of the loading test it can be observed that the material has linear behavior until the cracks appear in the structure. The existence of multilayer of glass fiber reinforcements leads to a step by step failure which shows some ductility of the overall behavior. This means that even if the strength was exceeded large displacements are allowed. From the numerical results it can be observed the distribution of stress in the wall with stress concentration in the corners of the window with a maximum value of 9.7 N/mm^2.

CONCLUSIONS

Recent research showed that there is a possibility of using a green mineral matrix achieved by partial replacement of Portland cement with a binder from industrial waste in construction industry.

The intended use directions relate to the development of structural walls made of mineral matrix reinforced with fiberglass meshes. Another possibility for using this

material is the utilization for structural rehabilitation of the load bearing elements from traditional building materials.

However the efficient use of these mineral matrix composites depends on a thorough knowledge of the strength and stiffness characteristics required for the design process; therefore a comprehensive experimental program has been designed and performed.

In this article some experimental and numerical results regarding the maximum laterally load carrying capacity of a composite structure made entirely of glass fiber reinforced mineral matrix has been presented. The built structural model has a total mass of 6 tones. The stiffness behavior and the low weight have proved a good seismic performance in several tests on a shaking table.

The main objective of laterally load carrying capacity experimental tests was the identification of the failure mechanism. The loading schema was numerical modelled with FEM and a map distribution of the stress and strain were analyzed. These results confirm the real behavior of the structural model. The failure mechanism was the result of high tensile stresses in the corners of the windows and high shear stresses occurred in the parapets under the window also.

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PERFORMANCES OF A POLYSILICON BYPRODUCT-SILICON TETRACHLORIDE ON WET BLUE PREPARATION

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Sodium silicate was prepared with a polysilicon byproduct-silicon tetrachloride through hydrolyzation and alkalization. Meanwhile, a new process for wet blue preparation was carried out, including enzyme dehairing, sodium silicate swelling, pickling and semi-chrome tanning. Properties of the wet blue such as mechanical properties, thermal stability and surface color were tested following the standard method. Chrome distribution of the wet blue was investigated with a inductively coupled plasma (ICP). A scanning electron microscope (SEM) and a atomic force microscope (AFM) were also used to illustrate the histological features of the wet blue. Finally, environmental impact of both swelling and tanning process were studied through COD, chrome contention, total solids and turbidity test. The results showed that, the standard requirements for shoe upper leather were met by the wet blue while the dosage of chrome agent was 1.0% (half of the traditional chrome tanning method). Meanwhile, for the wet blue prepared with new method, lighter surface color was presented, less Cr(III) was detected and also equally distributed. More compact fiber bundles were shown on the SEM images. No obvious damages were detected in tropocollagen fiber structures through AFM analysis. Furthermore, compared with the traditional process, COD, total solids and turbidity data of both swelling and tanning effluent provided a lower value. The results could provide a feasible way for silicon tetrachloride recycling, also provide valuable references for clean production of leather industry.

Keywords: silicon tetrachloride; silicon-chrome combination tanning; leather

INTRODUCTION

For recent decades, solar industry has gain a great support from society and has been considered as an important part of clean energy. Production of polysilicon also achieved a considerable scale. It is believed that, about ten tons of silicon tetrachloride will be generated while one ton of polysilicon is produced (Lv *et al.*, 2008). The silicon tetrachloride were highly acidic and corrosive fluid and often causes a serious of environmental problems. Generally, this byproduct can be applied for fumed silica preparation through combustion and hydrolyzation, or for organosilicon manufacture through alcoholysis and hydrolyzation, or even applied in trichlorosilane preparation through Siemens process and reused in polysilicon industry (Barthel *et al.*, 1998). However, it is hard to put the reuse into practice due to the sophisticated requirements of the reactions and the expensive cost of recycling. Therefore, how to deal with this byproduct in a proper way, has became a significant obstacle for the development of solar industry.

Just as with the solar industry, leather chemists are striving for narrowing the problems list which is caused by liming and tanning process. Many cleaner liming and tanning techniques have been developed and widely used so far in leather manufacture. However, there are still some problems for these techniques, such as lime and sulfide cannot be avoided completely, quality of the wet blue was not good as the ones prepared with traditional methods. Such problems always trouble the leather chemists.

In order to solve the problems from both leather and polysilicon industries. A byproduct-silicon tetrachloride obtained from polysilicon industry was used as a raw material. A hydrolyzation and a alkalization process were provided successively. Then, a new wet blue preparation process was carried out. Properties of the wet blue were investigated, environmental impact of this process was also studied. The results will offer a economical way for silicon tetrachloride recycling, also provide valuable evidences for employing sodium silicate into leather industry.

MATERIALS AND METHODS

Preparation of Sodium Silicate

0.5 kg of silicon tetrachloride was slowly added into 2.0kg of water and properly mixed, then hydrolysation reaction was happened following equation (1):

$$SiCl_4+H_2O \rightarrow SiO_2 \cdot xH_2O+HCl \quad (1)$$

After silica gel was formed, it was transferred into an oil bath and heated at 110°C for about 4h. The heating process was stopped until ammonia test showed no hydrochloric gas releasing from the mixture, then silicic acid was prepared.

0.27kg of sodium hydroxide was added into the silicic acid meanwhile stirred at 80°C for 30min, then the alkalization reaction was happened according to equation (2):

$$SiO_2 \cdot xH_2O+NaOH \rightarrow Na_2O \cdot nSiO_2+H_2O \quad (2)$$

Properties of this sodium silicate were tested following the standard methods (Chinese standard: GB/T 4209-2008), and the results showed that in the sodium silicate, content of Na_2O and SiO_2 was 9.77% and 14.28% respectively, modulus was 1.51.

Process for Wet Blue Preparation

Wet salted sheepskins were cut into two sides along the back line. For one side, a enzyme unhairing process(0.25% of 2709 protease, 1.5% of ammonium sulfate, and 0.2% of sodium sulfite, based on salted hide weight), a sodium silicate swelling process (5.0% of sodium silicate, based on unhaired hide weight), a traditional pickling process (pH 2.5), and a semi-chrome tanning process (4.0% chrome tanning agent, based on the pickled hide weight) was carried out successively and taken as sample prepared with new method. The other side was prepared with a traditional leather making process, which was liming (5% of lime), unhairing (3% of lime and 3% of sodium sulphide, based on the wet salted hide), deliming, bating, pickling (pH 2.5) and chrome tanning (8.0% chrome tanning agent) successively and taken as control. Samples for analysis were taken from standard section, meanwhile, effluents from both swelling and chrome tanning process were properly collected.

Analysis of the Wet Blue

Shrinkage temperature, tensile strength and tear strength were tested following the standard method (Chinese standard: QB/T 2713-2005, QB/T 2711-2005), surface color was investigated with a 8200 color master (X-rite, American).

Cr(III) Distribution in the Wet Blue

Even pieces (0.5cm×0.5cm, 0.2cm of thickness) of wet blue were cut from standard section, then the grain side, flesh side and core of the wet blue were separated with a freezing microtome. After a standard wet digestion process, the Cr(III) distribution were investigated with a Optima 2100 ICP-AES instrument (Perkin Elmer, American).

SEM and AFM Analysis

Histology properties of the wet blue were investigated with a JSM-7500F scanning electron microscope (JEOL, Japan) at 5.0kv. Meanwhile, a accessorial energy dispersive spectrometer (EDS) was used to detect the silicon and chrome contention.

Then, the wet blue was sliced from grain side to flesh side with a freezing microtome. Thickness of the slices were 50 um, samples were collected between every 300um. After air dried for 24hs, the character of procollagen was studied with an SPM 9600 atomic force microscope (SHIMADZU, Japan).

Analysis of Spent Liquor

The effluents form both swelling and chrome tanning process were analyzed for CODcr, total solid content and chrome content following standard procedures. Turbidity of the effluents were tested with a WGZ-2000 turbidimeter (scientific equipment co., LTD., China) after diluted for 100 times with distilled water and stilling for 2hrs.

RESULTS AND DISSCUSSIONS

Quality of the Wet Blue

Table 1. Properties of the wet blues

	Shrinkage temperature °C	Tensile strength N/mm^2	Tear strength N/mm	Elongation at break%	Color* L	a	b
New method	105.2±1.5	33.9±1.7	39.5±1.5	35.2±2.0	79.2±0.6	-3.1±0.1	0.5±0.3
Control	113.0±1.0	20.2±1.5	33.6±2.1	32.3±1.8	75.8±1.5	-4.6±0.2	-1.6±0.1
Chinese standard: QB 1873-2004	≥100	≥15	≥35	25-60	-	-	-

* L: "+" indicated a brighter color, "-" indicated a darker color; a: "+" indicated more red, "-" indicated more green; b: "+" indicated more yellow, "-" indicated more blue.

Table 1 indicates that, the mechanical properties and thermal stability of the wet blue presented proper values. Meanwhile quality standard for shoe upper leather were also met. Moreover, compared with the control, better tensile strength and tear strength were obtained by the new method. In addition, because of the reduction of dosage of chrome agent, surface color showed a lighter value. Therefore, in aspect of leather qualities, sodium silicate prepared with silicon tetrachloride can be used in leather industry.

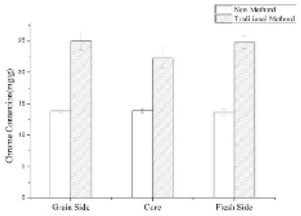

Figure 1. Cr(III) distribution in the wet blue

Figure 1 indicated that, compared with the traditional method, the Cr(III) was more equally distributed in grain side, core and flesh side of the wet blue. Furthermore, due to the reduction of chrome application, chrome contention was obviously less than control.

Histology Properties of the Wet Blue

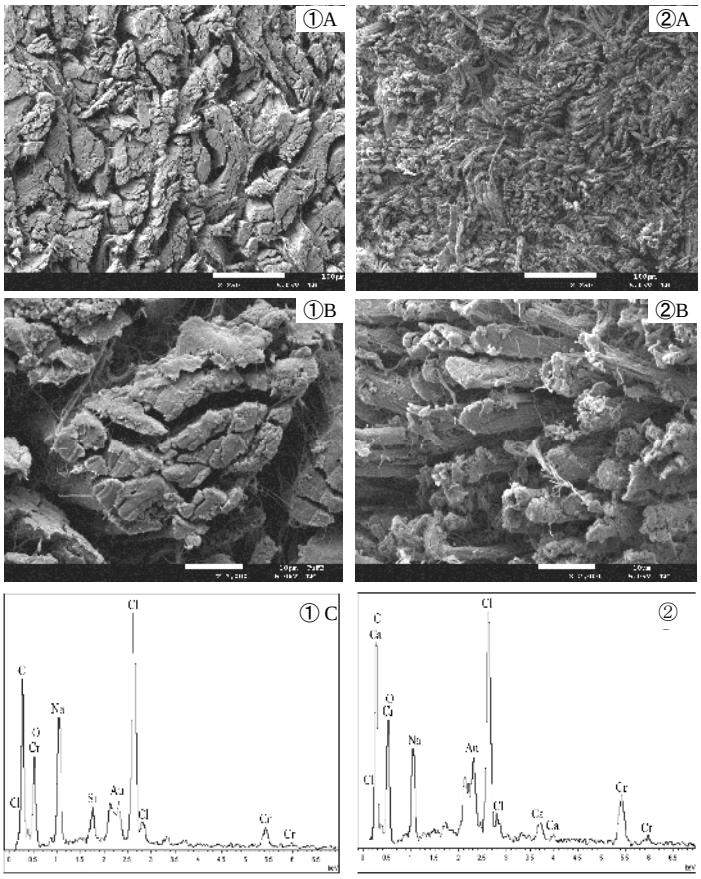

Figure 2. SEM and EDS images of the wet blue (1: wet blue obtained from new method, 2: control A: ×250, B: ×2000, C: EDS spectra)

Figure 2 indicated that, differences between sample and control are obviously existence. For the wet blue obtained from new method, more tightening characters could be discovered. Fiber bundles in sample were heavier and thicker. Meanwhile, lager distances were also shown between these fiber bundles. The sensory property of the wet blue presented a full and compact character. Both silicon and chrome were detected in the wet blue, indicated the sodium silicate was penetrated into the hide through swelling, and still remained after pickling and tanning process.

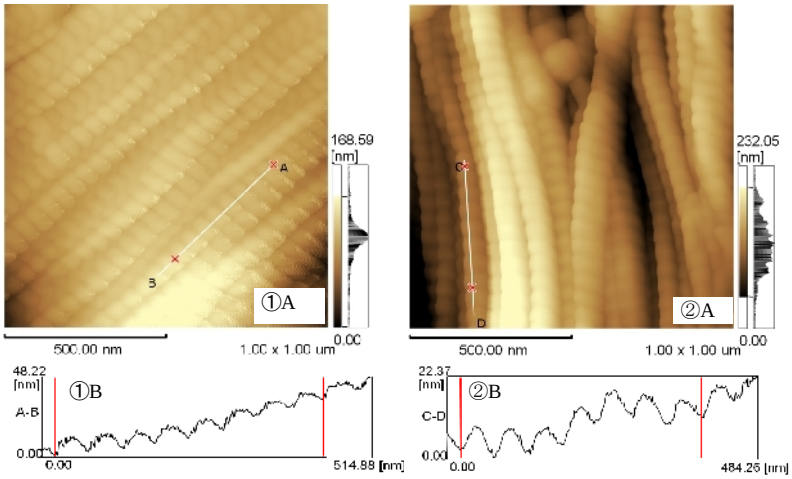

Figure 3. AFM images of the wet blue (1: wet blue obtained from new method, 2: control□ A: procollagen images, B□ D-period)

Table 2. D-period in each layer in wet blue

	New method (nm)	Control (nm)
First layer (grain side)	56.88±2.31	52.89±0.93
Second layer	56.23±2.34	52.82±1.04
Third layer	52.24±1.05	51.94±0.61
Fourth layer	52.05±0.90	51.18±2.99
Fifth layer (flesh side)	54.76±0.83	55.68±1.03

Based on the Schmitt model, there is a cross striations with 67nm length existed in microfibrils which is also called ‘1/4 stagger’ or ‘D-period’. The cross striations were clearly shown on the AFM images of both sample and control. Furthermore, table 2 shows that, the D-period of microfibrils in each layer of the wet blue were presented a similar value compared with the control. It indicated no obvious collagen structural damages were caused by the new method.

Pollution Load from Swelling and Tanning Process

Table 3. Environmental impact of new method

	Swelling effluent		Chrome tanning effluent	
	New method	Control	New method	Control
Chrome content(mg/L)	--	--	1396±63	3207±91
CODcr(mg/L)	2076±34	2187±62	1634±48	1821±66
TS(mg/L)	2312±44	3068±36	1474±52	2219±94
Turbidity	6.7±0.1	149.7±10.3	3.2±0.6	56.1±7.3

Table 3 shows that compared with traditional leather making process, the environmental influence of the new method were obviously reduced, in terms of lower chemical oxygen demand (CODcr), lower total solid content (TS) and lower turbidity of both swelling and tanning effluents. Reduction of TS and turbidity was contributed to the better solubility of sodium silicate.

But for the tanning effluents, the TS and turbidity difference was mainly due to the tanning effect of silicon agent, which enhanced the mechanical strength of collagen fibers. Therefore, the effluent from tanning process presented a lower TS and turbidity value.

CONCLUSIONS

A polysilicon byproduct-silicon tetrachloride was hydrolyzed and alkalized successfully, and then sodium silicate was properly prepared. A new process for wet blue preparation was carried out. Properties of the wet blue and environmental impact of the new process were also studied. The results showed that, the standard requirements for shoe upper leather were met by the wet blue while the dosage of chrome agent was 4.0% (half of the traditional chrome tanning method). Lighter surface color was also presented. Meanwhile, less Cr(III) were detected and equally distributed in the wet blue. More compact fiber bundles were shown on the SEM images. No obvious damages were detected in tropocollagen fiber structures. Furthermore, compared with the traditional process, COD, total solids and turbidity data of both swelling and tanning effluent provided a lower value. The results could provide a feasible way for silicon tetrachloride recycling. Also provide valuable references for clean production in leather industry.

Acknowledgement

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COMPLETE CHARACTERISITC CURVE OF CONCRETE AND RUBBERIZED CONCRETE

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The complete stress-strain curve, also known as characteristic curve, of brittle materials is difficult to get due to sudden failure of the testing sample shortly after the peak load. The need for a full stress-strain curve is of paramount importance especially since all of today's FEA packages employ such information in their analysis. Concrete is by far the most widely used construction material worldwide. It is inherently brittle and its complete characteristic curve is, to some extent, not yet available. The paper presents the experimental results that led to obtaining all the data required for tracing a full stress-strain curve of traditional concrete. Furthermore, the information is compared to that obtained for rubberized concrete where fine aggregates were replaced by rubber crumbs resulted from tire recycling. The rubberized concrete exhibited a more ductile behavior after peak load but with a penalty on the ultimate strength. The higher the percentage of aggregate replacement, the more ductile the behavior but with smaller compressive strength. Additionally, a decrease in the slope of the linear elastic range of the stress-strain curve was observed. The experimentally obtained curves were compared with the theoretical ones from the existing scientific literature. It was concluded that even though a close match was found, there is room for improvement in the consideration of the post-peak behavior of concrete.

Keywords: stress-strain curve, rubberized concrete, post-peak behavior

INTRODUCTION

The solid waste disposal is a major environmental concern of our days. Considerable amounts of non-biodegradable materials are annually stockpiled in landfills because there are no specific regulations regarding their recovery or because their recycling is not considered an economic alternative (Toma *et al.*, 2013).

Waste generation from post-consumer tires in the EU countries is estimated to stand over 3.5 million tons per year (ETRA, 2010). Only a third of this amount is actually recycled and a similar proportion is incinerated for energy recovery in cement kilns (Siddique and Naik, 2004). However, the remaining waste tires disposed in landfills are an important source of pollution especially when fires break out producing significant air and water pollution.

The use of waste automobile tires in civil engineering applications dates back to the very early ages when automobiles were first invented. Waste tires became natural candidates for construction materials, such as landfills and cushion materials. However, large scale recycling of waste tires in civil engineering applications did not happen until recently. Material recycling tends to be considered all over the world as an alternative solution instead of waste tires disposal. More than 36% of post-consumer tires are recycled in the EU countries and the recycled tire materials are used in more than fifty industries. Less than 10% is used for tire manufacturing.

Compared to their applications in asphalt paving mixtures, the use of recycled tires in Portland cement concrete has been limited (Azizian *et al.*, 2003). The size of waste tires used in concrete ranges from rubber chips (25 mm to 50 mm) to crumb rubber (1 mm to 8 mm) to powders (0.075 mm to less than 1 mm). Waste tire materials replace

part of coarse or fine aggregates. The addition of waste tire rubber significantly alters the properties of the concrete (Liu *et al.*, 2013). Due to the hydrophobic nature of rubber, the bond between the untreated rubber particles and hydrated cement is weak, resulting in significant reduction of both compressive and tensile strength of rubber concrete (Topcu, 1995; Huang *et al.*, 2004). On the other hand, concrete becomes more ductile, as illustrated by higher post failure toughness (Li *et al.*, 2004).

Several analytical and laboratory based experimental studies have been performed lately in order to evaluate the mechanical performances of concrete mixes obtained by replacing the mineral (fine or coarse) aggregates with various volume fractions of rubber particles (Ganjian *et al.*, 2009). Generally, a reduction of compressive strength has been observed, but the increase of the ductility recommends the rubberized concrete mix for the construction of highly deformable, but strong enough structural elements (Son *et al.*, 2011). However, a massive replacement of aggregate with rubber particles (more than 15-20%) is not indicated due to the significant reduction of compressive strength (Khaloo *et al.*, 2008).

The obtained results reported in the literature showed that the use of the rubber crumb in construction applications can be a viable solution for post-consumer tires recycling (Pacheco-Torgal *et al.*, 2012).

The paper presents the experimental results that led to obtaining all the data required for tracing a full stress-strain curve of traditional concrete. Furthermore, the information is compared to that obtained for rubberized concrete where fine aggregates were replaced by rubber crumbs resulted from tire recycling. The rubberized concrete exhibited a more ductile behavior after peak load but with a penalty on the ultimate strength.

MATERIALS AND EXPERIMENTAL SET-UP

Materials

The cement used was a type I, 42.5R cement, with rapid hardening. It is a general purpose cement being suitable for all uses in works requiring high strength values at early ages. The target concrete strength class was C28/35, normally used for screeds.

The aggregates consisted of river gravel, having rounded edges. The aggregates were used in two different sorts based on their size: 4-8 mm and 8-16mm. For the rubberized concrete, 10% volume fraction of the sand (0-4mm) was replaced by rubber crumbs resulted from tire shredding process and subsequent sieving. The rubber crumbs were inspected for the presence of steel beads and traces of textile materials that were part of the original tire but the samples provided by the manufacturer were clean of those impurities. The mix proportions considered at this stage of the research are presented in Table 1.

Experimental Set-Up

A total number of 120, 100×200 mm, cylinders were cast for the two mix proportions shown in Table 1. After demoulding, the cylinders were kept in water until the day of testing, in accordance with SR EN 12390-2 (2009) specifications. Prior to testing, each cylinder was measured and weighed in order to determine the hardened density of normal and rubber-concrete.

The mechanical and elastic properties of concretes made with the mix proportions shown in Table 1 were determined as follows: the uniaxial compressive strength was determined on 30 specimens following the recommendations of SR EN 12390-3 (2009) code, the static modulus of elasticity was determined on 29 of the 30 cylinders used for uniaxial compression according to BS EN 12390-13 (2013) recommendations, the splitting tensile strength was determined on 20 cylinders in accordance with SR EN 12390-6 (2010) code. The complete stress-strain curve was determined on the remaining 10 cylinders.

Figure 1 shows the equipment used to assess the modulus of elasticity in compression, whereas Figure 2 presents a typical load-displacement curve used to evaluate the modulus of elasticity.

Table 1. Mix proportions for normal and rubberized concrete

Mix name	Cement	Sand	Aggregates		Rubber	Water	W/C ratio	Superplasticizer
			4-8 mm	8-16mm				
	[kg/m^3]	[kg/m^3]	[kg/m^3]	[kg/m^3]	[kg/m^3]	[l/m^3]	-	[l/m^3]
Ref.	335	847	491	532	-	185	0.55	3.35
Mix10%	335	830.87	491	532	16.13	185		

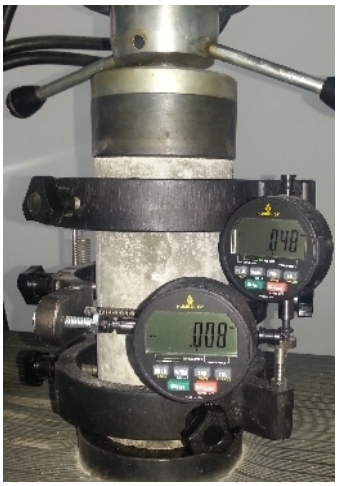

Figure 1. Measuring modulus of elasticity in compression

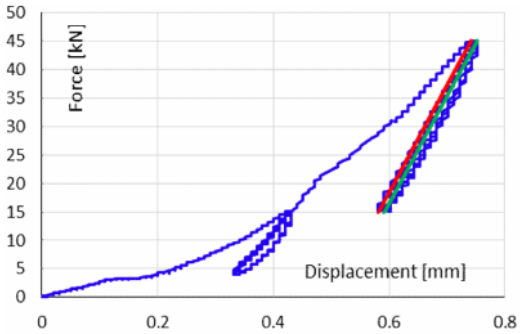

Figure 2. Load-displacement curve according to BS EN 12390-13 (2013)

The complete stress-strain curve was determined by using a special patented device (Negoita *et al.*, 1980), the purpose of which is to transform a force-controlled testing procedure into a displacement-control. The latter is a more suitable testing method in case in brittle materials, especially when the post-peak behavior is of interest.

RESULTS AND DISCUSSIONS

Strength and Elastic Characteristics

Table 2 presents the mechanical characteristics of the considered mix proportions. It can be observed that by replacing the sand with rubber crumbs leads to a penalty in terms of both compressive and splitting tensile strength by 7.38% and 15.64%, respectively. The obtained results are in line with the ones obtained on similar mix proportions and reported in the scientific literature (Issa and Salem, 2013). The traceability of the experimental results is quite good considering that the values of the coefficient of variation (COV) is below 10% and the number of data entries used for the statistical processing was at least 30.

Figure 3 shows the evolution of the modulus of elasticity as function of the curing age of the cylinders. It can be observed that in the case of the reference mix, the modulus of elasticity reaches its prescribed value for the corresponding concrete class at the age of 14 days and remains almost constant until the age of 28 days.

Table 2. Mechanical characteristics of normal and rubberized concrete

Mix name	Density [kg/m^3]	COV [%]	Compressive strength, f_{ck} [N/mm^2]	COV [%]	Splitting tensile strength, f_t [N/mm^2]	COV [l/m^3]
Ref.	2435	0.63	33.46	6.2	2.75	8.91
Mix10%	2350	1.05	30.99	5.4	2.32	6.33

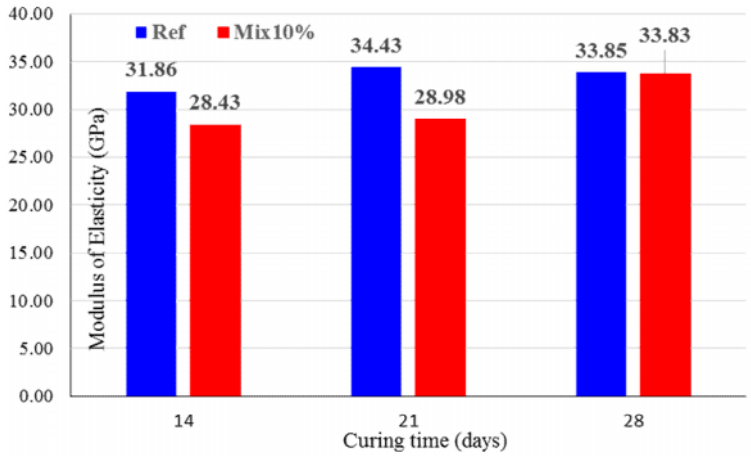

Figure 3. Modulus of elasticity as function of curing time

The rubberized concrete, however, exhibits a continuously increasing trend in the values for the modulus of elasticity from 14 to 28 days, reaching a similar value to the reference mix.

Based on the obtained results it can be concluded that the influence of rubber addition on the elastic properties of concrete is significant only during the early stages of curing when the concrete is not completely hardened. Once the hardening process is complete, the influence of rubber upon the modulus of elasticity is very small. This is,

however, valid for small percentages of rubber crumbs taking into account that in this case the rubber content is less than 4%, volume fraction, of the total volume.

Stress-Strain Curve

The complete stress-strain normalized curves of normal and rubberized concrete are shown in Figure 4. As expected, the rubberized-concrete is able to dissipate more energy, especially in the post-peak region. The strain energy stored in the samples made of rubberized concrete is 10.5% larger than the ones stored in the cylinders made from the reference mix. This means that structural elements made of rubberized concrete can dissipate more energy compared to their counterparts made of traditional concrete. More energy dissipation would improve seismic safety of concrete structures such as bridges and buildings. Similar results were reported by Xue and Shinozuka (2013).

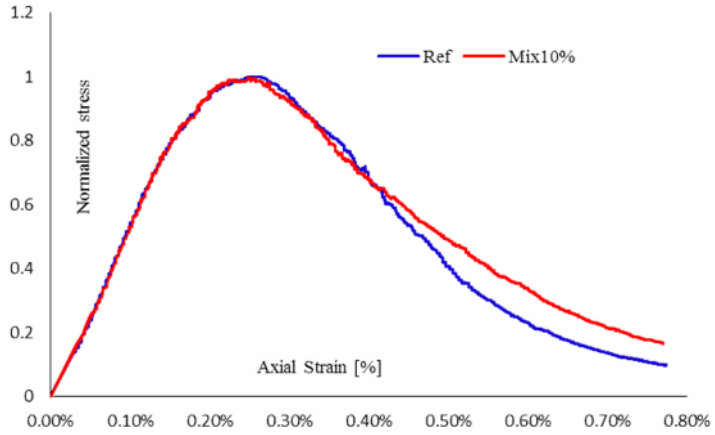

Figure 4. Normalized stress-strain curves for concrete and rubberized concrete

CONCLUSIONS

The ever-increasing volume of rubber waste in landfills from the disposal of used tires has grown into a serious environmental problem. For both environmental and economic reasons, there is renewed interest in developing alternatives to disposal. The paper presents preliminary findings, from a long series of experimental investigations, on the complete stress-strain curve of normal and rubberized concrete as well as their mechanical properties. Based on the obtained results, the following conclusions can be drawn:

The addition of rubber particles, in small percentages, does not seem to significantly alter the compressive strength and the modulus of elasticity at the age of 28 days. The latter has a continuously increasing trend compared to the one obtained for the reference mix which exhibits an almost constant value between 14 days and 28 days. The splitting tensile strength, however, decreases by as much as 15% for rubberized concrete.

The real benefit from adding tire rubber crumbs comes in the form of decreased density and a 10.5% higher energy dissipation capacity. More energy dissipation would improve seismic safety of concrete structures such as bridges and buildings.

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ELASTIC PROPERTIES OF MINERAL MATRICES WITH HIGH CONTENT OF ENVIRONMENTALLY SUSTAINABLE BINDER

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Concrete made with hydraulic binders, the vast majority of which are based on Portland cement, is by far the most widely employed construction material worldwide in terms of volume. The biggest advantage of modern concrete is the possibility of including other industrial by-products into the mix. The use of waste materials becomes more and more attractive as an alternative in the construction industry mainly due to the increasing cost of raw materials and the continuous reduction of natural resources. The present paper brings its contribution to the investigation on the use of a new binder, the anhydrous calcium sulphate in its β anhydrite III' form, a new Cementitious material, as partial replacement of the ordinary Portland cement in concrete. The binder is obtained exclusively from industrial wastes and can be entirely recycled after its expiration date. Its influence on the elastic properties of mineral matrices, at various curing ages, is experimentally investigated. The results show a slight decrease in the values of the longitudinal modulus of elasticity and no change in Poisson's ratio. Given the fact that up to 40% of Portland cement was replaced by the environmentally sustainable binder, the obtained results recommend the new binder as a viable solution in replacing Portland cement in construction works.

Keywords: sustainable mineral binder, elastic properties, cement replacement.

INTRODUCTION

Mortars have been used for a variety of applications since ancient times using mainly three types of binders: mud, probably the oldest type of binder used for preparing the mortar, gypsum and lime. The binder contributes to the workability of the mortar, whereas the aggregate influences the mechanical properties and helps in controlling shrinkage related problems. With the first introduction of the Portland cement the strength of mortars and, subsequently, concrete became higher and higher, following the trend of constantly increasing expectations related to their performance (Bentur and Mitchell, 2008).

The innovation in the construction industry is continuously driven not only by the need of reducing the environmental footprint (Toma *et al.*, 2013) but also by the increasing demand of reducing the costs in compliance with the evolution of the global market. This can only be achieved by a constant and sustained innovation process which should also anticipate the future environmental constraints (Schneider *et al.*, 2011; Shi *et al.*, 2011).

A major step in the direction of sustainable development has been done with the development of alternative cementitious binders (Juenger *et al.*, 2011). An important source of such materials is the industry itself. Fly ash, a by-product of the coal-fired power plants and municipal incinerators, has been successfully used in construction industry to improve the properties of concrete, such as strength and durability (Lee, 2009). Ground granulated blast furnace slag has been used since 1950 either as partial replacement of ordinary Portland cement or as fine aggregate in concrete mix design with highly improved resistance to aggressive environments (O'Connell, 2012). Silica

fume, a very reactive pozzolan due to its chemical and physical properties, has a relatively short period of time since its application in concrete industry. Significant improvements in the early age strength properties of mortars have been reported when silica fume was used (Sezer, 2012).

In view of the new stricter regulations in terms of CO2 emissions (Rehan and Nehdi, 2005) as well as continuous developments in both electric power and steel production one can only expect a significant decrease in the quantities of resulted fly ash and blast furnace slag. There are, however, other sources of production for the supplementary cementitious materials (SCM), some of them used for centuries (Yang *et al.*, 2007) but still requiring mining the natural deposits and some of them more recent, obtained from the treatment of industrial by-products such as phosphogypsum (PG) (Singh, 2002).

The present paper presents the results of a research work focused on the suitability of using a new mineral binder as partial replacement of ordinary Portland cement in concrete. Its effect on the elastic properties of mortars, when used in large percentages, is presented. The main parameters of the research were the type of cement and the replacement percentage with environmentally sustainable binder.

MATERIALS AND EXPERIMENTAL PROCEDURE

Cement

Two types of cement were used as this stage of the research, both of them being produced according to standard specifications. The first type was a CEM I 42.5R cement with high early strength. It is a general purpose cement being suitable for all uses in works requiring high strength values at early ages. The other type of cement was a CEM II B-M (S-LL) 32.5R cement also with high early strength. It is a composite cement with 65-75% Portland cement and 25-35% ground granulated blast furnace slag and lime. This second type of cement was considered in view of the fact that almost two thirds of the European cement market corresponds to CEM II cement. Both cement types are readily available on the market and widely used, this being the main reason of their selection for this research.

Environmentally Sustainable Mineral Binder

The binder is obtained from industrial by-products, most of them being disposed in landfills, such as phosphogypsum, lactogypsum, a.s.o. and can be entirely recycled after its expiration date (Kandco, 2010). Taking into account that gypsum results from the production process of many organic and inorganic fertilizers, pigments, metals etc. it has become a significant ecological problem (Singh and Garg, 2000). It is the belief of the authors that the use of a new mineral binder based on the anhydrous calcium sulphate in the β-anhydrite III' form as SCM has both environmental and economical advantages and justification (O'Rourke *et al.*, 2009). The opportunity for using such industrial unrecyclable wastes in construction industry, especially the phosphogypsum, has recently been recognized by researchers as having net benefits for the environment (Taher, 2007; Garg *et al.*, 2011).

Experimental Procedure

Table 1 presents the mix proportions considered for the mortars in this research. The mineral binder was assumed to occupy 50% of the total volume and consisted either only of Portland cement or as a mixture between the Portland cement and the environmentally sustainable binder (ESB). The water to mineral binder ratio of 0.4 was kept constant throughout the experimental works. Only tap water was used without any super-plasticizers or setting retarders. The mortar paste was cast in 50 × 100 mm cylinders.

The cylinders were used to determine the static modulus of elasticity in compression according to ASTM C469 specifications at the age of 28 days. The axial strain was measured with the help of a 50 mm gage length extensometer, Figure 1, whereas the transverse strain was measured by means of a circumferential extensometer, Figure 1. Six cylinders were cast for each age and mix proportion mentioned in Table 1, resulting in a total number of 48 cylinders.

RESULTS AND DISCUSSIONS

Nowadays the emphasis is more and more on the utilization of cementitious materials to produce sustainable cements (Gartner and Macphee, 2011; Zhang *et al.*, 2012).

Table 1. Mix proportions and specimen designation

Specimen designation	Cement type		Binder Environmentally Sustainable Binder (ESB)	Sand	Water / Binder ratio
	CEM I	CEM II			
	[%]	[%]	[%]	[%]	-
C1ESB0	50		-		
C1ESB30	20		30		
C1ESB35	15	-	35		
C1ESB40	10		40		
C2ESB0		50	-	50	0.4
C2ESB30		20	30		
C2ESB35	-	15	35		
C2ESB40		10	40		

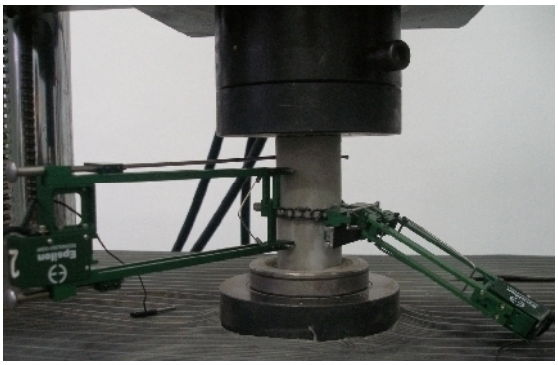

Figure 1. Loading set-up for the determination of elastic properties of mortar mixes

Their efficient utilization (Felekoglu *et al.*, 2009) has captured the interest of the scientific community due to their net benefits for the environment. However, there is still little information on their influence upon the elastic properties of the mineral matrix.

The modulus of elasticity, together with strength characteristics, is a key material property in design practice. Its assessment, especially for cement based materials, is quite difficult to be predicted because it is influenced by the properties and quantities of the constituent parts in the mix proportion.

The experimentally obtained values are presented in Table 2 for all mix proportions. The difference between the values of the modulus of elasticity of the control specimen and the other mortars, at the age of 28 days, is a mere 4% for high volumes of environmentally sustainable binder.

The mix proportions made with CEM II cement seemed to be less sensitive to the variation of cement percentage in terms of the values for the modulus of elasticity. When the volume of calcium sulphate mineral binder increased beyond 30% the obtained moduli of elasticity were 0.95% ~ 2% lower than the reference mix. The difference in behaviour compared to the mixes made with CEM I could be attributed also to the presence of other mineral admixtures in the CEM II cement. Even though further research should be conducted in this direction, the obtained results are encouraging and lead to the conclusion that the new ESB could be successfully used as replacement for the Portland cement.

Since there are no specially derived equations for the prediction of the modulus of elasticity of mortars, a few of the currently available equations for concrete have been used in order to check whether they are suitable to use in this particular case. The values are also presented in Table 2 for the equations proposed in ACI 363R-92 report and BS 8110-85 Part 2 and ACI 318M-05 codes. Previous research works have shown good agreement between the equation proposed in ACI 363R-92 and the experimentally obtained results for sprayed concrete on similar sized specimens with the present work (Goodier *et al.*, 2008). The other two equations, although derived for concrete, lead also to good approximations of the experimental results.

However, equations used in Table 2 should not be applied for any given case as they were not derived for mortars but for concrete.

Table 2. Elastic properties of mortars (average values determined on 6 cylinders)

Specimen	Experimental values (E) [GPa]	ACI363R-92 Eq. [1)] (E) [GPa]	BS8110-Part2 Eq. [2)] (E) [GPa]	ACI318M-05 Eq. [3)] (E) [GPa]	Poisson's Coefficient
C1ESB0	32.55	36.16	35.53	41.42	0.199
C1ESB30	31.24	28.20	28.23	30.15	0.202
C1ESB35	31.12	28.56	28.52	30.67	0.201
C1ESB40	31.27	28.49	28.45	30.56	0.201
C2ESB0	28.28	33.91	33.24	38.24	0.198
C2ESB30	27.69	27.10	27.40	28.59	0.2
C2ESB35	28.01	27.17	27.45	28.69	0.2
C2ESB40	27.89	27.09	27.40	28.59	0.203

[1)] $E_c = 3.32\sqrt{f_c'} + 6.9$; f_c' is the compressive strength [MPa]; valid for $21MPa \le f_c' \le 83MPa$

[2] $E_{c,t} = E_{c,28}\left(0.4 + 0.6\frac{f_{cu,t}}{f_{cu,28}}\right)$; for t ≥ 3 days and $E_{c,28} = K_0 + 0.2f_{cu,28}$ where K_0 is a constant related to the modulus of elasticity of the aggregate, taken as 20 [GPa] for normal-weight concrete; $f_{cu,28}$ is the characteristic cube strength at 28 days [MPa]; $f_{cu,t}$ is the characteristic cube strength at any given age, provided the sample is at least 3 days old.

[3] $E_c = 4.7\sqrt{f_c'}$ where f_c' is the compressive strength in MPa

They can be used to obtain a quick estimation of the results but should not substitute the latter. Further research work should be conducted in this field in order to propose a similar equation that can be used to estimate the modulus of elasticity of mortars.

The obtained values for the Poisson's ratio are also presented in Table 2. It can be observed that there are no significant variations in the values of the ratio among the mix proportions. The obtained values of the Poisson's ratio are in line with the predicted values for high-strength concrete. Herve *et al.* (2010) obtained similar values for Portland cement based mortar made with different types of round siliceous sand having fine, medium and coarse grain sizes.

CONCLUSIONS

The paper presents the findings of a research work focused on the evaluation of the elastic properties of mortars made with high percentages of a new environmentally sustainable cementitious material. The construction industry has to look for alternatives to Portland cement if the concept of sustainability was to be fully implemented.

Substituting the Portland cement by the new calcium sulphate based mineral binder results in a drop in the values of the modulus of elasticity by an average value of 4% at the age of 28 days for specimens made with CEM I cement. On the other hand, the mixtures made with CEM II cement showed less scattering of the results for the modulus of elasticity. However, it can be observed that increasing the volume of ESB in the mix the values become closer to that of the reference mix. The specimens made with CEM II cement seemed to be less sensitive to the variation of cement percentage in terms of the values for the modulus of elasticity.

Some of the existing equations for predicting the values of the elasticity modulus for concrete succeed in quite accurately estimating the modulus of elasticity of mortar. However, a clear trend is difficult to establish given the small number of specimens available at this stage.

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THE APPLICATION OF A PHOSPHORUS-NITROGEN FLAME RETARDANT RETANNING AGENT

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In order to improve the fire resistant of leather to meet the condition of higher fire-safety requirement, a phosphorus-nitrogen flame retardant retanning agent (M-THPS-U for short) was synthesized. The vertical flame test, oxygen index test, smoke density, mechanical properties, thicken rate, and shrinkage temperature were used to evaluate the property of M-THPS-U, and the dosage of M-THPS-U was optimized at the same time. Furthermore, the hide powder acting with M-THPS-U was used to study the flame resistant effect in detail by thermogravimetry (TG). The results showed that the fire resistance of leather was improved obviously by using 5% of M-THPS-U. With the increase of M-THPS-U dosage, the mechanical properties were dropped, but the fire resistance, thicken rate, and shrinkage temperature were raised. The thermogravimetry results indicated that the fire retardant could promote the fire resistance of leather by accelerating leather fiber into char. In short, not only does M-THPS-U improve the fire resistance of leather, but it also has retanning and filling effects.

Keywords: leather, phosphorus-nitrogen flame retardant, retanning

INTRODUCTION

Leathers have a better fire resistance than cloth and plastic materials in normal situation. The oxygen index of the leather untreated by flame retardant is between 21% and 27%, which is a self-extinguishing material (Ou, 2002). There is no melt dropped during the leather burning process, but the flameless combustion time is long and a lot of smoke together with nasty smell is emerged. The demands for fire-safety are increasing, so the leather must be treated by fire retardant to improve flame resistance (Huang, 2005).

A lot of flame retardant had been synthesized and applied to improve fire resistance of leather (Ling *et al.*, 2012), especially phosphorus-nitrogen flame retardant (Wang *et al.*, 2006), because it had the synergistic effect on fire resistance with two fire retardant elements, which was a hot area of research in recent years (Huang *et al.*, 2004). In this research, a phosphorus-nitrogen flame retardant retanning agent (M-THPS-U for short) was synthesized by using tetrakis hydroxymethyl phosphonium sulfate (THPS), urea, formaldehyde and sodium bisulfite. Because there were two fire retardant elements in M-THPS-U, the flame resistance was excellent. On the other hand, a lot of active groups such as hydroxymethyl were existed, which made it had good performances of retanning and filling.

EXPERIMENTAL

Materials

Shaved goat wet blue with average thickness of 1.0mm was made in the lab as a

common process. Other chemicals used for leather processing were commercial grade, and reagents used for analysis and synthesis were research grade.

Preparation of M-THPS-U

72g urea and 81.3g THPS were put into a round-bottom flask, then some distilled water was added into the system and the flask was stirred at 80°C for 2 hours. After cooling down to the room temperature, a 0.5mol/L NaOH solution was used to adjust the pH to about 8.5. 59.1g formaldehyde and some water were added, then the system was stirred at 65°C and reflux condensation was processing at the same time. Another 31.2g sodium bisulphite was added again for 50min to get the M-THPS-U. The M-THPS-U was colorless oily liquor and the solid content was about 45%.

Preparation of Fire Resistant Leather

The shaved goat wet blue was treated by M-THPS-U as shown in Table 1.

Table 1. The process of flame retardant leather

Process	Materials	T/°C	Dosage %	t/min	Remark
Washing	Water	35	200		
	Penetrant agent		0.3	10	
	Degreasing agent		0.2	20	Drain
Washing	Water	25	200	10	Twice, Drain
Neutralizing	Water	35	200		
	HCOONa		0.5	20	
	NaHCO3		0.9	60	pH: 6.0, Drain
Washing	Water	25	200	15	Drain
Fire retardant*	Water	35	150		
	M-THPS-U		X	60	
	HCOOH		1.2		pH: 4.0～4.2, Drain
Washing	Water	25	200	10	Twice, Drain
Drying					

*The leather was not treated by M-THPS-U used as a control.

Preparation of Fire Resistant Hides Powder

The hide powder (slightly chromed, chrome content was about 0.1%) and water (the mass was about 500% of hide powder) were mixed in a flask at room temperature for 4 hours to allow hide powder swelling. 10% M-THPS-U (based on the solid content) was acted with hide powder in thermostatic water bath oscillators at 30°C for 3 hours. After washing by distilled water some times and filtration, the hide powder treated by M-THPS-U was dried in a vacuum drying oven at 40°C for 5 hours. The hide powder was not treated by M-THPS-U was used as control.

Testing Methods

FT-IR Test of M-THPS-U

The M-THPS-U was purified by absolute alcohol, and after drying in a vacuum drying oven, the white solid was obtained. The samples were ground with KBr and made into pellets, then a Nicolet10 FT-IR (American Thermo Scientific Corporation)

was used to scan in the wavelength range of 400-4000cm^{-1} for 32 times, and the data was recorded.

TG/DTG of Hide Powder

The treated and control hide powder were dried at 40°C for 24 hours. A NETZSCH TG 209 F1 thermogravimetric analyzer (Germany) was used for analysis. The samples were put into Al_2O_3 crucibles and heated at 10°C/min in a N_2 atmosphere (flow N_2:100mL/min); the range of temperature was from 40 to 800°C. After the test, the TG curve was derivative, and the DTG curve was obtained.

Sampling for Flammability Test

A set of samples, including 5 pieces for the vertical flame test, 5 pieces for oxygen index test and 3 pieces for smoke density test were taken from each treated and control leather pieces. The samples for the vertical flame test were 51 mm by 317.5 mm, and their long axis was perpendicular to the backbone. Oxygen index test samples were 52 mm by 140 mm and samples for smoke density test were 50 mm by 52 mm. The prepared samples were stored in a chamber at 20±1°C and 65±2% relative humidity for 48 h and then the thickness of each sample was measured.

Flammability Test

The vertical flame, the oxygen index and the smoke density of all the samples were tested according to ALCA Method E50, ASTM D 2863-77 and GB/T 8627-1999 respectively.

Mechanical Properties and Shrinkage Temperature (Ts) Test

The leathers were sampled and conditioned as the standard method. The tensile strength and tear strength of leather were tested by tensile machine (AI-7000S, China) following the standard method. The shrinkage temperature was tested by Shrinkage Temperature Tester (MSW-YD4, China) with the bath of glycerin (75%). Each value was an average of two which were along and across the backbone.

RESULTS AND DISCUSSIONS

FT-IR of M-THPS-U

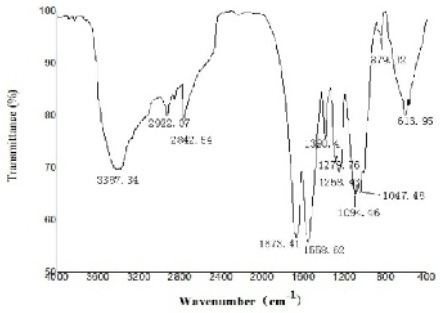

Figure 1. The FT-IR of M-THPS-U

Figure 1 showed the main absorption of M-THPS-U. The peak at 1047cm^{-1} was the -CH_2OH, which indicated that the hydroxymethyl was appeared in M-THPS-U. The peak at 3387cm^{-1} was due to the C-N stretching vibration of M-THPS-U, showing the urea acting with formaldehyde. The peak at 1258cm^{-1} was the characteristic absorption peak of –OH. The peak at 879cm^{-1} was due to the S=O stretching vibration of M-THPS-U, indicating sulphonation reaction happening. All these results indicated that M-THPS-U had been synthesized as expected.

Thermogravimetry of Hide Powder

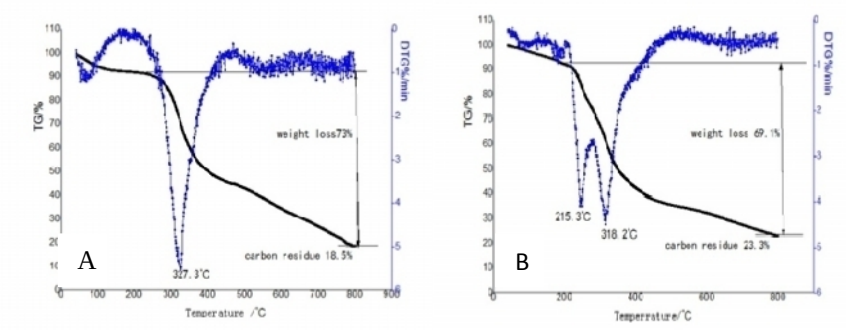

Figure 2. The TG curves of the hide power (A was treated sample, B was control)

As shown in figure 2, control hide powder was break down rapidly at 327.3°C, the weight loss was 73% during the process, and the carbon residue was only 18.5% at 800°C. The flame retardant hide powder had two rapid weight loss stages; there were 215.3°C and 318.2°C, and the carbon residue was 23.3% at 800°C. These results indicated that M-THPS-U could increase the carbonization of collagen fiber, and decrease the weight loss. According to Van Krevelen' theory, there was a liner relation between flame resistant effect and carbonization, the higher carbonization, the better flame resistant effect. The carbon residues were observed by optical microscope also proved these proofs. Furthermore, the DTG curve showed that M-THPS-U could slow down the decomposition velocity of leather and enhance the thermal stability of fire resistant leather.

The Flammability of Fire-Resistant Leather

Table 2. The vertical combustion of leather

Dosage of M-THPS-U	Flame combustion (s)	Flameless combustion (s)	Char length (cm)	Weight loss (%)	Oxygen index (%)	Smoke density (%)
0%	2.68	16.48	0.89	4.19	27.5	54
3%	1.06	0	0.64	3.32	30.6	48
5%	0	0	0.56	3.08	32.1	45
7%	0	0	0.52	2.91	32.9	44

As shown in Table 2, with the increasing of M-THPS-U, the flame combustion, flameless combustion, char length and weight loss were all decreasing, and the oxygen

index of leathers treated by M-THPS-U were increasing. When the dosage of M-THPS-U was 5% (based on the weight of wet blue), the flame combustion, flameless combustion were zero second, the char length and weight loss were 0.56cm and 3.08%, which were decreasing by 37% and 26% compared with control respectively.

Compared with control, the oxygen index was increasing by 11%~20% respectively. The smoke density was decreasing with the using of M-THPS-U, especially the dosage of M-THPS-U was 7%. During the combustion process, the collagen fiber was dehydrated by the M-THPS-U to form a loose carbon to isolate heat and oxygen, which could cut down the smoke and raise the oxygen index. The dosage of M-THPS-U was higher, the flame resistant effect was better, indicating that M-THPS-U could be used for making high quality fire resistant leather.

The Mechanical Properties, Ts and Thicken Rate of Leather

Table 3. The mechanical properties, Ts, and thicken rate of leather

Sample	Tensile strenth (Mpa)	Tear strenth (N/mm)	Elongation (%)	Ts (°C)	Thicken rate (%)
Control	25.4	73.5	32.7	109.1	0
M-THPS-U 3%	23.1	63.7	33.4	112.8	6.3
M-THPS-U 5%	21.8	59.5	31.2	114.3	8.1
M-THPS-U 7%	19.7	57.5	30.6	113.7	8.9

As shown in Table 3, with the dosage of M-THPS-U increasing, the mechanical properties were decreasing, but the Ts and thicken rate were rising. M-THPS-U was consisted of many active groups which could act with collagen. Stress concentration was created by these cross-links between collagen fibers, which could lower the mechanical properties. The thicken rate reflected filling effect and the increasing of Ts expressed the tanning effect. The leather treated with M-THPS-U was thicker and had higher Ts, showing that M-THPS-U had filling and tanning effect.

CONCLUSIONS

After treated with M-THPS-U, the smoke density, flame combustion, flameless combustion, char length and weight loss of leather were decreasing and oxygen index was rising, indicating M-THPS-U had excellent flame retardance; the shrinkage temperature and thicken rate of leather were increasing, showing M-THPS-U had filling and tanning effect. In sum, M-THPS-U could use for making high performance fire resistant leather.

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II.
BIOMATERIALS

SYNTHESIS AND CHARACTERISATION OF MICROCAPSULES BASED ON NATURAL BIOPOLYMERS AND LAUREL ESSENTIAL OIL

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The aim of this paper was to obtain and characterize some microcapsules based on natural polymers, collagen hydrolysate and sodium alginate and laurel essential oil. The composition of Laurel essential was determined by GC-MS. By varying different synthesis parameters, water-oil emulsion method was choosing for obtaining the liquid microcapsules. The microcapsules were dried by lyophilisation. Collagen hydrolysate and microcapsules based on polymers were characterized by FT-IR spectroscopy, optical and scanning electron microscopy and particle size.

Keywords: natural polymers, essential oil microcapsules, collagen hydrolysate, sodium alginate.

INTRODUCTION

Microcapsules obtained from natural polymer especially gelatin/collagen, sodium alginate that contain active principles like essential oils with therapeutic actions, are widely studied by researchers worldwide (Devi *et al.*, 2012; Kaya *et al.*, 2012; Kim *et al.*, 2006; Martins *et al.*, 2014; Ocak, 2012) being the top issues at this time. In this paper the water-oil type solution was the method to obtain the microcapsules and characterization methods were optical and electron microscopy, FT-IT(ATR), GC-MS, size dimension.

MATERIALS AND METHODS

Materials

Collagen hydrolysate was obtained from calf pelt by alkaline hydrolysis of at 125°C, 2 atm during 8 hours by currently used technology in Collagen Department (Sirbu *et al.*, 2009).

Sodium alginate (100,12 molecular mass) and glutaraldehyde was purchased from Sigma - Aldrich (Germany). Acetic acid was provided by Chimactive (Romania).

Laurus nobilis L essential oil was provided by the Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops (Kaya *et al.*, 2012). *Laurus nobilis L* essential oil was obtained from dried leaves that were collected from Amanos Mountain (Anatolia region) during flowering season, which was dried at room temperature. The essential oil was obtained after 100-150 g of dried leaf were subjected to steam distillation for 3-4 hours. The resulting essential oil was dried with anhydrous sodium sulfate and stored at -20°C.

Synthesis of Microcapsules

In order to obtain the microcapsules, the compositional recipes from Table 1 were used.

Table 1. Compositional recipes for syntesized microcapsules

No.	Code	Collagen hydrolyzate	Sodium alginate	Glutaraldehyde	Acetic acid	Essential Oil, ml
		g/100 ml water				
1	MC-CH 1	3	-	0.4	2.5	1
2*	MC-CH 2	3	-	0.4	2.5	1
3	MC-SALG	-	1	0.4	2.5	1
4	MC-CH-SALG	3	1	0.4	2.5	1
5	MC-SALG-CH	1	3	0.4	2.5	1

MC – MicroCapsule; CH – Collagen Hydrolysate; SALG – Sodium Alginate
* 1 hrs stirring

Initially 3% CH was solved in water and heated between 50-60°C. The solution was subjected to a fast stirring. At the desired temperature the essential oil was added, in drops and a water solution (3%) of SALG, also in drops. The pH of solution was corrected with acetic acid until pH 3.75 was achieved. After the pH correction, the solution was cooled down between 5-10°C in order to hardness the microcapsules. The obtained microcapsules were cross-linked using 0.4% glutaraldehyde. After crosslinking, the temperature was increased at 50°C and continuing stirring another 3 hrs (Kaya *et al.*, 2012).

Methods

The essential oil components were analyzed by GC/MS – Qualification and quantification was carried out by using a Finnigan-Trace GC–MS equipped with an auto sampler. One micro liter of sample volume was injected using split method with 50 split ratio. Chromatographic separations were accomplished with a Zebron ZB-5 capillary column (5% phenyl–95% dimethylpolysiloxane, 0.25mm i.d. × 60 m, film thickness 0.25 m) with injections in the split mode with 50 split ratio. Analysis was carried out using helium as the carrier gas, flow rate 1.0 mL/min. The column temperatures were programmed from 50 to 240°C at 3°C/min. The sample sizes were 2 µL. The injection port temperatures were 250°C. The ionization voltages applied were 70 eV, mass range m/z 41–400 a.m.u. The separated components identified by matching with GC–MS results of National Institute of Standards and Technology (NIST) mass spectral library data. The quantitative determination was carried out based on peak area integration.

FT-IR spectrometry analysis was performed with Jasco 4200 FT-IR spectrophotometer, equipped with an ATR - diamond sensor.

Optical microscopy analysis was performed with a Leica S8AP0 stereomicroscope for 20-160X magnification and Leica CME microscope for 40-1.000X magnification.

Electron microscopy analysis was performed using Quanta Fei 200 scanning electron microscope that has the magnification range between 1.000 X - 1000.000 X.

Particle size was determined using Malvern ZetaSizerNano equipment that has the possibility to determine dimension between 0.6 nm - 6 µm.

RESULTS AND DISCUSSION

Optical and Scanning Electron Microscopy of Collagen Hydrolysate and Sodium Alginate

Optical microscopy images show that the collagen hydrolysates have spherical form, white color and sizes of the grain vary between 8 and 25 µm (Figure 1a) and sodium alginate has granular form, translucent, white color and sizes of the grain vary between 20 µm and 400 µm (Figure 1c). Scanning electron microscopy images reveal that the collagen hydrolysate grains have spherical shapes, which confirm the observation made by optical microscopy analysis (Figure 1b) and sodium alginate grains have an irregular morphology with smaller grains on top (Figure 1d).

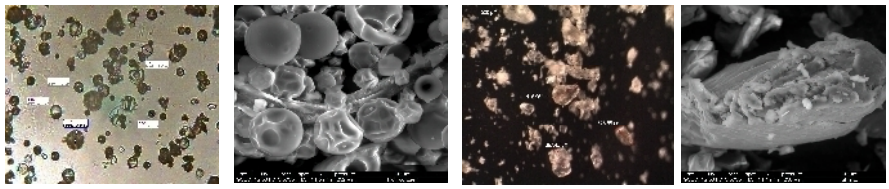


Figure 1. a) Optical microscopy image of CH (400X); b) SEM image of CH (5000X); c) Optical microscopy image of SALG (80X); d) SEM image of SALG (5000X)

Composition of Laurel Essential Oil

Table 2 presents the components of essential oil determined by GC-MS chromatography. The essential oil has 23 components and the majors ones are eucalyptol – 54.00%, alpha-terpinenyl acetate – 10.93% and sabinene – 7.96%.

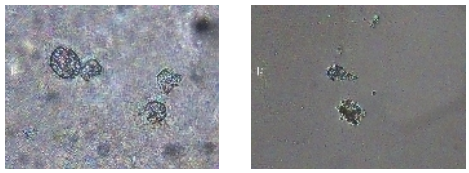
Table 2. Essential oil (*Laurus nobilis L*) components

No.	Component	Retention time	Aria (%)
1	alpha-pinene	11.33	4.68
2	Sabinene	12.86	7.96
3	2-beta-pinene	13.18	3.44
4	p-cymene	15.12	1.34
5	dl-limonene	15.28	1.22
6	1,8-Cineol (Eucalyptol)	15.52	54.10
7	gama-terpinene	16.54	0.77
8	Linalool	18.31	0.77
9	4-Terpineol	22.47	2.55
10	Beta-Fenchyl alcohol	23.16	1.52
11	Ocimenyl acetate	28.31	0.63
12	alpha-terpinenyl acetate	29.80	10.93
13	Eugenol	30.38	0.08
14	Beta-elemene	31.66	1.21
15	Methyleugenol	32.26	0.49
16	trans-caryophyllene	33.20	0.81
17	alpha-caryophyllene	34.76	0.14
18	Germacrene-D	35.80	0.22
19	Beta-Selinene	36.22	0.11
20	Beta-Bisabolene	36.42	0.26
21	Cis-alpha-Bisabolene	37.88	0.22
22	Caryophyllene oxide	40.13	0.21
23	Beta-Eudesmol	42.99	0.11

Characterisation of Microcapsules

The microcapsules obtained according with methods previously described in presented in Table 1 were characterized by optical and scanning electron microscopy, particle size and FT-IR spectroscopy.

The figure 2 shows the difference between MC-CH 1 and MC-CH 2.

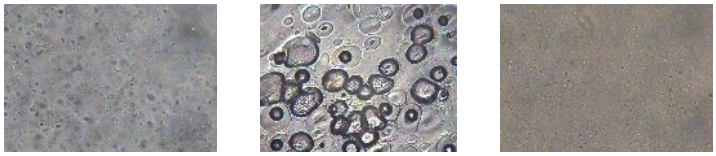


a) b)

Figure 2. Optical microscopy images of microcapsules: a) MC-CH 1 and b) MC-CH 2

Optical microscopy for MC-CH 1 – Figure 2a shows the presence of some aggregates that have different shapes and sizes. The aspect of the microcapsules indicates that they start to form a microcapsule incipient structure but are not complete. Because MC-CH 2 was obtaining in a similar condition like MC-CH 1, optical microscopy shows the presence of some aggregate in a more incipient form than MC-CH 1 - Figure 2b.

Figure 3a shows the optical microscopy image for MC-SALG but the sizes of the microcapsules are too small in order to be observed in good condition.



a) b) c)

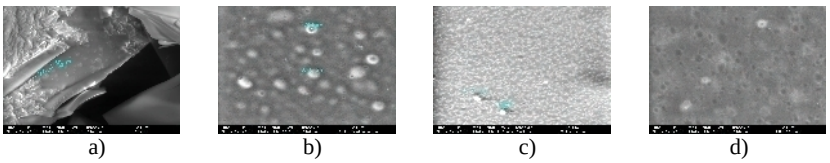
Figure 3. Optical microscopy images of microcapsules: a) MC-SALG; b) MC-CH-SALG; c) MC-SALG-CH

Optical microscopy image for MC-HC-SALG (Figure 3b) indicates the presence of microcapsules; the outer shell and also the internal component – essential oil – can be noticed more clearly.

The MC-SALG-CH microcapsules image (Figure 4c) indicates that they are too small to be seen in optical microscopy.

In order to observe them in more detail, the microcapsules solution was dried by lyophilization to transform them from liquid into solid state.

Even if the SEM of microcapsules can provide more information about their morphologies, the exact forms of the microcapsules from Figure 4 cannot be identified.



a) b) c) d)

Figure 4. SEM for microcapsules: a) MC-HC 1; b) MC-ALGS; c) MC-HC-ALGS and d) MC-ALGS-HC

In Figures 4 b, d some ovoidal shapes can be observed, but the images are not eloquent. Figures 4 a, c show some formations of microcapsules, very different even optical microscopy clearly indicate microcapsules form.

Size of Microcapsules

The microcapsules sizes are presented in Table 1.

Table 3. Size of microcapsules

No	Name	Dimension (nm)	Intensity (%)
1	MC-CH 1	6025 385.6	52.7 47.3
2	MC-CH 2	2673	100
3	MC-SALG	229 10.28	92.2 7.8
4	MC-HC-SALG	1020	100
5	MC-SALG-HC	1164	100

The size analysis of microcapsules indicates that the size distribution of microcapsules is in a large interval, from several to hundred micrometers.

FT-IR (ATR) Spectroscopy

Even if the MC-CH 1 and MC-CH 2 solutions were made by varying the stirring time, the difference between them is not so significant.

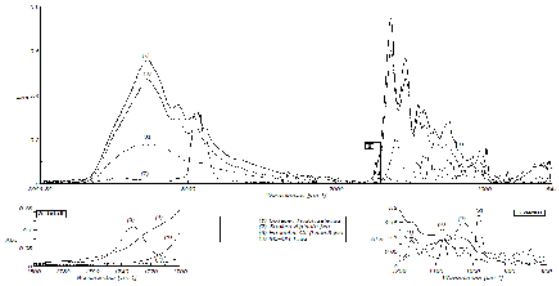


Figure 5. FT-IR (ATR) overlays spectra for collagen hydrolysate, sodium alginate, essential oil, MC-CH 1

The FT-IR (ATR) overlay spectra for collagen hydrolysate, sodium alginate, essential oil, MC-CH 1 (Figure 5) show that the existence of peak at 1732 cm^{-1} in the essential oil determine structural changes in the microcapsules structure – figure 5 (left). In figure 5 (right) is also possible to see some interaction between collagen hydrolysate and sodium alginate.

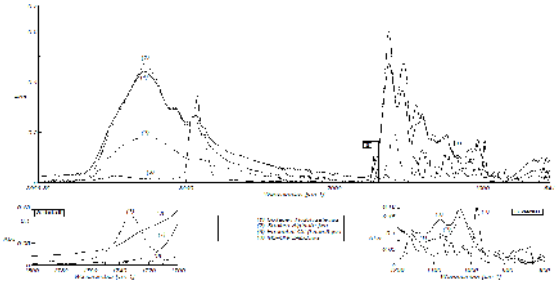


Figure 6. FT-IR (ATR) overlays spectra for collagen hydrolysate, sodium alginate, essential oil, MC-CH-SALG

FT-IR (ATR) spectrum for the MC-HC-SALG indicates major similarities with MC-CH 1 and MC-CH 2 spectra.

A more detailed analysis highlights the fact that the addition of sodium alginate in synthesis solution leads to changes of 1031 cm^{-1} peak intensity (0.0629) of collagen hydrolysate to MC-HC-SALG peak intensity (0.1686) – figure 6 right detail.

The presence of peak at 1732 cm^{-1}, in essential oil FT-IR (ATR) spectra leads to a structural change in the chemical composition of the microcapsules and can be observed in Figure 6 - left side detail.

CONCLUSION

The microcapsules obtained from collagen hydrolyzate, sodium alginate and essential oil (*Laurus nobilis L*) were obtained and characterized by FT-IR spectroscopy, optical and SEM microscopy and size particles. The results showed that the obtained microcapsules had micrometer sizes and the essential oil influenced the microcapsule structures.

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BIOCOMPATIBILITY - REVOLVING ISSUE FOR BIOMATERIALS IN CONTAMINATED FIELDS: NOVEL THERAPEUTIC SOLUTIONS FOR COMPLICATED INCISIONAL HERNIAS

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Incisional hernias occur in nearly 20% of all abdominal procedures. Emergency repair is challenging and has limited the use of prosthetics in the past, especially if the operating field is contaminated to a certain degree. On the other hand, primary repair of abdominal wall defects has a high recurrence rate, ranging between 10 and 50% because of intrinsic parietal tension and myocutaneous flap necrosis. Growing interest for minimally invasive surgery and reduced hospital stay when repairing abdominal wall defects has led to research and development in the field of prosthetics that can serve those aims. Biomaterials, such as collagen impregnated meshes, seem to offer new possibilities for prosthetic repair of complicated incisional hernias, emergency incisional hernia surgery and mesh placement in contaminated fields. The article presents a retrospective study on biocompatibility and late tissue reactions, determined for complicated incisional hernias. The study relies on a 5 years' experience (2009-2013), analyzing 195 emergency prosthetic repairs for complicated incisional hernias versus 195 repairs for uncomplicated incisional hernias. The assessment of postoperative complications for the study parameters showed no significant differences between the two groups. The results promoted the development of a protocol for parietal prosthetic repair using biomaterials (collagen impregnated meshes) in complicated incisional hernias (i.e. Altemeier class III surgical wounds).

Keywords: biomaterials, biocompatibility, complicated incisional hernias

INTRODUCTION

Incisional hernias occur, according to different authors (Alaedeen *et al.*, 2007), in up to 20% of all abdominal procedures. Emergency repair of incarcerated incisional hernia with or without bowel obstruction, in potentially contaminated fields is challenging due to edematous, inflamed and friable tissues with occasional need for concurrent procedures (small bowel, colonic resections, stoma revisions or take-down) and therefore with higher rates of postoperative complications, infectious or otherwise (Davies *et al.*, 2007).

Primary repair of incisional hernias has high recurrence rates ranging between 10% and 50% primarily because of the tension created and myocutaneous flap necrosis (Burger *et al.*, 2004). Many techniques have been proposed over time to reduce tension, such as relaxing incisions and compartment release. Results are far from being optimal. In addition, large, full thickness abdominal wall defects secondary to wide resection of cancer, traumatic injuries or congenital abnormalities, cannot be closed primarily. The use of prosthetic meshes has become necessary. Along with the traditional open techniques of mesh implantation, the recent laparoscopic techniques have gained popularity because of the decrease in wound infection, recurrence rates and recovery time.

There is constantly growing interest for minimally invasive surgery and reduced length of hospital stay. This has stimulated the medical industry in the development of new materials that support these aims. Biological materials are an important component

of surgical treatment of incisional hernias. The ideal biological material must allow a perfect biological interaction with the environment in which it is implanted, and must therefore possess high biological compatibility and biodegradability. The biological materials currently on the market exhibit total resorption and are biologically compatible, carrying out four important physiological functions: adhesion, hemostasis, sealing and repair. The repair of complex contaminated abdominal wall defects is even more challenging. The fear of fibrosis, erosions, infection and fistulas with the prosthetic meshes commonly used has led engineers and doctors to investigate biological meshes. Biological grafts seem to offer a solution. Their aim is to provide a collagen and other extracellular matrix scaffold, in which the host fibroblasts can create angiogenesis and deposit new collagen. The non-synthetic nature of these products allows them to be more resistant to infections. Several biological grafts are available on the market. Their classification is based on the species of origin (allogenic, xenogenic), type of collagen matrix utilized (dermis, pericardium, intestinal submucosa), decellularization process, presence or absence of cross-linking, storage requirements (need for refrigeration, need for rehydration) (Cavallaro *et al.*, 2010). Porcine dermal collagen is now indicated in the following situations: complicated incisional hernias with septic or contaminated surgical fields, contact of the mesh with the bowel, stomal hernias, cases with associated bowel resections and anastomosis, patients with infected previously placed synthetic meshes (Armellino *et al.*, 2006).

According to the literature, the use of meshes reduces the recurrence rate but is also associated with serious complications in 10%-15% of cases. Infection, fistula, skin erosion often lead to mesh removal (Buinewicz and Rosen, 2004). Using meshes in contaminated wounds leads to removal in 50% to 90% of cases (Szczerba and Dumanian, 2003). Ideal meshes should posses proper strength, should be compatible with host tissues and have an ability to avoid infections. Many synthetic and biological mesh tissues have been proposed over time but no single material, nor newer biosynthetic mesh, has fulfilled these requirements and gained universal acceptance (Cavallaro *et al.*, 2010).

Incisional hernia repair in the setting of surgical field contamination is a delicate subject. Advocated mesh use for incisional hernia repair in order to lower recurrence rates changes in cases with contamination. The principles of repair are removal of the source of contamination and reconstruction of the abdominal wall.

Associated colonic procedures (contaminated and infected, class III-IV Altemeier classification) at the time of repair strongly discourage the use of meshes (Machairas *et al.*, 2008). The use of meshes has been discouraged by authors if open bowel is encountered during repair (Morris-Stiff and Hughes, 1998). The risk of using a foreign body (mesh) for incisional hernia repair was highlighted by Korenkov *et al.* (2002) who found high rates of chronic postoperative pain and wound infection. Some authors feel that hernia repair should be postponed and done separately if intestinal resection is required (Temudom *et al.*, 1996).

The paper presents descriptive retrospective study based on authors' recent experience with incisional hernias.

MATERIALS AND METHOD

The paper compares incisional hernia treatment and outcome in 390 patients, 195 presenting for elective surgery and 195 presenting for emergency procedures with complicated incisional hernias. The patients were all operated on by a team of surgeons

using standardized techniques (on-lay, in-lay, under-lay) and a prosthetic mesh (polypropylene, collagen impregnated composite dual-mesh) was used in all cases. Data was recorded over a period of 5 years (2009-2013) from patient charts, operative notes and follow-up records. Follow up was attempted in all cases for a period of one year.

The three types of meshes used for incisional hernia repair are shown in Table 1: synthetic polymers, composites and biological prosthesis. The prosthesis can be placed in a pre-fascial site (subcutaneous), intra-parietally (pre-peritoneal) or in an intra-peritoneal site. The choice of prosthesis thus depends on the site where it will be implanted - a reticular mesh (polypropylene or polyester) in a pre-fascial and intra-parietal sites (Chevrel or Rives procedure), a laminar (ePTFE) or a composite prosthesis intra-peritoneally since they avoid adhesion formation with the intra-abdominal viscera.

Table 1. Types of prosthetic materials for incisional hernias

Synthetic	
Non-absorbable polymers	Polypropylene
	Polyester
	Expanded polytetrafluoroethylene (ePTFE)
Absorbable synthetic polymers	
Composites	
Biologic prosthetics	Human
	Bovine, Swine

EXPERIMENTAL AND RESULTS

During the five years of the study (2009-2013), 390 patients operated for incisional hernias were evaluated preoperatively and postoperatively. The studied population consisted of 257 (65.9%) female subjects and 133 (34.1%) male subjects showing an approximately 2:1 female to male ratio. Of the 390 patients 195 presented for elective incisional hernia surgery and 195 were operated for complicated incisional hernias in an emergency setting. Mean age was comparable for both groups, 56 years old overall.

Various sites for incisional hernias were recorded, as follows: for the elective surgery group (uncomplicated incisional hernias) 112 (57.4%) cases were in the lower abdomen on the midline, 72 (37%) cases were in the upper abdomen on the midline and 11 (5.6%) cases were flank hernias; for the emergency surgery group (complicated incisional hernias) 76 (39%) cases were in the lower abdomen on the midline, 69 (35.4%) cases were in the upper abdomen on the midline, 43 (22%) cases presented with flank hernias and 7 (3.6%) cases presented with para-stomal hernias. All para-stomal hernias were considered complicated due to the inherent septic nature of the procedure regarding mesh placement. A slight overall predominance of lower midline incisional hernias was observed. Mean diameter of the abdominal wall defect observed was 7.9 cm for the uncomplicated group and 6.3 cm for the complicated group.

The 195 patients in the uncomplicated group all had clean wounds and required no additional septic procedures during surgery. The patients in the complicated group were selected so that their Altemeier wound class was no greater than class II. They presented with incarcerated hernias and problems concerning bowel integrity. Only patients without bowel necrosis that required no procedure or patients with small bowel necrosis but no spillage were selected for the study group. In the case of patients with bowel necrosis enterectomy (either mechanical or hand-sewn) was performed. No patients with colonic necrosis and associated colon resections were included. Of the 195 patients

of the complicated group, 127 presented without bowel necrosis and 68 patients presented with small bowel necrosis and required enterectomy with no spillage of bowel content. All patients in the complicated group had clean (Altemeier class I) or clean-contaminated (Altemeier class II) wounds.

ASA score for the uncomplicated group was distributed as follows: ASA I in 52 (26.4%) cases, ASA II in 78 (40%) cases and ASA III in 65 (33.3%) cases. For the complicated group of patients ASA IE was recorded in 19 (9.7%) cases, ASA IIE in 53 (27.3%) cases, ASA IIIE in 97 (49.7%) cases and ASA IVE in 26 (13.3%) cases. The procedures performed can be classified in onlay techniques (mesh placed above the aponeurosis), sublay techniques (mesh placed over the closed posterior rectus sheath) and inlay techniques (mesh placed intraperitoneally). All cases were operated in an open fashion. The meshes used for repair were monofilament polypropylene for the onlay and sublay techniques and a composite mesh impregnated with bovine collagen - polyester and absorbable hydrophilic film for the intraperitoneal technique. Mean operating time was 130 minutes for the onlay technique, 190 minutes for the sublay technique and 125 minutes for the intraperitoneal approach. Procedures were divided between the two studied groups as follows: for the uncomplicated group 113 (57.9%) onlay procedures, 46 (23.5%) sublay procedures and 36 (18.5%) intraperitoneal approaches; for the complicated group 82 (42%) onlay procedures, 51 (26.2%) sublay procedures and 62 (31.8%) intraperitoneal approaches. Of the 68 patients that required enterectomy, the abdominal wall was repaired in 53 cases with an intraperitoneal approach and in 15 cases with a sublay technique.

Mean hospital stay was 3 days for the patients in the uncomplicated group. The complicated group had a mean hospital stay of 4 days for patients that did not require enterectomy and of 6 days for patients that presented with bowel necrosis. Postoperative pain was handled adequately in both groups; bowel function return was day 2 on average for uncomplicated hernias and day 4 for complicated ones with no difference regarding bowel necrosis or not. Length of hospital stay was increased in the group with bowel necrosis and enterectomy probably due to surgeon preference.

Table 2 shows the types of complications and their occurrence. A slight increase in complication rates can be observed between the group that required enterectomy and the group that did not. Overall complication rates, however, remain comparable. Prosthesis infection was managed in all cases with mesh removal and an alternate repair without mesh was used. One year follow-up was possible in 373 patients and chronic pain and recurrence were investigated at this point. 13 patients died during the immediate postoperative period, 5 patients from the group with enterectomy, 3 from the complicated group that did not require enterectomy and 5 patients that presented with uncomplicated incisional hernias. 8 deaths were related to sepsis and 5 to pre-existing comorbidities.

Table 2. Complications following surgery

Complication	Uncomplicated incisional hernias n (%)	Complicated incisional hernias Enterectomy (-) n(%)	Enterectomy (+) n(%)
Seroma	65 (33.3%)	34 (17.4%)	41 (21%)
Hematoma	22 (11.2%)	12 (6.1%)	7 (3.6%)
Prosthesis infection	11 (5.64%)	2 (1%)	7 (3.5%)
Fistula	4 (2%)	5 (2.5%)	9 (4.6%)
Skin necrosis	2 (1%)	0	1 (0.5%)

Complication	Uncomplicated incisional hernias n (%)	Complicated incisional hernias Enterectomy (-) n(%)	Enterectomy (+) n(%)
Chronic po pain	36 (18.46%)	21 (10.7%)	17 (8.7%)
One year recurrence	27 (13.8%)	13 (6.6%)	20 (10.2%)

Various techniques for mesh placement are currently used without generalized consensus, the most common being onlay, sublay and intraperitoneal underlay. The sublay Rives-Stoppa technique has been advocated to have low infection rates but it also comes at the expense of longer operating times which could prove to be ever important in cases that require emergency surgery. Veillette *et al.* recorded mean operating times of 131 minutes for primary repair, 141 minutes for onlay procedures and 231 minutes for Rives-Stoppa (Veillette *et al.*, 2001). The present study found a clearly longer mean operating time for Rives-Stoppa recorded at 190 minutes compared to the other mesh procedures that required 130 and 125 minutes. Emergency surgery for patients with incarcerated incisional hernias and bowel obstruction sometimes places the surgeon in the setting of hemodynamically unstable patients and the time-consuming Rives-Stoppa procedure might prove costly. Our study showed a slight preference for intraperitoneal mesh placement in the setting of complicated hernias when compared with the uncomplicated group. Zafar *et al.* advocated the use of an onlay technique and an open wound treated with daily dressings until neoepithelization for contaminated wounds (Zafar *et al.*, 2012). Consensus is lacking in regards to the best technique to be used for the treatment of incisional hernias, especially in the context of wound contamination.

DISCUSSIONS AND CONCLUSIONS

The study shows that synthetic meshes are relatively safe to use in clean or clean contaminated incisional hernia repairs. It also shows that a preference to use collagen composite dual-mesh with higher rates of surgical field contamination is justified. Although complication rates are higher for clean-contaminated wounds, the overall rates do not differ from complicated to uncomplicated. A preference was noted for intraperitoneal repairs with composite meshes in the setting of clean contaminated wounds. Length of hospital stay was larger for patients with complicated hernias although not always supported by objective factors. The new additions of biological materials to the market have driven us to elaborate a protocol for mesh placement in contaminated surgical wounds (i.e. Altemeier class III). Still, mesh removal after infection and a high recurrence rate regardless of the procedure or mesh used, are problems that need to be resolved in the following period.

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RESEARCH ON OBTAINING NUTRITIONAL SUBSTRATES FROM PROTEIN BIOCOMPOSITES

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Organic biocomposites are a source of raw materials for agriculture, as their composition provides enough elements to improve physical-chemical properties of degraded soils and plant growth. Using biocomposites obtained from compounding collagen hydrolysates from pelt waste with various biodegradable polymers stimulates enzymatic substances in the plant, favours development of the root system and increases germination capacity of seeds, favouring rootlet development. The paper presents the development of nutritional substrates with protein biocomposites from pelt waste by means of an experimental facility of manufacturing biodegradable nutritional substrates. The advantages of using these nutritional substrates derive from the fact that, in comparison with currently used plastic substrates, they are biodegradable.

Keywords: leather waste, biocomposites, nutritional substrate.

INTRODUCTION

The application of innovative biotechnologies to valorize pelt waste from tanneries leads to the development of biodegradable nutritional substrate.

As Figure 1 shows, processing 1000 Kg raw hide (raw material) results in 750 Kg leather waste of which 592 kg is pelt waste, which can be further processed into fertilizers for agriculture (Zainescu *et al.*, 2014).

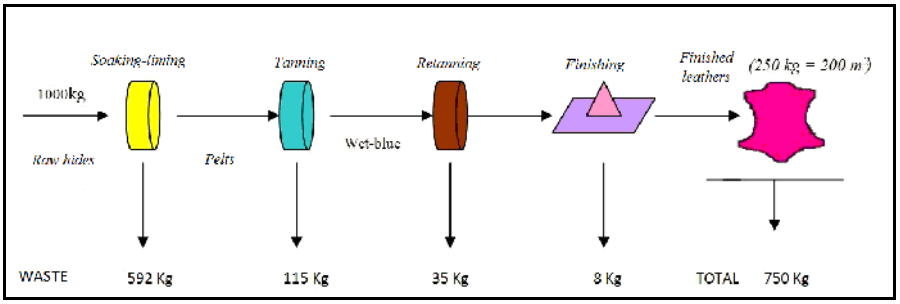

Figure 1. Balance of materials from processing 1000 kg raw hides

The composition of biocomposites obtained from collagen hydrolysate comprises macroeluents - nitrogen, phosphorus, potassium and a wide range of microelements essential for plant metabolism. Therefore, they are chelate complexes containing Fe, Cu,

Zn, Mo, B, Mg, Mn, S, as well as organic protein substances made up of proteins and protein hydrolysates (Pillai and Archana, 2012; Bajza and Vrucek, 2001). Biocomposites can be diversified depending on vegetation phases of plants and on the type of crops, being able to correct nutritional deficiencies and scarcity of nutrient compounds of the soil (due to the technological factor or climatic stress) (Thanikaivelan *et al.*, 2004; Zainescu *et al.*, 2013).

The advantages of using these nutritional substrates derive from the fact that, in comparison with currently used plastic substrates, they are biodegradable and contain fertilizers with long-term action.

Biodegradable nutritional substrates belong to a new generation of transplant substrates and are designed so as to perform two distinct functions: substrate for the seedling for a variable time period depending on the plant; and active biological material, which provides biostimulating effects by gradual degradation in the soil (Zainescu *et al.*, 2011).

EXPERIMENTAL

In this study pelt waste from fleshing and trimming bovine hides (weighing 35 kg) were used, from SC Pielorex Jilava tannery, Ilfov County. Raw hide contains 50-68% proteins, 0.6-9% fat, 15-50% ash and less than 5% water, reported to dry weight.

An innovative process was proposed for the treatment of pelt waste by hydrolysis of protein waste in acid medium, resulting in a protein biopolymer which, combined with other polymers (polyvinyl alcohol, corn starch, polyacrylamide, maleic anhydride, celullose, etc.) will be used in agriculture (for remediation of degraded/eroded soils and plant growth in greenhouse or in the field).

Figure 2. Technological process of obtaining biocomposites from pelt waste

Hydrolysis of raw hides is applied, especially in medicine, to obtain natural collagen which is a natural polymer formed by polymerization of 20 amino acids arranged in

specific sequences for the collagen molecule, which has a unique triple helix conformational structure. Thus, in the collagen composition, the glycine amino acid (Gly) is about 33%, and the proline (Pro) and hydroxyproline (Hyp) amino acids are about 22%.

The polypeptides in the collagen hydrolysate form chelate complexes with metal ions, especially Fe, Ca, Mg, Cu, Zn by means of reactive carboxyl, hydroxyl and nitrogen groups such as NH-pyrrolidine and -CO-NH- peptide bond. The lower the average molecular weight of the hydrolysate or the lower the metal ion concentration, the more stable the chelates formed (Gu and Lee, 2009).

To obtain nutritional substrates in the form of pots it was necessary to design and implement an experimental facility for manufacturing biodegradable nutritional substrates.

Thus, an experimental model facility was developed with a high degree of flexibility, the principle of which consists in forming and dewatering biodegradable nutritional substrates on a media die-punch system using a vacuum. We opted for a single post mold and mold shape and size have been determined based on the following criteria: geometry, technology, possibility of mechanization, packaging, transportation, economic efficiency and productivity. The mold facility has the advantage of using high consistency and enables the possibility to obtain finished products with the desired size and shape without requiring other operations and finishing materials. The optimum solution selected in order to develop the mold for nutritional substrates was the cone, which has the advantage of better stability of the substrate and has a high degree of flexibility regarding the operating parameters, allowing different compositional variants for biodegradable nutritional substrates.

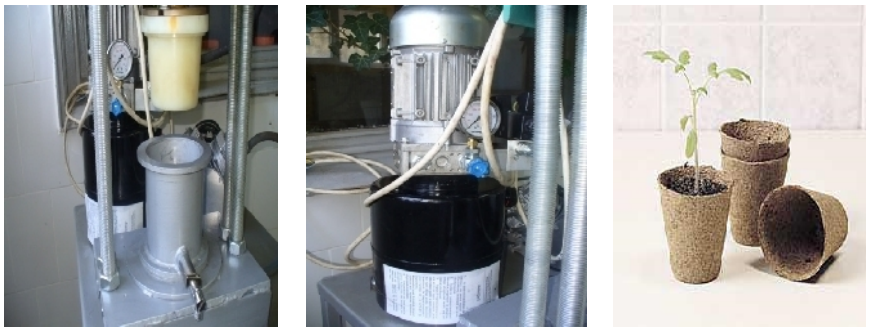

Figure 3. Experimental facility for obtaining nutritional substrates

The experimental facility for obtaining nutritional substrates is composed of the following elements: stainless steel laboratory table which supports the facility, vacuum pump and the tapered single-post (nest) mold (equipped with sieve) in which the material is introduced, the mobile die-punch mounted on a fixed steel support (for centering) is connected to a power press (100 atm).

The advantages of using these nutritional substrates derive from the fact that, compared to currently used plastic substrates, they are biodegradable, as they are made of protein biocomposites from leather waste. Biodegradable nutritional substrates belong to a new generation of culture media and are designed so as to perform two distinct functions:

- substrate for the seedling for a variable time period depending on the plant;
- active biological material, which provides biostimulating effects by gradual degradation in the soil.

RESULTS AND DISCUSSIONS

Research has shown that the hydrolysis of protein waste leads to new products acting as state-of-the-art organo-mineral protein fertilizers, designed to transport nutrients to and into the plant, resulting in metabolism stimulation, speeding the production phase, stimulation of the plant defense system and optimization of mechanisms responsible for the health of the fruit.

The technology for developing biodegradable nutritional substrates is based on key properties that these materials must meet in the process of growth and development of seedlings, namely:

- to form a reservoir of mineral nutrients (biologically active material);
- to completely degrade physically during a life cycle of the plants transplanted into the soil;
- to ensure availability of water transfer to plants;
- to provide support to the plant;
- to allow exchange of gaseous compounds (oxygen, CO_2);
- the products resulting after degradation of the composite structure (protein hydrolyzate, polymers, additives) should not be toxic to the soil;
- to be biodegradable and contribute to soil bioremediation.

The composition of nutritional substrates consists of:

- protein hydrolysate from pelt waste mixed with different soils (clay, peat). The type of soil must ensure a controlled porosity in the substrate structure so that the physicochemical processes of mineralization, upon contact with the soil, would lead to regulating the duration of biodegradability;
- auxiliaries with the role of regulating the biodegradation capacity of the nutritional substrate, controlling tensile strength, establishing an optimal balance of nutrients and providing plant prophylaxis; they are obtained by die-punch molding and dehydration.

Application of the protein biocomposite from pelt waste corrects micronutrient deficiencies, prevents and corrects weak fruiting and leaf malformations. It also significantly increases phosphorus uptake, improves vegetative development and cell wall formation, increasing pollen fertility and plant resistance to low temperatures.

CONCLUSIONS

The established technology provides a cost-effective and environmental solution for protein waste recovery from the leather industry in the form of composite materials that can be used in the development of biodegradable nutritional substrates.

Biodegradable nutritional substrates based on protein biocomposites from leather waste and peat, with addition of protective and stimulating materials constitute a superior form of transportation used in current technologies for producing plant seedlings; therefore these nutritional substrates have the following advantages:

- they allow direct transfer of the plants into the soil without disturbing the roots;
- when using this type of transplant no solid waste is generated as in the case of using plastic or ceramic substrates;
- they are biodegradable, being made of natural compounds;

- they have a good permeability to water and air;
- due to their porous structure, they present an increased capacity of being penetrated by plant roots.

Acknowledgements

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RHEOLOGICAL STUDY OF BIODEGRADABLE LUBRICATING GREASES

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In the recent years, the lubricating market is demanding new biodegradable products based on renewable resources as a consequence of progressively more strict environmental regulations. This work is focused on the rheological study of some dispersions, potentially applicable as biodegradable lubricating greases obtained by dispersing calcium soap in vegetable base oils. The calcium soap is obtained during the grease preparation by saponification reaction between stearic acid, CH_3-$(CH_2)_{16}$-COOH, and calcium hydroxide, $Ca(OH)_2$. The vegetable oils were olive, palm and corn oil. For this study 15 grease samples have been prepared and each of them was analyses at 20, 30, 40, 50 and 60^oC. The effects that concentration of calcium soap and temperature exert on the rheological properties of these greases were studied. The evolution of shear stress with shear rate was very similar to that found for traditional lubricating greases. The rheological curves indicate that the greases have non-Newtonian behaviors which are better described by the Bingham model. In general, the values of plastic viscosity increase with calcium soap concentrations and decrease with temperature.

Keywords: calcium soap, biodegradable greases, rheological properties

INTRODUCTION

Lubricating greases are highly structured suspensions, usually consisting of a thickener (5–30% wt) dispersed in mineral or synthetic oil (70–95% wt). Fatty acid soaps of lithium, calcium, sodium, aluminium or barium are most commonly used as thickener agents. Although the lubricating component is the base oil, the thickener is a key component, added to increase the consistency of the lubricant, in order to solve some difficulties that lubricating oils cannot cover properly in specific applications (Sanchez *et al.*, 2011; NLGI, 2006). The thickener forms an entanglement network, which traps the oil and confers the appropriate rheological and tribological behaviour to the grease (Mas and Magnin, 1994).

The main consumer market of lubricating greases is the automotive market. The grease used is conventional lithium greases, conventional calcium greases, and often sodium greases. This type of greases usually has very good water resistance, adhesive properties, corrosion resistance and oxidation stability. Greases for lubricating machines used in food processing or in drinking-water systems, in which incidental and unavoidable contact between food and lubricant can occur, must fulfill specific requirements relating to food legislation, human health protection, taste, and odor (Mang and Dresel, 2007).

Researches concerning the effects of compositions and texture of thickeners agents on the greases wear and friction properties have been reported on many studies (Franco *et al.*, 2005; Martin-Alfonso *et al.*, 2011; Nunez *et al.*, 2012; Sanchez *et al.*, 2011). These studies demonstrated that the greases composition and processing conditions have a great influence on their microstructure and rheology.

Because of increasingly stringent environmental regulations, most of the last years studies (Adhvaryu *et al.*, 2005; Dresel, 1994; Nunez *et al.*, 2012) are mainly focused on the replacement of mineral oils by vegetable ones. Even the vegetable oils present poor oxidative stability, in comparison to some of the mineral oils, they have good lubricity and ability for adhering to metal surfaces, low volatility, small viscosity–temperature dependence and, of course, non-toxicity and high biodegradability (Adhvaryu and Erhan, 2002; Adhvaryu *et al.*, 2004; Dicken, 1994; Erhan and Asadauskas, 2000; Erhan *et al.*, 2006).

Taking into account these considerations, the main goal of the present work is focused on the development of new renewable and biodegradable lubricating greases, using calcium stearate soap as thickener agents dispersed in vegetables oils like corn, olive and palm oils. The effects that temperature and concentration of the thickener agent exert on the rheological properties of the corresponding biolubricating greases have been analyzed.

EXPERIMENTAL

Greases Synthesis

The oil (three types: olive oil, corn oil and palm oil) stearic acid and $Ca(OH)_2$ were loaded from the beginning in the mixing vessel in proportions shown in Table 1. During the preparation of the grease, the soap was obtained by reaction between stearic acid and calcium hydroxide:

$$2\ CH_3\text{-}(CH_2)_{16}\text{-}COOH + Ca(OH)_2 = (CH_3\text{-}(CH)_{16}\text{-}COO)_2Ca + 2\ H_2O$$

The manufacture methodology was reported elsewhere (Sterpu *et al.*, 2010) and consisted briefly of the warming up and mixing at 120^{o}C for an hour to eliminate the water from the product in order to improve its viscosity. There were prepared 15 greases corresponding to soap concentrations of 10%; 15%; 20%, 25% and 30% for each type of oil, according to the formulas in Table 1. At the end of the preparation, the mixture was cooled down by natural convection. The benchmark grease was prepared from the paraffin oil with 20% calcium soap, in the same conditions and was characterized in a previous work (Sterpu *et al.*, 2010).

For simplification, the greases were named after the base oil name and the concentration of calcium soap: O10%; O15%; O20%, O25% and O30% for grasses produced from olive oil, P10%; P15%; P20%, P25% and P30% for grasses produced from palm oil and C10%; C15%; C20%, C25% and C30% for grasses produced from corn oil.

Table 1. The greases preparation formula for different concentration of calcium soap

Soap concentration	Stearic acid, [g]	$Ca(OH)_2$ [g]	Obtained soap, [g]	Oil, [g]
10%	46.86	6.11	50	450
15%	70.29	9.15	75	425
20%	93.73	12.21	100	400
25%	117.15	15.26	125	375
30%	140.59	18.32	150	350

Viscosity Determination

The preparation of the lubricant greases was performed in an open vessel with a helix agitator provided by Petrotest, Germany, in batches of 0.500 kg. For the rheological behavior determination of the grease G20% at different temperatures a rheoviscometer, HAAKE VT 550, Germany with cone and plate geometry was utilized. The grease samples were analysed at 5 different temperatures: 20°C, 30°C, 40°C, 50°C and 60°C, with both increasing and decreasing shear rate, in the range of 9.97– 4500 s^{-1}.

RESULTS AND DISCUSSIONS

The reports containing two variation curves (ascending and descending) of shear stress with shear rate (rheograme) were provided automatically by the software included in the rheoviscometer system. Every variation curve was built in 100 points. The rheograms of the grease samples at different temperatures are presented in Figs.1-3. To simplify the diagrams of this work, only the descending curves were represented.

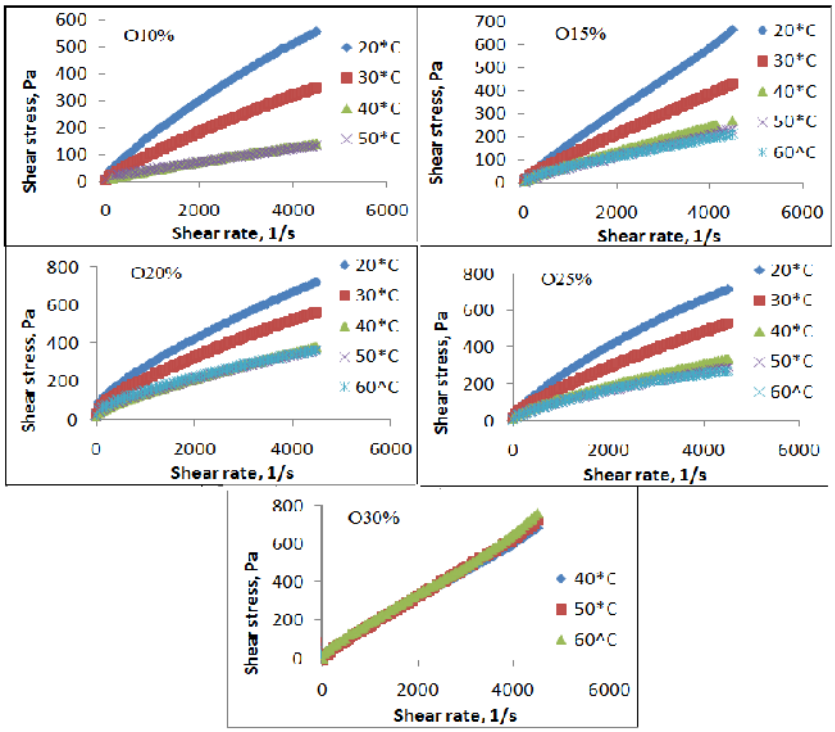

Figure 1. Variation of shear stress *vs.* shear rate of greases based on olive oil at different temperature

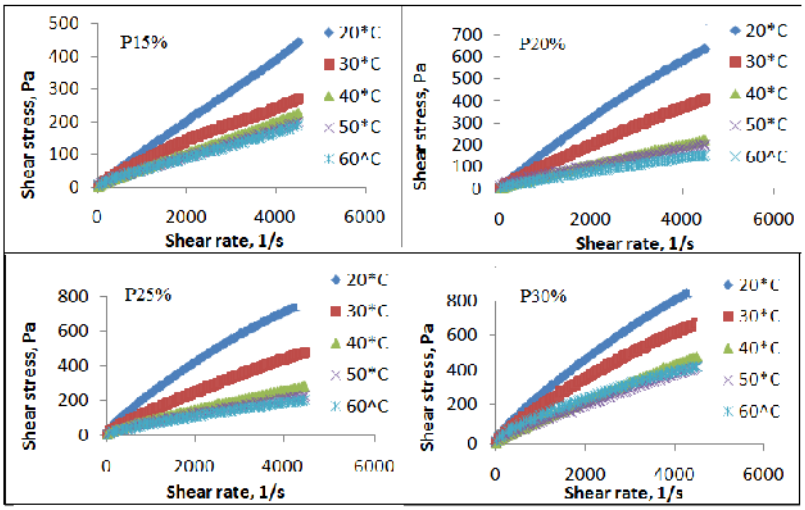

Figure 2. Variation of shear stress *vs.* shear rate of greases based on palm oil at different temperature

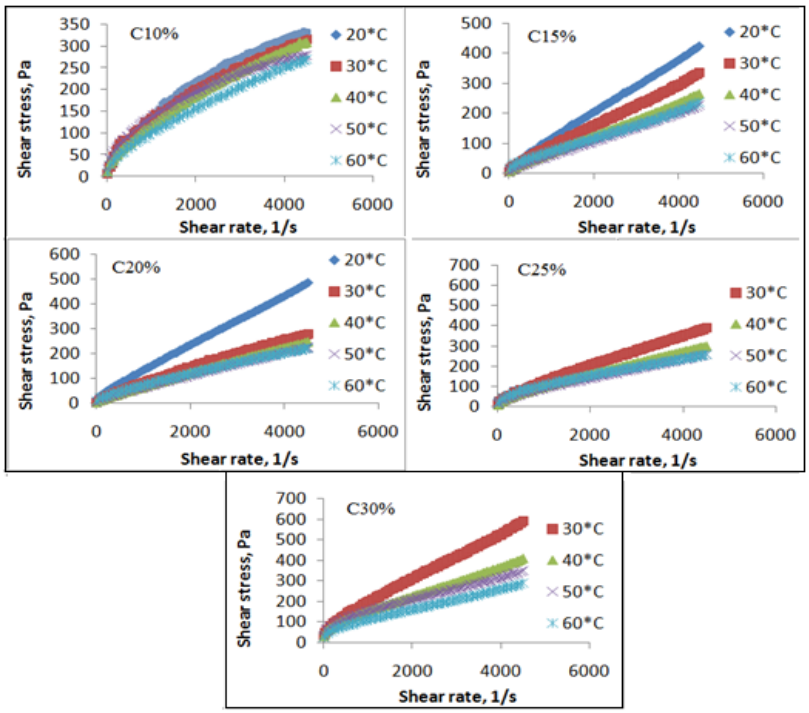

Figure 3. Variation of shear stress *vs.* shear rate of greases based on corn oil at different temperature

By processing the rheological data, it resulted that the curves are best described by the Bingham model (Eq.1):

$$\tau = a \cdot \dot{\gamma} + b \quad (1)$$

where τ is the shear stress, γ is the shear rate and a, b are constants. In Eq.1, *a* represents the plastic viscosity (η_p) and *b* is the yield stress (τ_0). These are the two rheological properties describing the flow behavior of a Bingham plastic fluid. This type of fluid acts like a solid for small values of shear stress and then as a fluid at higher shear stress.

The Bingham model described with Eq.1 has positive constants a and b and always 0 < a < 1. The rheological curves are accurately described by the linear function taking into account that the values of correlation factors (R^2) are between 0.949 and 0.999, as seen in Table 2.

Table 2. The values of coefficients *a*, *b* and the correlation coefficients R^2 of the Eq.1

Soap conc.	Temp. °C	Greases based on olive oil			Greases based on palm oil			Greases based on corn oil		
		a	b	R^2	a	b	R^2	a	b	R^2
10%	20				-	-	-			
wt		0.116	55.29	0.993				0.088	50.26	0.998
	30	0.074	28.36	0.995	-	-	-	0.058	44.52	0.996
	40	0.028	15.23	0.997	-	-	-	0.053	36.94	0.994
	50	0.026	14.91	0.997	-	-	-	0.047	36.06	0.994
	60	-	-	-	-	-	-	0.044	54.82	0.984
15%	20									
wt		0.139	79.80	0.999	0.135	10.12	0.999	0.108	27.39	0.999
	30	0.089	48.10	0.998	0.076	21.98	0.991	0.072	21.15	0.995
	40	0.056	31.87	0.998	0.044	6.152	0.999	0.055	11.58	0.998
	50	0.046	29.12	0.997	0.035	8.666	0.999	0.049	14.15	0.997
	60	0.042	46.00	0.993	0.03	10.81	0.998	0.045	25.1	0.995
20%	20									
wt		0.137	91.86	0.990	0.14	30.15	0.996	0.13	24.63	0.999
	30	0.107	66.43	0.990	0.089	15.31	0.998	0.077	18.2	0.999
	40	0.067	47.92	0.992	0.046	8.931	0.998	0.06	12.29	0.999
	50	0.058	40.27	0.990	0.039	23.1	0.998	0.05	12.42	0.999
	60	0.054	67.08	0.985	0.033	9.224	0.997	0.046	24.21	0.997
25%	20							-	-	-
wt		0.147	112.9	0.988	0.161	81.29	0.990			
	30	0.108	80.30	0.993	0.101	37.78	0.996	0.096	63.77	0.972
	40	0.074	59.45	0.991	0.058	26.83	0.995	0.065	52.11	0.983
	50	0.069	55.80	0.989	0.046	25.84	0.993	0.052	43.93	0.989
	60	0.068	77.03	0.972	0.041	23.02	0.993	0.049	80.86	0.949
30%	20	-	-	-				-	-	-
wt					0.183	51.76	0.993			
	30	-	-	-	0.11	37.16	0.991	0.119	104.8	0.985
	40	0.059	14.51	0.998	0.063	14.94	0.998	0.089	77.37	0.996
	50	0.057	8.898	0.999	0.054	19.44	0.998	0.074	71.31	0.995
	60	0.056	13.18	0.997	0.048	40.02	0.986	0.058	86.19	0.998

The grease proceeding from palm oil containing 10% wt soap wasn't analysed because it was unstable and the oil was separated after a week. Also, the grease with 10% soap proceeding from olive oil wasn't analysed at 60°C, because it was too soft

and started to leak. The greases with 30% soap from olive oil and with 25% and 30% from corn oil, which are solid at room temperature, couldn't be analysed at 20 ^{o}C and/or 30^{o}C.

CONCLUSIONS

In this work we evaluated some dispersions formulated with calcium stearate as thickener agent in olive oil, palm oil and corn oil to be applied as lubricating greases. The evaluation was made from the rheological point of view.

The said greases can be characterized as soft, and they rheological behaviour is non-newtonian, described with accuracy by the Bingham model (R^2 = 0.949 - 0.999).

The highest values of plastic viscosity (a or η_p) are those of greases from palm oil and the lowest values are those of greases from corn oil; this indicates that palm oil greases are the most consistent and corn oil greases are the softest.

The finding of rheological equation for every grease at different temperatures is important for the study of mixing with applications in the processing of these products.

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ANTIBACTERIAL ACTIVITY OF *GLYCYRRHIZIC ACID* AGAINST MULTI DRUG RESISTANT BACTERIA AND FUNGUS

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Glycyrrhizic acid (GA) is a kind of triterpene glycoside obtained from Liquorice (*Glycyrrhiza glabra*). The aim of this study was to search the antimicrobial activities of *Glycyrrhizic acid* against bacteria some drug resistant bacteria (*E.coli*, *A.baumanii* and *P.aeruginosa*) and fungus (*C.albicans*). To determine the non-cytotoxic concentration of GA, HEp-2 cell line was used. The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum 1% (w/v). Cells were incubated in a humidified atmosphere at 37 ºC in 5% CO_2. Antimicrobial activity of GA was screened by broth microdilution procedures and principles of the CLSI. Stock solutions of GA at the concentration of 1000 µg/ml were prepared in ethanol. GA concentration range used in the antimicrobial tests was 1.92; 3.8; 7.8; 15.6; 31.2; 62.5; 125; 250 and 500 µg/ml prepared for bacteria in Mueller-Hinton broth and for yeast in Saboraud Dextrose broth. Minimal inhibitory concentrations for GA were investigated against both standard bacterial strains; *E.coli* (ATCC 25922), *P.aeruginosa* (ATCC 27853), *A.baumanii* (ATCC 17978), and yeast-like fungus; *C.albicans* (ATCC 90028) and clinical isolates of multi drug resistant isolates. It was established that *glycyrrhizic acid* inhibited the growth of bacteria with MIC values ranging between 15.6 and 31.2 µg/ml and showed anti-yeast activity with MICs at 62.5 µg/ml. While the GA exhibited significant antibacterial activity against multidrug resistant Gram negative bacteria, it was found to be slightly less effective against *C.albicans* isolates. We think that GA may be new hope for the treatment of diseases caused by multidrug resistant bacterial isolates.

Keywords: *Glycyrrhizic acid*, resistant, bacteria, fungus.

INTRODUCTION

Globally, widespread use of antibiotics leads to the development multi-drug resistant microorganisms. Multidrug-resistant microorganims are among the major causes of hospital-acquired infections over the last 20 years (Laxminarayan and Heymann, 2012; CDCP, 2013).

Infections due to multi-drug resistant microorganisms are sources of significant medical problems in hospitals and healthcare facilities, because these kinds of infections are the major causes of both morbidity and mortality. The main multi-drug resistant Gram negative bacteria related to antimicrobial resistance are extended-spectrum beta-lactamase (ESBL)-producing gram-negative bacteria such as *Escherichia coli*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa* (Datta *et al.*, 2012; Napier *et al.*, 2013; Nordmann *et al.*, 2011).

On the other hand, another major problem in drug resistance is the resistance to antifungal drugs. Drug-resistant isolates of fungal infections have been reported to reach notable levels depending on immunocompromised patients including those who have undergone organ transplants, patients receiving chemotherapy or HIV treatments. In recent years, drug resistance in fungal infections has been reported to increase due to *C. albicans* (Goffeau, 2008; Cowen, 2008).

Because antibiotic resistance is a rapidly evolving problem in both bacteria and fungi, new drug research is proceeding fast around the World. Recently, studies especially on natural products are remarkable in this area. *Glycrrhiza glabra* is one of

the most important natural products in new drug research. It has many pharmacological activities such as antimutagenic activity (Alekperov, 2008) anti-ulcer effects (Dey *et al.*, 2009), protective action against hepatotoxicity (Wan *et al.*, 2009), antitumor promoting activity (Rafi *et al.*, 2002), etc. *Glycyrrhizic acid* is the most important component of *Glycrrhiza glabra* which have a wide range of pharmacological properties.

This study aims to search for antimicrobial activities of *Glycyrrhizic acid* against bacteria including drug resistant bacteria (*Escherichia coli*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa*) and fungus (*Candida albicans*).

MATERIALS AND METHODS

Microorganisms & *Glycyrrhizic Acid*

Escherichia coli, *Acinetobacter baumanii* and *Pseudomonas aeruginosa* and *Candida albicans* strains were obtained from Mustafa Kemal University culture collections, in Hatay province. For the culture of Gram negative bacteria (*Escherichia coli*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa)* we used EMB agar and Mueller-Hinton broth (Difco, USA).

Glycyrrhizic acid was obtained from commercially from Sigma (Sigma, USA). To determine the non-cytotoxic concentration of *Glycyrrhizic acid*, HEp-2 cell line was used. The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum 1% (w/v). Cells were incubated in a humidified atmosphere at 37 ºC in 5% CO_2.

The Effect of DMSO on the HEp-2 Cells

DMSO (dimethyl sulfoxide) was used as a solvent for *Glycyrrhizic acid.* To determine the non-toxic concentration of DMSO on HEp-2 (human larynx epidermoid carcinoma cells), 1×10^6 cells were inoculated into each well of 12-well plates containing RPMI-1640, and incubated for 48 hrs in the presence of decreasing amounts of DMSO (8%, 4%, 2%, 1%, 0.5%). The nontoxic concentration was determined up to 2%. The 0.5% and 1% concentrations of DMSO did not affect the growth of the microorganisms. Therefore, *Glycyrrhizic acid* were dissolved in 1% DMSO in experiments.

Cell Culture Tests for Cytotoxicity

To evaluate the cytotoxicity of *Glycyrrhizic acid* for human cells, (HEp-2) cell line was selected. The cells were cultured in RPMI-1640 medium with 10% (w/v) FCS. Incubation of the cells was performed at 37°**C** with 95% air and 5% carbon dioxide.

Glycyrrhizic acid was dissolved in DMSO (Sigma, USA). Stock solutions of *Glycyrrhizic acid* were prepared in DMSO at the concentration of 1%. The 250; 125; 62.5; 31.2; 15.6; 7.8; 3.9; 1.92; 0.96; 0.48 µg/ml of concentrations of *Glycyrrhizic acid* were tested.

To determine the non-cytotoxic concentration of *Glycyrrhizic acid* on HEp-2 cells, 1×10^5 cells were inoculated into each well of flat-bottomed plates, and were cultured for 8 hrs at 28°C. Next, they were allowed to grow for an additional period 96 hours. *Glycyrrhizic acid* were diluted, whereupon decreasing amounts (from 250 to 0.48 µg/ml) were placed per well. All experiments were performed in triplicate, and the results were expressed as log number cells per milliliter on the percentage of growth inhibition.

The cytotoxicity of the *Glycyrrhizic acid* was determined using a conventional hemocytometer and the trypan blue exclusion (Burlesson et al., 1992). The highest noncytocidal (on HEp-2 cells) concentration of the tested samples was determined to be 125 µg/ml. Hence, *Glycyrrhizic acid* concentrations were lower than that of non-cytotoxic concentration.

Antimicrobial Activity

Multi-drug resistant clinical isolates including both bacteria (*Escherichia coli*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa*) and fungi (*Candida albicans*) were used in the experiments. Also, the following standart microorganisms were cultured simultaneously for antimicrobial activity test: *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 17978, *Pseudomonas aeruginosa* ATCC 27853 and *C.albicans* strais (ATCC 90028). Antimicrobial activity was evaluated with broth microdilution method. Minimal inhibition concentration ranges were determined according to the CLSI (Clinical and Laboratory Standards Institute) guidelines (CLSC, 2008).

Mueller-Hinton broth (Difco, USA) and Saboraud Dextrose broth (Difco, USA) were used when testing bacterial and *Candida albicans* strains, respectively. The inoculum density was $1x10^6$ cfu/ml for bacteria and $1x10^5$ cfu/ml for fungi. *Glycyrrhizic acid* was dissolved in DMSO. Solutions in the test medium provided the required concentration ranging from 1024-0.5 µg/ml. The inoculated plates were incubated at 35°C and evaluated after 24 hours. For *Candida albicans* this incubation period was selected as 48 hours. Minimum inhibitory concentration values were determined as the lowest concentrations of the substances that had no visible turbidity. For bacteria amikacin was selected as a positive control, while, in the case of yeasts, flucanozole was used.

For antimicrobial activity of *Glycyrrhizic acid*, stock solutions of *Glycyrrhizic acid* at the concentration of 125 µg/ml were prepared in ethanol. *Glycyrrhizic acid* concentration range used in the antimicrobial tests was 0.48; 1.92; 1.56; 3.9; 7.8; 15.6 31.2; 62.5 and 125 µg/ml prepared for bacteria in Mueller-Hinton broth and for yeast in Saboraud Dextroz broth. Minimal inhibitory concentrations for *Glycyrrhizic acid* were investigated against both standard bacterial strains; *E.coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), Acinetobacter baumanii (ATCC 17978), and yeast-like fungus; *C. albicans* (ATCC 90028) and clinical isolates of multi drug resistant isolates.

Statistical Analysis

All data is represented as mean ± standard error of mean (SEM) for triplicate set of experiments. Statistical analyses were performed using Student t-test. The p value <0.05 was considered significant. All statistics in the present study were done using SPSS for Windows, version 17.5 SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Cell cultures were incubated with DMSO alone (without any supplement) in the DMSO control group. In this group, we compared the effects of DMSO with cell control group on the viability of HEp-2 cells. As given in Figure 1, DMSO at the 1% (w/v) concentration did not showed no toxic effect for the cells. This concentration of DMSO (1% w/v) did not inhibit the growth of cells. At the end of incubation, there

were no statistically significant difference in the cell number between the cell control and the DMSO ontaining groups ($p>0.05$). Furthermore, nor were any cytopathological changes observed in DMSO group compared with the cell control group (Figure 1).

Glycyrrhizic acid was evaluated *in vitro* for antimicrobial activity against both standart strains of *E. coli, P. aeruginosa, Acinetobacter baumanii and C. albicans* and drug-resistant clinical strains of these microorganisms. As the control drugs, flucanazole and amikacin were selected (Tables 1-4).

Antimicrobial test results performed with the standard strains (*E. coli, P. aeruginosa, Acinetobacter baumanii and C. albicans*) are given in Table 1 and 2. In Table 1 and 2, MIC values of *glycyrrhizic acid* against drug-resistant clinical isolates (*E. coli, P. aeruginosa, Acinetobacter baumanii and C. albicans*) are given. As can be seen in the table, while standart drugs inhibited the microorganisms growth between 15.6 and 31.2 µg/ml, *glycyrrhizic acid glycyrrhizic acid* proved to inhibit the growth of bacteria with MIC values ranging between 15.6 and 31.2 µg/ml and showed anti-yeast activity with MICs at 62.5 µg/ml.

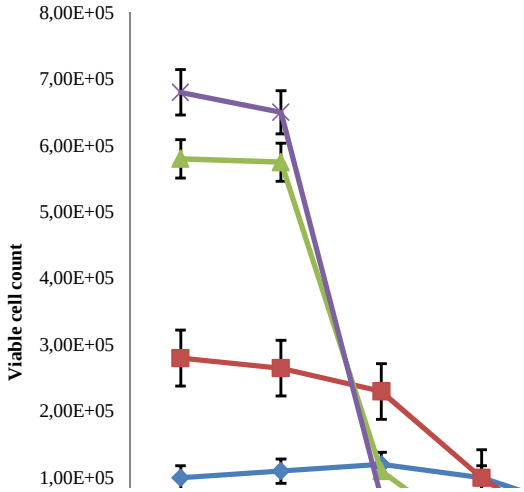

Figure 1. Effects of *Glycyrrhizic acid* on the proliferation of HEp-2 cells

Table 1. MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration) values (µg/ml) of glycyrrhizic acid and amikacin for *E.coli*, *P. aeruginosa*, *A. baumanii* and *C. albicans*

	Drug resistant isolates		*E.coli (ATCC 25922)*	
	MIC	MFC	MIC	MCF
Glycyrrhizic acid	15.6	31.2	3.9	15.6
Amikacin	4	8	0.5	2
	Drug resistant isolates		*P. aeruginosa* (ATCC 27853)	
	MIC	MFC	MIC	MCF
Glycyrrhizic acid	15.6	62.5	7.8	31.2
Amikacin	8	16	1	2
	Drug resistant isolates		*A.baumanii* (ATCC 17978)	

	MIC	MFC	MIC	MCF
Glycyrrhizic acid	62.5	125	15.6	31.2
Amikacin	8	16	1	2

In Table 3 and 4, the synergistic effects of the standard drugs with *Glycyrrhizic acid* were investigated. As is clear in these tables, *Glycyrrhizic acid* significantly raised the effectiveness of the standart antimicrobials (amikacin and flucanazole). A synergistic activity was determined between *Glycyrrhizic acid* and amikacin, while an additional activity was found between *Glycyrrhizic acid* and flucanazole.

Table 2. MIC and MFC values (µg/ml) of *Glycyrrhizic acid* and flucanozole for *Candida albicans*

	Drug resistant isolates		*C.albicans strais (ATCC 90028)*	
	MIC	MFC	MIC	MCF
Glycyrrhizic acid	62.5	125	15.6	31.2
Flucanozole	2.5	5	2.5	5

Table 3. Synergistic effects of *Glycyrrhizic acid* and Amikacin on growth inhibition of *E.coli*, *P. aeruginosa* and *A. baumanii*

	Glycyrrhizic acid (µg/ml)					
	Drug resistant strains			*E.coli* (ATCC 25922)		
Amikacin (µg/ml)	1.56	3.12	6.24	1.56	3.12	6.24
0.5	G	G	NG	G	G	NG
1	G	NG	NG	NG	NG	NG
2	G	NG	NG	NG	NG	NG
	Glycyrrhizic acid (µg/ml)					
	Drug resistant strains			*P. aeruginosa* (ATCC 27853)		
Amikacin (µg/ml)	1.56	3.12	6.24	1.56	3.12	6.24
0.5	G	G	NG	G	G	NG
1	G	G	NG	G	G	NG
2	G	G	NG	NG	NG	NG
	Glycyrrhizic acid (µg/ml)					
	Drug resistant strains			*A.baumanii* (ATCC 17978)		
Amikacin (µg/ml)	1.56	3.12	6.24	1.56	3.12	6.24
0.5	G	G	G	G	G	NG
1	G	G	NG	NG	NG	NG
2	G	NG	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth.

Table 4. Synergistic effect of *Glycyrrhizic acid* and flucanozole on growth inhibition of *Candida albicans*

	Glycyrrhizic acid					
	Drug resistant strains			*C.albicans* strains (ATCC 90028)		
Flucanozole	1.56	3.12	6.24	1.56	3.12	6.24
1	G	G	G	NG	NG	NG
2	G	G	NG	NG	NG	NG
4	G	G	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth.

CONCLUSIONS

In conclusion, *Glycyrrhizic acid* alone was confirmed to be active against multi drug resistant isolates of *E.coli*, *P.aeruginosa, Acinetobacter baumanii* and *C.albicans*. In this study, *Glycyrrhizic acid* was shown to be more active than amikacin and flucanozole *in vitro* when combined with amikacin and flucanozole. Our results suggest a therapeutic potential of glycyrrhizic acid for the treatment caused by *E.coli*, *P. aeruginosa, Acinetobacter baumanii* and *C.albicans* infections. Further studies to be performed should evaluate to explore its therapeutic potential and the beneficial effect of glycyrrhizic acid. We think that *Glycyrrhizic acid* may be new hope for the treatment of diseases caused by drug resistant microbial isolates.

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ANTIBACTERIAL ACTIVITIES OF SELECTED MEDICINAL PLANTS AGAINST MRSA STRAINS ISOLATED FROM SURGICAL WOUND INFECTIONS

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Methicillin resistance in *Staphylococcus aureus* is one of the most important antibiotic resistance. In this study, we aimed to investigate the antibacterial activities of some medicinal plants *(Laurus nobilis, Salvia officinalis, Thymbra spicata)* against MRSA strains isolated from surgical wound infections. Firstly, non-cytotoxic concentrations were determined in cellculture. In order to determine the non-cytotoxic concentrations of essential oils, HEp-2 cell line was selected. Antimicrobial activity studies were carried out under the non-cytotoxic concentrations for cells. Mueller-Hinton broth was selected to test the bacterium strain. The inoculum density was 1x 10^6 cfu/ml. The essential oils of plants were dissolved in absolute ethanol. The ratio of essential oils in the test medium furnished the required concentration ranging from 1000-7.8 (7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000) µg/ml. The plates were incubated at 37°C and visually read after 48 hours. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity. The antibiotic susceptibilities of MRSA isolates were determined by microdilution method according to the CLSI (Clinical and Laboratory Standards Institute) criteria. MIC for essential oils were investigated against both standard and clinical isolates of Methicillin-resistant *S.aureus*. In this study, the essential oils of these three plants have been confirmed the antibacterial effect against methicillin resistant *S. aureus*. Also, while the essential oils of *L. nobilis* and *S. officinalis* were found to exhibita significant synergistic activity with antimicrobial drugs, *T. spicata* showed limited synergistic activity compared the others.

Keywords: *S.aureus*, methicillin, resistance, essentialoils, cellculture.

INTRODUCTION

Antibiotic resistance is a major public health problem. *S. aureus* is an opportunistic pathogen microorganism that is asymptomatically carried on different parts of the human body including skin and nasal passages. In recent years, the significance of Gram-positive microorganisms in surgical site infections has come to the fore worldwide. Among Gram-positive bacteria is *S. aureus*, one of the most significant infectious agents, whose infections range from mild to life threatening degree (CDC, 2006; Wasserman and Taljaard 2011; Jonson, 1998). Especially, *S. aureus* in patients who undergo surgery may lead to life-threatening infections. Methicillin resistance in this microorganism is reported to be more difficult to treat than ordinary staph infections. MRSA ratio was reported to vary from 10 to 65% in hospitals. This rate is reported to be significantly higher in intensive care units. MRSA is resistant to many conventional drugs. It is resistant to most beta-lactams in addition to aminoglycosides, erythromycin, clindamycin, fluoroquinolones, co-trimoxazole and rifampin (Fraise, 1998; Alexander *et al.*, 2011; CLSI, 2010).

Despite effective antimicrobial options in staphylococcal infections, increasing drug resistance leads to serious problems for the treatment of staphylococcal infections. To this end, new drug research is underway. Nowadays, natural products continue to be an important source of novel drugs. Among these natural products, are *L. nobilis, S. officinalis* and *T. spicata*, which have various pharmacological properties (Derwich *et al.*, 2009; Lawrence, 2005; Alarcon *et al.*, 2002; Akin *et al.*, 2010; Kilic, 2006).

In this study, we aimed to search the antibacterial activities of some medicinal plants *(L. nobilis, S. officinalis, T. spicata)* against MRSA strains isolated from surgical wound infections.

MATERIALS AND METHODS

Microorganisms

In this study, MRSA ATCC and clnical MRSA strains isolated from surgical wound infections were used. MRSA strains were obtained from Mustafa Kemal University culture collections, Hatay. For culture of microorganisms Mueller-Hinton broth (Difco, USA) and Blood agar (Difco, USA) were used. The essential oils of the *L. nobilis, S. officinalis* and *T. spicata* were obtained from Department of Field crops, Agriculture Faculty of Mustafa Kemal University. Firstly, non-cytotoxic concentrations were determined in cell culture. In order to determine the non-cytotoxic concentrations of essential oils, HEp-2 cell line was selected. Antimicrobial activity studies were carried out under non-cytotoxic concentrations for cells. Mueller-Hinton broth was selected to test the bacterial strains. The inoculum density was $1x\ 10^6$ cfu/ml.

Cell Culture

Firstly, non-cytotoxic concentrations were determined in cell culture. In order to determine the non-cytotoxic concentrations of essential oils of *L. nobilis, S. officinalis* and *T. spicata*, HEp-2 cell line (human epithelial cells derived from a larynx carcinoma) was selected. Antimicrobial activity studies were carried out studied under thenon-cytotoxic concentrations for cells. As a culture medium RPMI-1640 was selected. The cell culture medium consisted of RPMI-1640 with fetal calf serum (Seromed, Germany) at a ratio of 10% as the growth factor. The cells were incubated in an atmosphere of 5% carbon dioxide at 37°C.

The Effect of Ethanol

Ethanol was used as a solvent for the essential oils of *L. nobilis, S. officinalis* and *T. spicata.* To determine the non-toxic concentration of ethanol, 1×10^6 cells were inoculated into each well of flat-bottomed plates containing RPMI-1640 and incubated for an 48 h in the presence of decreasing amounts of ethanol (15%, 12.5%, 10%, 7.5%, 5%, 2.5%, 1%). The non-toxic concentration was determined up to 10%. The 1-20% concentrations of ethanol did not affect the growth of the cells. Therefore, the essential oils were dissolved in 5% ethanol in experiments.

The essential oils of plants were dissolved in absolute ethanol. The ratio of essential oils in the test medium furnished the required concentration ranging from 1000-7.8 (7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000) µg/ml. The plates were incubated at 37°C and read visually after 48 hours. The MIC (minimal inhibitory concentrations) values were recorded as the lowest concentrations of the substances that had no visible turbidity. The antibiotic susceptibilities of MRSA isolates were determined by the microdilution

method according to the CLSI (Clinical and Laboratory Standards Institute) criteria. Minimal inhibitory concentrations for essential oils were investigated against both standard and clinical isolates of methicillin-resistant *S. aureus* (CLSI, 2008).

Cell Culture Tests for Cytotoxicity

In order to test the effect of the essential oils of *L. nobilis, S. officinalis* and *T. spicata* on HEp-2 cells, 5×10^5 cells were seeded into each well of flat-bottomed plates, cultured for 6 h at 28°C and the cells were allowed to grow for an additional 48 h. The essential oils of the plants were placed per well at decreasing amounts (1000-7.8 (7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000 µg/ml). The cytotoxicity of the essential oils was determined using a conventional haemocytometer and the trypan blue-exclusion method (Burlesson *et al.*, 1992). The highest noncytocidal (on HEp-2 cells) concentration of the The essential oils of *L. nobilis***,** *S. officinalis* and *T. spicata* were determined to be 125, 250 and 250 µg/ml, respectively. Therefore, up to 250 µg/ml was used for the determination of the antimicrobial activities.

Antimicrobial Activity

The essential oils of *L. nobilis*, *S. officinalis* and *T. spicata* were prepared by dissolving in ethanol and then diluting in Mueller-Hinton broth to give an initial concentration of 1000 µg/mL. Further dilutions of the essential oils and standard drug in the test medium were prepared at the required quantities at concentrations of 7.8; 15.6; 31.2; 62.5; 125; 250, 500 and 1000) µg/ml µg/ml. The MIC for each compound was investigated against standard (methicillin resistant *S. aureus* (MRSA, ATCC 33591) and clinical methicilline resistant bacteria. The cultures were prepared in Mueller-Hinton broth (Difco, USA) for methicilline resistant bacteria. Antimicrobial activity was evaluated by using broth microdilution method. The MIC range was determined according to the CLSI guidelines. The lowest concentration that showed no growth of microorganism was recorded as the MIC expressed in µg/mL. These experiments were triplicated to determine the MIC values. Ampicillin/Sulbactam was used as the control drug.

Statistical Analysis

The statistical analyses were performed using Student t-test. The p value <0.05 was considered significant. All statistics in the present study were done using SPSS for Windows, version 17.5 SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The essential oils were evaluated *in-vitro* for antimicrobial activity against both standart strains of MRSA and clinical strains of isolated from surgical wound infections. As the control drug, ampicillin/sulbactam was employed. Ethanol was used to solve the essential oils.

The cell cultures were incubated with ethanol alone (without any essential oils) in the ethanol control group. In the experiment, we compared the effects of ethanol with cell control group on the viability of HEp-2 cells. As given in Figure 1, ethanol concentration up to 10% (w/v) did not inhibit the growing of cells. At the end of incubation, there were not statistically significant difference in the cell number between the cell control and the ethanol containing groups ($p>0.05$). Nor were any cytopathological changes observed in ethanol group compared with the cell control group (Figure 1).

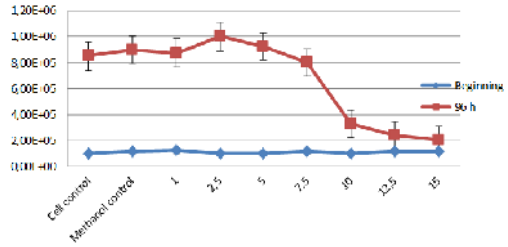

Figure 1. HEp-2 cell viability with different concentrations of ethanol

The cytotoxicity results of the essential oils were given in Figures 2-4. As can be seen from these figures, non-cytotoxic concentrations for *L. nobilis*, *S. officinalis* and *T. spicata* are up to 125 µg/ml, 250 µg/ml and 250 µg/ml, respectively.

Antimicrobial test results were given in Figures 5 and 6. As illustrated by these figures, while standart drugs inhibited the microorganisms growth between 2 and 8 µg/ml, the essential oils proved to inhibit the growth of bacteria with MIC values ranging between from 15.6 to 62.5 µg/ml (Figures 5 and 6).

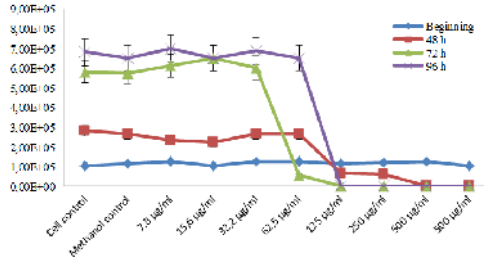

Figure 2. HEp-2 cell viability with different concentrations of the essential oils of *L. nobilis*

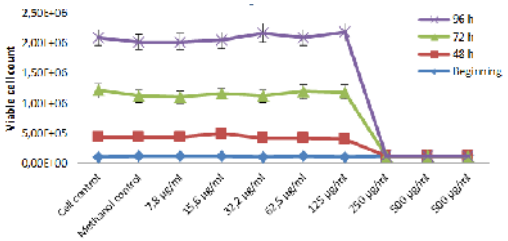

Figure 3. HEp-2 cell viability with different concentrations of the essential oils of *S. officinalis*

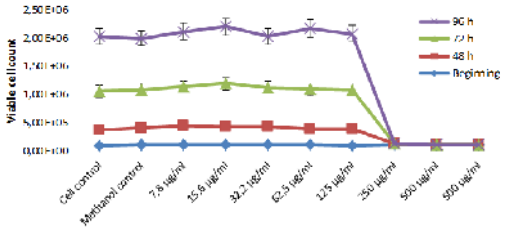

Figure 4. HEp-2 cell viability with different concentrations of the essential oils of *T. spicata*

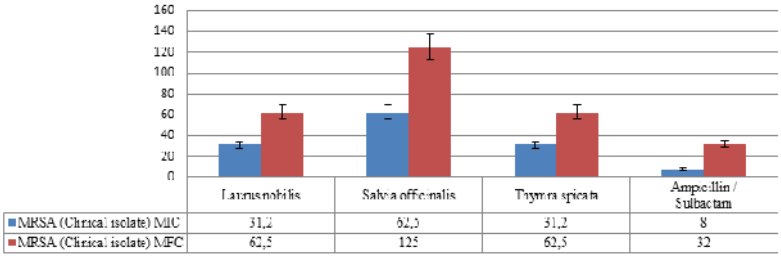

Figure 5. MIC values of the essential oils against MRSA isolated clinically

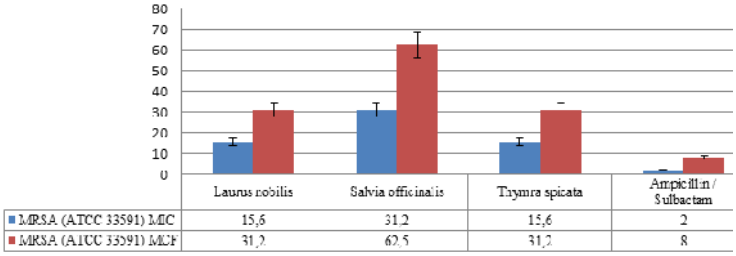

Figure 6. MIC values of the essential oils against standart MRSA strains

In this study, essential oils of these three plants have been confirmed to have antibacterial effect against methicillin resistant *S. aureus*. Also, while the essential oils of *L. nobilis* and *S. officinalis* were found to exhibit a significant synergistic activity with antimicrobial drugs, *T. spicata* showed limited synergistic activity compared with the others (Tables 1-4).

Table 1. MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration) values (µg/ml) of the essential oils and Ampicillin/Sulbactam for MRSA

	MRSA (Clinical isolate)		*MRSA (ATCC 33591)*	
	MIC	MFC	MIC	MCF
Laurus nobilis	31.2	62.5	15.6	31.2
Salvia officinalis	62.5	125	31.2	62.5
Thymbra spicata	31.2	62.5	15.6	31.2
Ampicillin/Sulbactam	8	32	2	8

Results obtained from three independent experiments; G: Growth, NG: No Growth

Table 2. Synergistic effects of *L. nobilis* and Ampicillin/Sulbactam on growth inhibition of MRSA strains

	Laurus nobilis (µg/ml)					
	MRSA (Clinical isolate)			MRSA (ATCC 33591)		
Ampicillin/Sulbactam	7.8	15.6	31.2	7.8	15.6	31.2
2	G	G	NG	NG	NG	NG
4	G	G	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth

Table 3. Synergistic effects of *S. officinalis* and Ampicillin/Sulbactam on growth inhibition of MRSA strains

	Salvia officinalis (µg/ml)					
	MRSA (Clinical isolate)			MRSA (ATCC 33591)		
Ampicillin/Sulbactam	7.8	15.6	31.2	7.8	15.6	31.2
2	G	G	NG	G	NG	NG
4	NG	NG	NG	NG	NG	NG
8	NG	NG	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth

Table 4. Synergistic effects of T. spicata and Ampicillin/Sulbactam on growth inhibition of MRSA strains

	Thymbra spicata (µg/ml)					
	MRSA (Clinical isolate)			MRSA (ATCC 33591)		
Ampicillin/Sulbactam	7.8	15.6	31.2	7.8	15.6	31.2
2	G	G	G	G	G	NG
4	G	G	NG	NG	NG	NG
8	G	G	NG	NG	NG	NG

Results obtained from three independent experiments; G: Growth, NG: No Growth

Acknowledgement

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NOVEL ANTICANCER COMPOUNDS OF PROPOLIS AGAINST THREE DIFFERENT CANCER TYPES

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Cancer is currently the second most common cause of death. The drugs used for conventional cancer therapies cause unwanted side effects. At best, these kinds of drugs used to treat cancer patients only extend the patient's lifespan by a few years. Therefore new drug research in cancer treatment is continuing rapidly. Natural products have recently been investigated as promising agents for the different types of cancer. Recently, propolis has attracted attention due to its various of pharmacological properties. In this study, we aimed to investigate the chemical charecterization of propolis samples collected from Hatay region. Besides this, the aim of this study was to estimate the proliferative effects of the propolis samples on three different cancer lines (A549; human lung adenocarcinoma, HeLa; human cervical carcinoma, A498 human renal carcinoma). The GC-MS (gas chromatography mass spectrometry) analyses were performed for the analyses of the constituents of the propolis samples. Cell culture. Human cell cultures (A549; human lung adenocarcinoma, HeLa; human cervical carcinoma, A498 human renal carcinoma) were maintained by weekly transfers in RPMI-1640 medium supplemented with 10% fetal calf serum with antibiotics (penicillin; 100 U/ml and streptomycin (100 µg/ml) at at 37 °C in 5% CO2. In this study, it was shown that these three propolis components (benzoic acid, phenylethyl alcohol, 9-octadecenoic acid) have to be remarkable antiproliferative effects against human lung adenocarcinoma (A549), human cervical carcinoma (HeLa) and human renal carcinoma (A498) cells. These components can be served as promising propolis compounds for further new drug development.

Keywords: propolis, anticancer, drug, cancer cells

INTRODUCTION

For many centuries, plants have provided a rich source of therapeutic agents. Currently 25% of prescribed drugs worldwide are still derived from plant sources, showing that plant species are still an important source of new drugs for diseases that continue to lack a cure, such as cancer. The use of natural products has been one of the most successful strategies for the discovery of new medicines; natural products have been used for folk medicine purposes throughout the world for thousands of years (Castaldo and Capasso, 2002; Duran, 2012; Duran *et al.*, 2006; Ferlay *et al.*, 2010; Siegel *et al.*, 2012).

Among these kinds of natural products, propolis has attracted increased interest because of pharmacological activities. Propolis is a natural product derived from plant resins collected by honeybees. It is used by bees as glue, a general-purpose sealer, and as draught-extruder for beehives. Propolis has been used in folk medicine for centuries. It is known that propolis possesses anti-microbial, antioxidative, anti-ulcer and anti-tumor activities (Castaldo and Capasso, 2002). Therefore, propolis has attracted much attention in recent years as a useful or potential substance used in medicine and cosmetics products. Furthermore, it is now extensively used in foods and beverages

with the claim that it can maintain or improve human health. The chemical composition of propolis is quite complicated. More than 300 compounds such as polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids and inorganic compounds have been identified in propolis samples. The chemical composition of propolis is quite complex. Over 300 different compounds such as polyphenols, phenolic aldehydes, terpenes, kinins, coumarins, amino acids, inorganic components and steroids have been identified so far in propolis (Castaldo and Capasso, 2002; Duran, 2012). Propolis has various components such as caffeic acid, caffeic acid phenethyl ester, artepilin C, quercetin, naringenin, resveratrol, galangin, genistein and other are the most promising as antitumor agents. The contents of propolis samples depend on the collecting location, time and plant source. Consequently, biological activities of propolis gathered from different phytogeographical areas and time periods vary greatly (Castaldo and Capasso, 2002; Duran, 2012; Duran *et al.*, 2006).

In our earlier studies, propolis samples collected from Hatay region have been identified antiproliferative activity on cancer cell lines (Duran *et al.*, 2011).

Search for new substances with antiproliferative activity towards cancer cells is very important because of the drug resistance and side effects used in conventional cancer therapy (Luqman and Pezzuto, 2010).

In this study, we aimed to investigate the chemical charecterization of propolis samples collected from Hatay region. Besides this, the aim of this study was to estimate the proliferative effects of the propolis samples on three different cancer lines (A549; human lung adenocarcinoma, HeLa; human cervical carcinoma, A498; human renal carcinoma).

MATERIALS AND METHODS

Preparation of the Cell Culture

To determine the cytotoxicity of propolis for human cells, HEK-293 (human embryonic kidney cells) cell line was used. The cells were cultured in RPMI-1640 medium with 10% (w/v) fetal calf serum. The cells was incubated at 37°C in air with 5% carbon dioxide.

To solve propolis samples, DMSO (Sigma, USA) was selected. Stock solutions of propolis samples were solved in the non-toxic DMSO concentration. For this purpose DMSO concentration of 1% was used. The concentrations of propolis were 10, 25, 50, 100, 200 and 400 µg/ml.

In order to test the effect of the propolis samples on HEK-293 cells, 1×10^5 cells were inoculated to the each well of flat-bottomed plates. Then, cultured for 8 hours at 30°C, and the cells were allowed to grow for an additional 96 hours. All experiments were performed in triplicate. Propolis components were determined to be non-toxic up to 150 µg/ml on HEK-293 cells. Therefore, antiproliferative activity studies were carried out in lower than 200 µg/ml concentration.

MTT Antiproliferative Assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used to determine the effects of propolis samples on cell proliferation in the A549, HeLa and A498 cell lines. Briefly, $1x10^5$ cells/well were evenly distributed to the flat bottomed plates and the plates incubated overnight at 37 °C. Then, each cancer cell

lines were treated with propolis at concentrations of 5, 10, 20, 40, 60, 80, 100, 150, 200 and 250 µg/ml incubated for 48 hours. Later, the medium in each well was replaced with 20 µl MTT (5 mg/ml in phosphate buffer saline) and incubated at 37 °C for 4 hours. The formazan crystals was dissolved in 100 µl dimethyl sulfoxide and it was read with a microplate reader at a 450 nm wavelength**.** The 50% inhibitory concentration (IC_{50}) was calculated as the concentration of test components that achieved a 50% inhibition of cell viability.

Antiproliferation Tests

To determine the antiproliferative activity of propolis on cancer cell lines, 1×10^5 cells were inoculated into each well of flat-bottomed plates, and were cultured for 8 hrs at 28°C. Later, the inoculated plates were incubated at 37°C up to 96 hours. Propolis components were diluted, whereupon decreasing amounts (100, 80, 60, 40, 20, 10 and 5 µg/ml) were placed per well. It was evaluated at the incubation of the 24, 48, 72 and 96 hours. All experiments were performed in triplicate, and the results were expressed as log number cells per milliliter on the percentage of growth inhibition.

Antiproliferative effects of propolis components were evaluated by viable cell counts. Viable cell count were determined by a conventional hemocytometer and the trypan blue exclusion (Burlesson *et al.*, 1992).

Statistical Analysis

The statistical analyses were performed using Student t-test. The p value <0.05 was considered significant. All statistics in the present study were done using SPSS for Windows, version 17.5 SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

In previous studies it has been demonstrated that some propolis components such as CAPE, caffeic acid, caffeic acid phenylethyl ester have anti-proliferative effects against various cancer types (Chen *et al.*, 1996; Coleman *et al.*, 2008; Counter *et al.*, 1998; Gunduz *et al.*, 2005; Motomura *et al.*, 2008; Motomura *et al.*, 2008). The components of propolis such as benzoic acid, phenylethyl alcohol, 9-octadecenoic acid are the most abundant components in Hatay propolis samples (Duran *et al.*, 2011).

In this study we analyzed the components of propolis samples collected from our region. In addition, we investigated the anti-proliferative properties of rich propolis components such as benzoic acid, phenylethyl alcohol, 9-octadecenoic acid.

We have shown that these three propolis components (benzoic acid, phenylethyl alcohol, 9-octadecenoic acid) have to be remarkable antiproliferative effects against human lung adenocarcinoma (A549), human cervical carcinoma (HeLa) and human renal carcinoma (A498) cells (Figure 1-3).

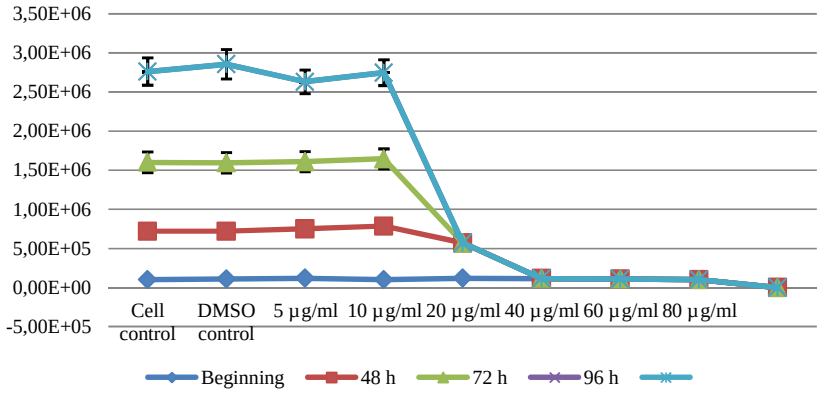

Figure 1. Effects of benzoic acid, phenylethyl alcohol, 9-octadecenoic acid on the proliferation of A549 cells

In the experiments, non-cytotoxic concentrations of the propolis samples was found to be up to 100 µg/ml. Antiproliferative results were given in Figure 1-3. As seen in figures 1 and 2, while propolis components inhibited the A549 and HeLa cell at the concentraton of 20 µg/ml, A-498 cells was found to be inhibited at the concentration of 40 µg/ml propolis (Figure 3).

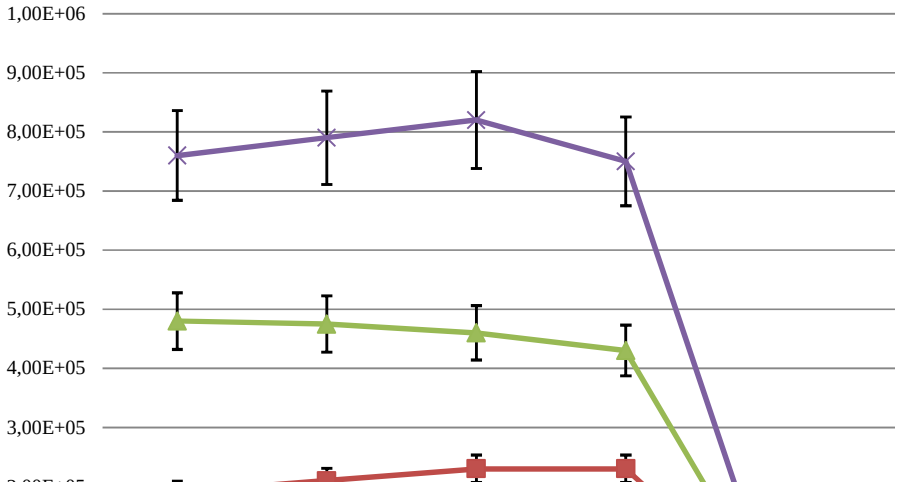

Figure 2. Effects of benzoic acid, phenylethyl alcohol, 9-octadecenoic acid on the proliferation of HeLa cells

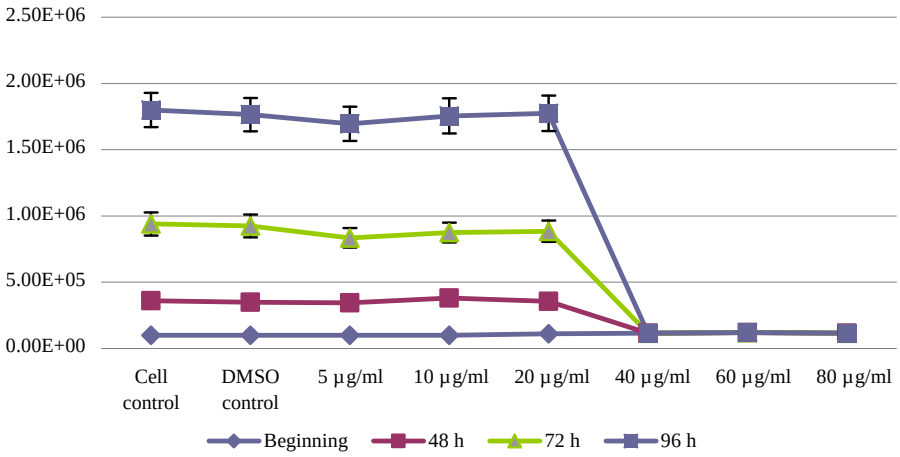

Figure 3. Effects of benzoic acid, phenylethyl alcohol, 9-octadecenoic acid on the proliferation of A-498 cells

CONCLUSIONS

In conclusion, three important components (benzoic acid, phenylethyl alcohol, 9-octadecenoic acid) of propolis samples were found to show an anticancer activity against human lung adenocarcinoma, human cervical carcinoma and human renal carcinoma cells. These components may be a potent drug active substance for these kinds of cancer types. However further researches such as in vivo studies are needed to clarify the effectiveness of these components.

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NANO-TIO_2 HYDROSOL/COLLAGEN-CHITOSAN COMPOSITE SCAFFOLD FOR WOUND REPAIRING

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Collagen-Chitosan (COL-CS) porous scaffolds have been widely used as a dermal equivalent to induce fibroblasts infiltration and dermal regeneration. To improve the anti-bacterial properties, nano-TiO_2 hydrosol was introduced into COL-CS scaffolds. The TiO_2/COL-CS composites scaffolds were prepared through freeze-drying. Their possible application in wound healing was tested in vitro. Scanning electron microscopy (SEM) was employed to study the micro-structure of the scaffolds. The swelling property and porosity of the composite were investigated. The results showed that the scaffold may provide good permeability and humid environment for wound healing. The SEM images of the scaffold showed a porous feature which would be favorable for cell migration and rapid ingrowth of host fibroblasts and endothelial cells. The nano-TiO_2/COL-CS composite scaffolds could be a promising candidate for wound healing dressing.

Keywords: collagen; chitosan; wound healing; nano-TiO_2

INTRODUCTION

Skin substitution with skin replacement materials can be a lifesaving measure in the treatment of acute burns and scald (Dainiak, 2010). Biologically active scaffolds used in skin-replacement play a critical role in wound healing. Their function is to provide mechanical support; to direct the growth of cells, either seeded onto the scaffold or migrating from surrounding tissue; and to enhance angiogenesis (MacNeil, 2007).

Collagen is the most abundant ECM constituent of nature. Dermis and scaffolds made from collagen exhibit weak antigenicity, biodegradability, and superior biocompatibility (haemostatic and cell-binding properties) (Lee, 2001; Chen, 2005). Chitosan, a natural-based polysaccharide, is a versatile biopolymer usually derived by partial deacetylation of chitin. It has been proved to possess variety of fascinating biological properties such as biodegradability, biocompatibility, non-antigenicity, nontoxicity, antimicrobial activity and hemostasis, making it a potential candidate in the wound management area (Ong, 2008; Ishihara, 2002; Anilkumar, 2011).

During the wound healing process, an appropriate dressing to cover the wound is required to avoid loss of heat and reduce the wound infection rate. However, most commercially available dressings do not have active antibacterial capabilities, which results in increased infection rates and ulcer formation. Moreover, the wound dressing must be frequently replaced to avoid inducing a secondary injury that would inevitably prolong the healing time (Yu, 2014). In recent years, various bacterial infection become more serious, inhibiting the growth and reproduction of bacteria has also become increasingly important (Zhu, 2011). TiO_2 nano-particles have been widely applied in biomedical and bioengineering fields, owing to their strong oxidizing properties, chemical inertness, anti-bacterial and non toxicity (Yan, 2011).

The main purpose of this study is to develop a TiO_2/COL-CS composite scaffold for wound repairing. Nanometer titanium dioxide was prepared by sol-gel method (Mao, 2005; Venkatachalam, 2007; Macwan, 2011). COL-CS composite scaffold

supplemented with different ratios of TiO_2 was prepared by freeze-drying technique and characterized by using SEM.

MATERIALS AND METHODS

Materials

Chitosan (>90% deacetylation) was from Jinhu Crust Product Co., LTD., China. Tetrabutyl titanate (M=340.36) was made by Tianjin Hedonghongyan reagent factory. Fresh pigskin was commercially available. All other reagents used were of analytical grade.

Collagen Extraction

Type I collagen used here was extracted from fresh pigskin. The extraction was performed according to the procedure of Feng Wen-po et al (2010). The purified collagen was freeze-dried and stored at − 20°C for subsequent use.

Nano-TiO_2 Hydrosol Preparation

Tetrabutyl titanate (TBT) was used as a raw material. The molar ratio of TBT, distilled water, ethanol, hydrochloric acid was 1:200:16:0.3. Firstly, hydrochloric acid (0.22g) was dissolved in distilled water(73.52g) to yield solution A. TBT (6.95g) was added to anhydrous alcohol (15.05g) while stirring and the pH was adjusted to 3.0 by HCl, yielding solution B. Solution B was added dropwisely to solution A while vigorously stirring for 2 h at 40°C.

TiO_2/COL-CS Composite Scaffold Fabrication

The blend of collagen and chitosan was prepared by mixing the solutions of collagen (0.5%) and chitosan (0.5%) in 0.5 M acetic acid at the ratio of 1:1. Similarly nano-TiO_2 blended with COL-CS composite was prepared by mixing different amounts of nano-TiO_2 hydrosol with the COL-CS solution to reach the concentrations of 0, 1, 3, 5, and 7% (w/w), respectively. The mixtures were then poured into a six-well culture plates and frozen overnight at − 30°C, after lyophilization dried for 24h. All the samples were stored at − 20°C for subsequent use.

Micro Structure of TiO_2/COL-CS Composite Scaffold

The cross sectional morphology of the TiO_2/COL-CS composite scaffold was characterized by a SEM (Quanta200). The cross sections were prepared by cryogenically fracturing the films in liquid nitrogen and then coated with aurum before observation.

PBS Solution Adsorption of TiO_2/COL-CS Composite Scaffold

The water absorption and equilibrium water content of TiO_2/COL-CS composite scaffold were determined. The samples were put in pH=7.4 phosphate buffered saline (PBS) at 35.0 ± 0.5^0C. The water absorption and equilibrium water content of the samples in the PBS media were calculated according equations (1) and (2) (Yan, 2011):

$$A(\%) = \left(\frac{W_S - W_0}{W_0} \right) \times 100 \quad (1)$$

$$B(\%) = \left(\frac{W_S - W_0}{W_S} \right) \times 100 \quad (2)$$

where A=Water adsorption, B=Equilibrium water content, W_s is the weight of swollen sample and W_0 is the initial sample weight. Three tries were done and the average data was reported.

Porosity Studies of TiO_2/COL-CS Composite Scaffold

The initial weights of scaffolds with the same size were examined by analytic balance, and then soaked in dehydrated alcohol at room temperature for 24 h. After the excess ethanol was carefully wiped from the surface, the scaffolds were weighted again. The interval porosity of the samples were calculated according to equation (3):

$$C(\%) = \left(\frac{W_t - W_0}{\rho_0 V_0} \right) \times 100 \quad (3)$$

where C=Porosity, W_0 is the initial weight of the scaffold, W_t is the swollen weight, V_0 is the initial volume of the scaffold, and ρ_0 is the density of dehydrated alcohol (0.79 g/ml).

RESULTS AND DISCUSSION

Micro Structure of TiO_2/COL-CS Composite Scaffold

The microstructure such as pore size and its distribution, porosity as well as pore shape prominently affect the cell attachment, proliferation, function and migration in tissue engineering. The cross section of scaffolds of COL-CS and COL-CS-TiO_2 (1,3,5,7%) was studied by SEM and the images are shown in Figure 1.

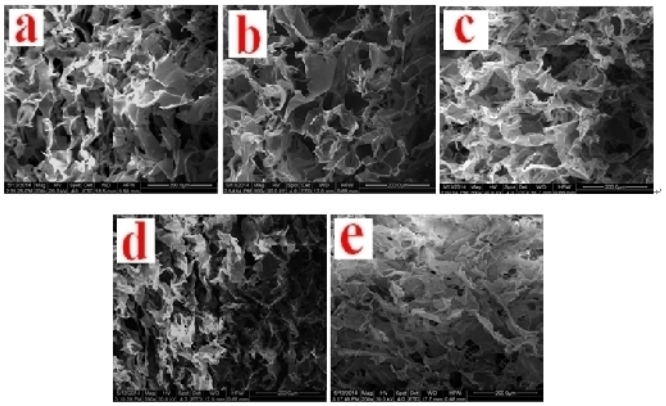

Figure 1. SEM images of cross sections of COL-CS scaffolds with various concentration of nano-TiO_2 (0,1,3, 5 and 7% represented as a, b, c, d, e, respectively)

From Figure 1, it was shown that the TiO_2/COL-CS has good pore size distribution, and there is a relation between the pores and aperture. The scaffold formed lamellar structure which may be conducive to cell attachment, proliferation, function and migration. No obvious change was found in the three-dimensional morphology of scaffold with the addition of TiO_2. With increasing the TiO_2 content, the pore structure becomes uniform and the density of the aperture also increases. At the TiO_2 content of up to 5%, the pore size and its distribution are better than others. Up to 7%, the aperture decreases and lamella structure has a portion of stack, which is not conducive to cell adhesion, migration and growth.

PBS Solution Adsorption of TiO_2/COL-CS Composite Scaffold

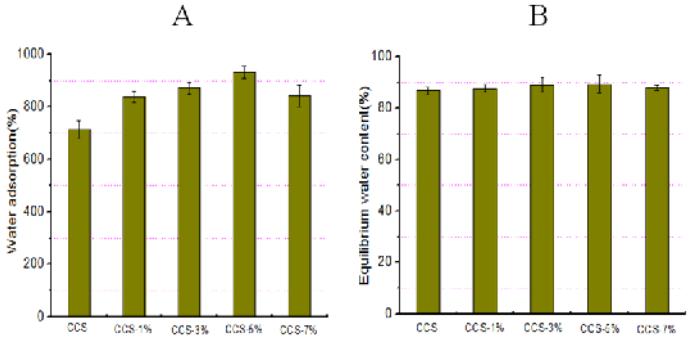

Figure 2. Water adsorption and equilibrium water content of COL-CS (CCS) scaffold containing different concentration of nano-TiO_2 (0,1,3,5,7%)

High water keeping ability of scaffold is favorable for cell adhesion and growth. It would make it easy to transport nutrients from the scaffold to cells in the culture system. In wound healing, it would prevent the fluid loss from the body while applied on the wound. The water keeping ability of the TiO_2/COL-CS scaffold could be attributed to both of their hydrophilicity and maintenance of their three-dimensional structure. From Figure 2, after being immersed in the PBS media for 24h, the water adsorption reach up to 715%, and the water adsorption gradually increased with increasing the nano-TiO_2 concentration from 1% to 5%, this may be due to the hydrophilic property of nano-TiO_2 that was included in the COL-CS composite scaffold. When reach out to 7%, little decrease was found in the water adsorption. The nanometer titanium dioxide is easy to reunite, which decreases its hydrogen forming ability. As a result, the water adsorption of the scaffold decreased.

Porosity of TiO_2/COL-CS Composite Scaffold

From Figure 3, the porosity of the COL-CS scaffold was about 82.07%, and the porosity of the TiO_2/COL-CS scaffold with different nano-TiO_2 concentrations was about 88.21%, 92.76%, 95.02%, 88.23%. With the addition of TiO_2, the porosity of the scaffold increases gradually to reach the maximum at 5%. Pore size and its uniformity, porosity determine the mechanical properties and the water retention. Thus the scaffold can easily absorb the culture medium to facilitate the cells for migration, adherence and proliferation in the porous structure.

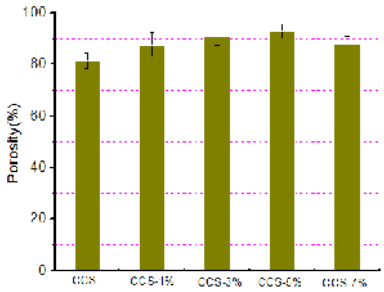

Figure 3. Porosity of COL-CS (CCS) scaffold with different concentration of nano-TiO_2 (0,1,3,5,7%)

CONCLUSIONS

Porous TiO_2/COL-CS scaffold was prepared by freeze-drying. Nano-TiO_2 affects the water adsorption and the porosity of the scaffold. No significant differences were found in the morphology. The SEM images of the scaffold showed a porous feature, favorable for cell migration and rapid growth of host fibroblasts and endothelial cells. The water adsorption of the TiO_2/COL-CS scaffold reached the maximum of about 1200%. The porosity of all COL-CS scaffold is more than 80%, with the maximum of about 93%, The scaffold may provide good permeability and humid environment for wound healing. The nano-TiO_2/COL-CS composite scaffolds could be a promising candidate for wound healing dressing.

Acknowledgements

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IBUPROFEN-COLLAGEN SPONGES FOR WOUND HEALING

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The aim of this paper was to design and characterize some collagen-ibuprofen sponges, potentially usable in the treatment of inflammation associated to cutaneous lesions and subsequently to the post-lession pain, the *in vitro* drug release evaluation and *in vivo* wound healing test. The collagenic matrices, obtained by collagen-ibuprofen hydrogels lyophilization, uncrosslinked and crosslinked with glutaraldehyde, were characterized by morphological (water absorption), goniometric (contact angle), and biological analysis (enzymatic biodegradation). *In vitro* ibuprofen release was performed with a transdermal sandwich device adapted to a dissolution apparatus. The *in vivo* wound healing test was determined using experimental animals (small rodents) with lesions induced with a special metallic device. Similar release profiles were obtained for the matrices with different composition and the kinetic mechanism was set. The matrices swelling capacity, surface wettability and resistance at enzymatic degradation are in accordance with kinetic results. The animal groups treated with collagen sponges and drug-loaded collagen sponges indicated a much faster wound healing effect compared to a non-treated control group. The study results showed that physical-chemical, biological and biopharmaceutical characteristics, and *in vivo* sponges efficiency are strongly influenced by their composition, the determination of the optimum formulation parameters for the new drug supports being possible by modulating the matrices composition.

Keywords: collagen sponges, ibuprofen delivery, anti-inflammatory effect.

INTRODUCTION

An important aspect to be considered in the healing of acute or chronic lesions with low, moderate or high exudate is the control of the post-lesion inflammatory response and implicitely of the associated pain which is a major discomfort for the patients, influencing markedly their life quality (Arapoglou *et al.*, 2011; Shemesh and Zilberman, 2014). Among the pain-killing agents, a special attention is given to the non-steroidal anti-inflammatory drugs (NSAIDs) which generally possess analgesic, anti-inflammatory and antipyretic properties. But, due to the side effects induced by these drugs, especially at gastro-intestinal level, an alternative to the oral administration route was studied (Albu *et al.*, 2012; Komatsu and Sakurada, 2012). Thus, the healing of cutaneous wounds of different etiologies may be optimized and supported through a rational approach based on the use of topical drug delivery systems with biopolymeric supports (Ghica *et al.*, 2012). NSAIDs release directly at lesion level, in a controlled manner to maintain a sufficient and effective drug concentration, is essential to combat the inflammation and subsequently the pain that occur during the healing process.

Collagen, a natural biopolymer, processed as spongious form is useful in the healing of different cutaneous wounds, as it absorbs large exudate quantities, preserves a moist environment, and enhances the formation of new granulation and epithelium tissue (Albu *et al.*, 2011; Ghica *et al.*, 2013; Lu *et al.*, 2014).

The goal of the present work was the design and characterization of some collagen-based spongious matrices ibuprofen-loaded as a NSAIDs drug model, the *in vitro* drug release evaluation and *in vivo* wound healing test using experimental animals (Aoyagi *et al.*, 2007; Sung *et al.*, 2010; Ramli and Wong, 2011; Xingang *et al.*, 2013).

MATERIALS AND METHODS

Materials

Type I fibrillar collagen (C) gel having a concentration of 2.11% (w/w) and pH 2.5 was extracted from calf hide as previously described (Albu 2011). Ibuprofen (IBU) was purchased from ICN Biomedicals Inc. (USA) and glutaraldehyde (GA) was supplied from Sigma-Aldrich (Germany). Sodium hydroxide, monobasic potassium phosphate and disodium hydrogen phosphate were obtained from Merck (Germany). All the reagents used were of analytical grade and the water was distilled.

Preparation of Collagen Sponges and Ibuprofen-Loaded Collagen Sponges

Collagen hydrogels were obtained by adjusting initial collagen gel to 1.0% and 1.2% and 7.4 pH, adding 0.5% ibuprofen, reported to the amount of collagen gel, cross-linked with glutaraldehyde. The composition of the designed collagen formulations is presented in Table 1.

Table 1. Composition of collagen-based hydrogels

Hydrogel	Collagen, C (g%)	Glutaraldehyde, GA (g%)	Ibuprofen, IBU (g%)
G1	1.0	0	0
G2	1.2	0	0
G3	1.0	0.0025	0
G4	1.2	0.0025	0
G5	1.0	0	0.5
G6	1.2	0	0.5
G7	1.0	0.0025	0.5
G8	1.2	0.0025	0.5

*the amounts of C, IBU and GA are reported to 100g hydrogel

The obtained hydrogels were then lyophilized using the Delta LSC 2-24 Martin Christ lyophilizer (Germany) using the method previously described (Lungu *et al.*, 2011) and the corresponding M1-M8 collagen sponges were obtained.

Water Absorption and Enzymatic Biodegradability Studies

The obtained collagen sponges were assessed by water absorption and enzymatic biodegradation according with the methods previously described (Lungu *et al.*, 2013).

Contact Angles Determination

The wettability capacity evaluation of sponges surface was quantified by the contact angle measurement, using a KSV Scientific Instrument (Finland) equipped with a video camera for images capturing and a CAM-101 software for data acquisition. For the determination of contact angle the pendant drop dynamic method was applied, and for its computing the Young-Laplace equation which describe the drop shape was used.

***In vitro* Ibuprofen Release Analysis**

Ibuprofen *in vitro* release from collagen spongious forms was performed using a transdermal sandwich device adapted to a dissolution apparatus as previously reported in our studies (Ghica *et al.*, 2013). Briefly, ibuprofen-loaded collagen sponge samples were placed into the release vessels, using as the release medium the phosphate buffer of 7.4 pH, maintained at 37°C. The absorbance of the solution extracted at predetermined period of time was spectrophotometrically determined at 264.4 nm (Perkin-Elmer UV-Vis spectrophotometer), using the calibration curve ($A_{1\%}^{1cm} = 19$, R = 0.9999). The Power law equation was applied for the ibuprofen release kinetics assessment from sponges as previously described (Ghica *et al.*, 2013).

***In Vivo* Wound Healing Test**

Experiments were performed on Wistar rats weighing 230±10g purchased from The Animal Biobase of The "Carol Davila" University of Medicine and Pharmacy, Bucharest. All animals used in the study were kept in standard laboratory conditions. They received water *ad libitum* and were not fed for 12h before the experiment. All experiments were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from February 2nd, 2002. The rats were distributed in 9 groups of 3 individuals each and were anesthetized with ether ethylic. The hair was removed from the dorsal area of the animal and an experimental wound was induced using a special metallic device of 1cm diameter. The device was heated in boiling water and applied on the shaved dorsal area for 25 seconds. The severe burns measuring 1cm diameter were treated with collagen sponges as it follows: Group 1 to Group 4 with M1-M4 collagen sponges, Group 5 to Group 8 with M5-M8 ibuprofen-loaded collagen sponges, and Group 9 is control group, no dressing applied on the wounded area. The experimental wounds were sterilized and the collagen scaffolds were applied and fixed with a plaster. The surface morphology of the wounds was recorded using a digital camera and the wound diameter was measured every two days for about 2 weeks. Any aspects of inflammation or infection of the wounds, as well as any modification on the animal health status were also monitored.

RESULTS AND DISCUSSION

The water absorption of spongious matrices was dependant on collagen / crosslinking agent concentration and drug content. Thus, the high concentrated matrices absorbed less water than the ones with 1% collagen (29% for M4 and 37% for M3) while the cross-linked sponges absorbed more water than the un-cross-linked ones (19% for M1 and 37% for M3). The drug content decreased the water absorption for all the samples with 1-2% biopolymer. Enzymatic degradation showed a good resistance to

collagenase for all the cross-linked samples. Moreover, samples M7 and M8 were degraded 38.85% and 33.79% respectively after 12 days.

The values recorded for the contact angle indicate a surface hydrophilicity both for collagen and drug-loaded sponges (45.55^o-88.21^o), which favors the wetting by the biologic fluids.

The released ibuprofen percent from M5-M8 sponges during 8 hours of experiment was plotted against time and the kinetic patterns of the swellable systems un- and cross-linked with glutaraldehyde are shown in Figure 1. The released drug percent varied between 54.59% (M8) and 72.99% (M5) (Table 2). A biopolymer concentration increase determines a released ibuprofen percent decrease of 1.16 – 1.21 times while the cross-linking leads to a decrease of 1.11 – 1.15 times respectively.

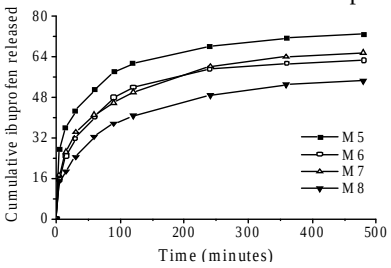


Figure 1. Cumulative release profiles of ibuprofen from M5-M8 collagen sponges as a function of time

The application of Power law model to the kinetic data indicated a non-Fickian drug diffusion mechanism, specific to the drug porous matrices for which various factors are involved: sponges wetting and swelling after contact with biological fluid and their transformation into gel, in the same time with drug diffusion through the gel formed, and eventual erosion of the polymeric gel matrix. The values recorded for the correlation coefficient (R) as well as the values obtained for the kinetic characteristics specific to the above model are listed in Table 2.

Table 2. Values for correlation coefficients and kinetic parameters characteristic for Power law model; released drug percent

Collagen sponges	Correlation coefficient	Kinetic constant ($1/min^n$)	Release exponent	Released percent (%)
M5	0.9942	0.219	0.202	72.99
M6	0.9859	0.135	0.260	62.67
M7	0.9948	0.137	0.262	65.81
M8	0.9947	0.098	0.285	54.59

After the treatment with the burning device, the affected skin area appeared as a white eschar with a hyperemic area on the periphery evolving in the following days to a full hyperemic area. The re-epithelialization process was higher in Group 7 followed by Groups 5, 6 and 8 (Figure 2). After the treatment with the collagen sponges, a small increase of the wound diameters was observed in Groups 1-4 and Group 9 in the first two days due to the inflammation of the local tissues. During the following days the wound diameter decreased in all treated groups and the re-epithelialization was faster compared to the control group which needed more than 25 days for a complete healing.

Collagen itself offers the advantage of a natural biomaterial with wound healing effect, explaining its own pharmacological action on the experimental animals. Also, in combination with ibuprofen a sinergic action occurs during lesion healing process.

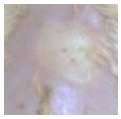
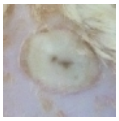
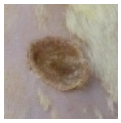
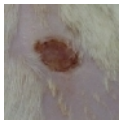
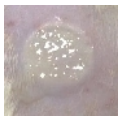
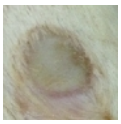
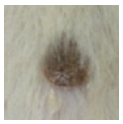
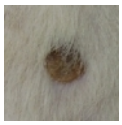
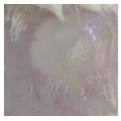
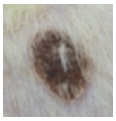
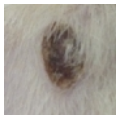
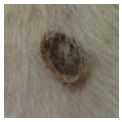
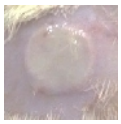
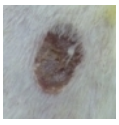
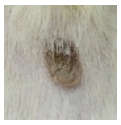
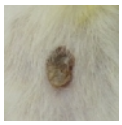
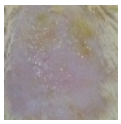
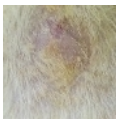
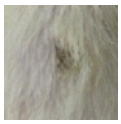
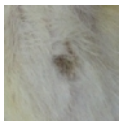
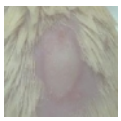
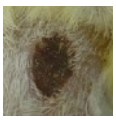
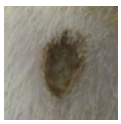
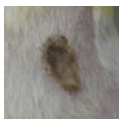
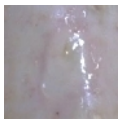
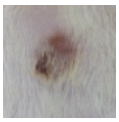
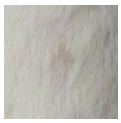
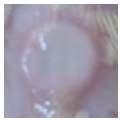
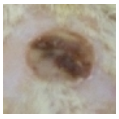
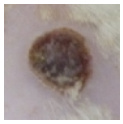
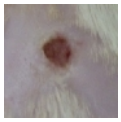
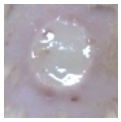
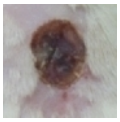
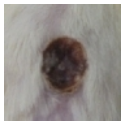
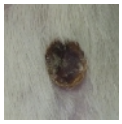
	DAY 1	DAY 4	DAY 8	DAY 12
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
Group 7				
Group 8				
Group 9				

Figure 2. The evolution of re-epithelialization process after the sponge application on wounds induced to the experimental animals

CONCLUSIONS

The study results showed that physical-chemical, biological and biopharmaceutical characteristics, and *in vivo* sponges efficiency are strongly influenced by their composition, the determination of the optimum formulation parameters for the new drug supports being possible by modulating the matrices composition. The ibuprofen-loaded sponge cross-linked with glutaraldehide and with 1% collagen (M7) could be potentially usable in the treatment of inflammation associated to cutaneous lesions and subsequently to the post-lession pain.

Acknowledgements

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PREPARATION AND CONTROLLED DRUG RELEASE OF SODIUM ALGINATE/MCC HYDROGEL BEADS

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In order to enhance the drug entrapment efficiency and to improve the swelling behaviors of drug delivery system, sodium alginate (SA)/microcrystalline cellulose (MCC) hydrogel beads were prepared with metformin hydrochloride (MH) as model drug. The hydrogel beads were crosslinked in Ca^{2+}, and the effects of MCC content and the crosslinking time on the properties of the beads were investigated. The chemical structure and morphology of the hydrogel beads were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM), respectively. The swelling and pH-sensitivity of the hydrogel beads were studied in both simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Results indicated that the MCC content of 20 wt% had the highest drug loading capacity and the lowest cumulative release percentage in 30 min in SIF. After prolonging the crosslinking time from 30min to 180min, cumulative release percentages of the beads with the MCC content of 60 wt % decreased by 30% in SIF.

Keywords: sodium alginate, microcrystalline cellulose, drug release.

INTRODUCTION

The ability of hydrogels to swell and regulate the release of encapsulated drugs by controlling cross-linking makes them attractive as materials in the controlled release (CR) of drugs (Graham and McNeil, 1984). Sodium alginate, widely used in food and pharmaceutical industries, is a water soluble salt of alginic acid, a naturally occurring non-toxic polysaccharide found in all species of brown algae (Rubio and Ghaly, 1994). Sodium alginate has a unique property of cross-linking in the presence of multivalent cations, such as calcium ions in aqueous media to form the 'egg box junctions' and insoluble calcium alginate (Smidsrod and Skjak, 1990). Calcium alginate beads can be produced by dropping a sodium alginate aqueous solution into a calcium chloride solution. Although this is a simple way of obtaining particulate drug carriers, drug loss and high permeability of pure SA hydrogel is considered a major limitation during drug-loaded beads preparation (Torre *et al.*, 1998). Hence, some researchers tried to circumvent this problem by preparing composite hydrogel beads such as SA/pectin (Liu and Krishnan, 1999), SA/chitosan (Anal and Stevens, 2005), SA/gelatin (Shinde and Nagarsenker, 2009) and even SA/PVA (Hua *et al.*, 2010).

In our study, we have tried to add a new member microcrystalline cellulose (MCC). It expected that the new kinds of hydrogel beads with improved structure and drug loading and release properties can be obtained by the combination of Gel, SA and MCC.

The aim of this work was to formulate a dual crosslinked SA/MCC matrix that effectively prolongs drug release, which is obtained by changing different proportion of MCC content. The structure and morphologies were characterized and the swelling properties, pH-sensitivity and their drug release behaviors were studied using metformin hydrochloride (MH) as the model drug.

MATERIALS AND METHODS

Materials

Sodium alginate (SA, a viscosity of 0.035Pa·s in 2% aqueous solution at 25°C) was from Paini Chemical Reagent Factory (Henan Province, China). Calcium chloride was purchased from Kermel Chemical Co. Ltd. (Tianjin, China). Microcrystalline cellulose was from Qufu Tianli Medical supplements Co. Ltd. (Shandong, China). Metformin hydrochloride was from Accela ChemBio Co. Ltd. (Shanghai, China). The simulated gastric fluid (SGF, pH 1.2) composed of 0.085M HCl, the simulated intestinal fluid (SIF, pH 7.4) composed of 0.05M potassium phosphate and 0.0395M sodium hydroxide. All the reagents used in this study were of analytical grade and used as received.

Preparation of SA/MCC Beads

Drug-loaded SA/MCC beads were prepared by droplet extrusion/precipitation of a sodium alginate/MCC aqueous mixture solution with metformin hydrochloride. The influence of ratios of alginate to MCC the drug loaded beads SM-0, SM-20, SM-40, SM-60 and SM-80 (0 wt.%, 20 wt.%, 40 wt.%, 60 wt.% and 80 wt.% of MCC, respectively) was investigated. The mixture was added into a gently stirred (100 rpm) 2.0% (w/v) calcium chloride aqueous using a syringe with the form of droplets, and the stirring was kept for additional 30min. After that, SM-20 and SM-60 were chosen to prolong the crosslinking time to 3hours and named SM-20-3h and SM-60-3h, respectively. The crosslinked spherical and homogeneous SA/MCC beads were obtained, and washed with distilled water three times. The resultant beads were dried at 45^{o}C for 9h.

Characterization

FTIR spectra were obtained at room temperature using a Nicolet Impacta 400 spectrometer (Nicolet iS10, USA) in the range of 4000-400cm-1 using KBr pellets. Surface morphology of the dried beads was characterized before release testing, samples of the beads were sputter coated with gold in a vacuum evaporator, and photographed using a scanning electron microscope (JSM-7500F, Japan) using an accelerating voltage of 20 kV.

Swelling Studies

Two aqueous media were used in swelling and drug releasing measurement: HCl buffer solution (SGF, pH1.2) and phosphate buffer solutions (SIF, pH7.4). Weighted dry beads were placed at 37.0±0.5˚C in conical flasks containing 200mL of buffer solution and magnetically stirred at 50 rpm. Swelling ratio was determined by measuring periodically the weight of swollen beads after wiping off excess of liquid with a filter paper. The weight change of the beads with respect to time was determined as follows:

$$\text{Swelling ratio}(\%) = \left(\frac{W_t - W_0}{W_0} \right) \times 100 \quad (1)$$

where W_t is the weight of the beads at time t; W_0 is the initial weight of beads.

In vitro Drug Release Profiles

The in vitro drug release tests were carried out using the magnetic mixer, the rotor was rotated at 50 rpm and 37.0±0.5˚C. The dissolution media used were PBS buffer at pH 7.4 and HCl buffer at pH 1.2. Weighted beads added to 200 mL dissolution medium. Samples (5 mL) were collected and replaced with the same fresh medium at various time intervals. The amount of drug released was analyzed spectrophotometrically at 230nm (UV-2550, Shimadzu, Japan). The UV standard absorbance curve for metformin hydrochloride was established in different buffer, and the UV absorbance obeyed the Beer's law in the concentration range from 1.2×10^{-5}-6×10^{-5}mol/L.

RESULTS AND DISCUSSION

Fig. 1 shows the wet and dried drug-loaded SA/MCC beads, generally, the beads are perfectly spherical and have a smooth surface, with a meas diameter of approximately 3.0 mm before oven drying. As expected, increasing of the MCC content leads to shape change of beads due to the viscosity increase of SA/MCC suspension and its filling effect. During oven drying, the initial spherical and oval shapes are lost and the particle shape changes significantly with MCC encapsulation. In addition, the beads change from transparent to white and the diameter decreases as the MCC content increasing.

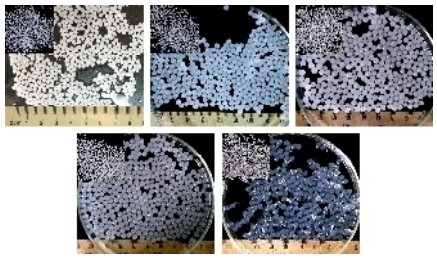

Figure 1. Photographs of wet and dry (upper left corner) with different proportions of SA/MCC drug-loading beads: (a) SM-80, (b) SM-60, (c) SM-40, (d) SM-20 and (e) SM-0

Fig. 2 shows the SEM images of sample SM-60 and SM-0 beads after dried by oven at 60^0C for 6h. The inclusion of MCC in the matrix creates beads with a rough surface and denser morphology (Fig. 2a, b). Fig. 2d reveals cracks caused by partial collapsing of the polymer network during dehydration. The results indicate that the addition of MCC caused the decrease of water evaporation from the beads during the drying period, then the collapse of polymers are less severe.

FTIR spectra of crosslinked SA/MCC beads and MCC were analyzed (Fig. 3). FTIR spectrum of beads showed the bands around 3412, 1636 and 1431 cm^{-1}, which was due to the stretching of −OH, asymmetric and symmetric stretching of -COO-. Compared with MCC, the absorption band of SM-60 around 3412 cm^{-1} shifted to a higher wavenumber at 3437 cm^{-1}, suggesting a possible break of hydrogen bonding intermolecular and intramolecular after the crosslinking. 1151 cm^{-1} was the asymmetric stretching of C-O-C from MCC. In order to recognize the possible effect of crosslinking scheme on drug release rate, a swelling study was conducted in advance of in vitro drug release study. Plots of dynamic swelling of beads in SGF (pH 1.2) and SIF (pH 7.4) buffer are given in Fig. 4.

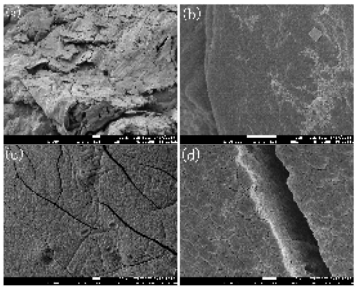

Figure 2. SEM micrographs of SM-60 (a, b) and SM-0 (c, d)

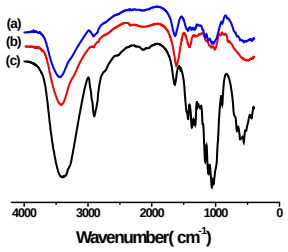

Figure 3. FTIR spectra of (a) SM-60, (b) SM-0 and (c) MCC

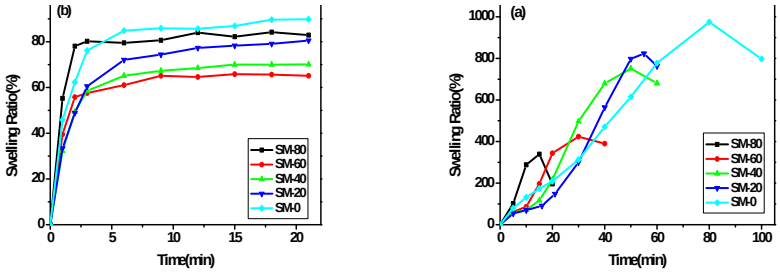

Figure 4. Swelling Ratio of dried beads made by different proportions MCC in SGF (a) and in SIF (b)

In the acidic environment of SGF, the SA/MCC beads began to swell immediately and reached the swelling equilibrium in 10min. As the MCC amount increased, the maximum swelling ratio at equilibrium decreased and then had a rise. It is obvious that in the neutral environment, maximum swelling time for beads was ranged from 15min and 80min, and the swelling ratio at equilibrium was 340% and 975%, respectively. The SA/MCC beads recovered their initial production spherical shape, and start to erode. At the neutral pH values, the affinity of phosphate present in PBS to calcium is higher than that of SA, and results in the breakage of Ca-SA beads. Besides this chelating action of the phosphate ions, the dissolving out of MCC made the phosphate ions infiltrated into the beads more quickly and improved the chelating action.

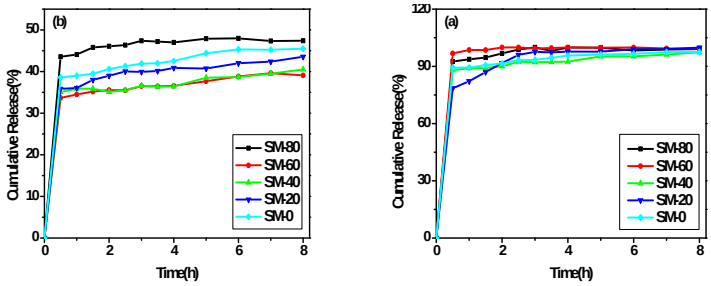

Figure 5. Release profiles of drug-loaded hydrogel beads of different proportions MCC in SGF (a) and in SIF (b)

Swelling behaviour of beads indicates the speed and easiness of a liquid to penetrate the alginate/MCC matrix, as a necessary step for drug release, whereas release tests show the evolution of bead structure during drug release. To study the release profiles of entrapped MH, dried test samples were immersed in SGF (pH 1.2) and SIF (pH 7.4) for 8h at 37^0C, respectively. In both buffer solutions, SM-80 drug release is rapid and most dissolution is attained within 3h. The MH adhered on the surface of the beads began to be dissolved and diffused into the buffer at the initially stage and showed quite brust release. Associated with the swelling behavior, MH total release from SM-60 was 40% in 8h, which was much lower than other formations (Fig. 5a). Under neutral conditions, 100% of MH was released in 8h. Meanwhile, SM-20 present a more stable and prolonged release profile, but as the MCC content continue to increase, the cumulative release began to be enhanced.

The comparison of Fig. 5 (a) and (b) showed that the release properties were associated with the swelling behavior of the beads and the beads present significant pH-sensitivity. MCC interpenetrated in the internal network and filled in the fracture and pore, meanwhile it had a certain effect with the drug adsorption which can slow down the drug release. The drug releasing process is further enhanced by the presence of phosphate ion, which acts as a calcium sequestrant. So the addition of MCC could show a sustained drug release at a certain extent.

With the purpose of improving the stable of the beads to control the drug release in PBS, the influence of crosslinking time was studied. After prolonging the crosslinking time, the swelling ratio at equilibrium decreased in both beads, and the swelling rate increased (Fig. 6). In SIF, increasing the crosslinking time reduced the swelling ratio at equilibrium and swelling rate for SM-60. The swelling ratio at equilibrium of SM-20 crosslinked for 3 hours is higher than for 0.5 hours.

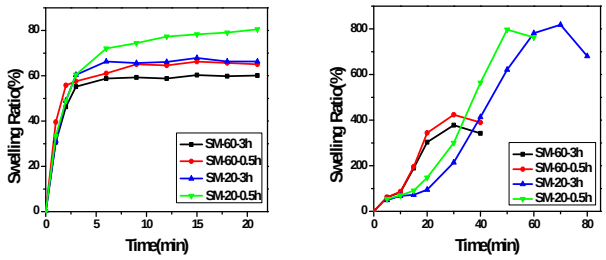

Figure 6. Swelling Ratio of dried beads made by different crosslinking time in SGF (a) and in SIF (b)

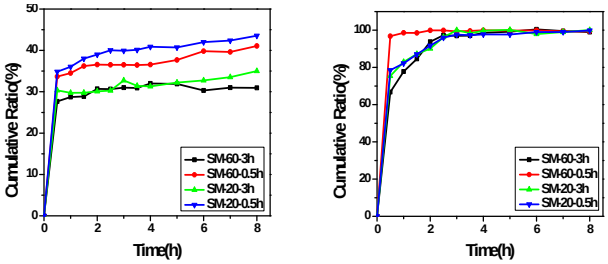

Figure 7. Release profiles of drug-loaded hydrogel beads of different crosslinking time in SGF (a) and in SIF (b)

In acidic medium, the beads presented a more stable network and was improved the release profile after extented the crosslinking time to 3h in Fig. 7. But in SIF, crosslinking time had no significant effect on SM-20. For SM-60, after prolong the crosslinking time, the cumulative release decreased from 97% to 66% in first half an hour and had obviously sustained release. MCC in the matrix absorbed water and swelled, preventing the further seeping of dissolution medium and thus helped to control the release of MH. But as the content increased, it leading to an easier water and phosphate ions (in SIF) permeation and swelling and, consequently, to faster drug release compared to the medium.

CONCLUSIONS

SA/MCC beads were successfully cross-linked by Ca^{2+} and used in the controlled release of metformin hydrochloride. All the materials used were environmentally friendly and the method developed was simple, fast and reproducible. A remarkable delay in the release of MH was observed for the beads with the MCC content of 20 wt% which had the lowest cumulative release percentage in first 30min in SIF and release slowly for 3h, but it had no significant effect on the swelling and release after prolong the crosslinking time for 3 hours. And SM-60 crosslinked for 3h presented obviously sustained release. Swelling and in vitro releasing behaviors demonstrate the formation of different kinds of SA/MCC matrix, it was observed that the release of MH was much higher in SIF compared to SGF, indicating that all the beads obtained were pH-sensitive and can be used as a release system for intestine specific drug delivery.

Acknowledgements

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A NOVEL COLLAGEN/HYDROXYAPATITE/MICROCRYSTALLINE CELLULOSE COMPOSITE FOR BONE TISSUE ENGINEERING

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A novel composite with collagen-hydroxyapatite and microcrystalline cellulose (MCC) was fabricated by biomimetic mineralization, sonication dispersion, dehydrothermal treatment (DHT), freeze-drying, and cold isostatic compaction technique. Fourier transform infrared spectroscope (FTIR), ultraviolet-visible spectrophotometer (UV), scanning electron microscope (SEM), and X-ray diffraction (XRD) were employed to analyze the structure and composition of the resultant composite. Swelling property, mechanical property and degradability of this novel composite were investigated. It is found that the composites prepared are hydrophilic and may swell in simulated body fluid. After being soaked in simulated body fluid for 40 days, it can still keep its original shape. Besides, the compressive strength of the composite is 99.05±1.74 MPa, reached the standards of artificial bone materials and is superior to some present used artificial bone materials. The workl may provide an efficient and alternative for bone tissue engineering.

Keywords: collagen; hydroxyapatite; microcrystalline cellulose

INTRODUCTION

With rapid development of biomedical engineering, chemical engineering, materials science and engineering, tissue engineering tends to flourish. This field is an interdisciplinary field that combines knowledge and technology of cells, biomaterials as well as suitable biochemical factors to fabricate artificial organs, tissues, or to regenerate damaged sites (Langer and Vacant, 1993). As the key branch of tissue engineering, bone tissue engineering potentially make alternative chances to bone substitudes instead of allograft, providing a framework for the cells to attach, proliferate, differentiate and form an extracellular matrix (ECM), and a carrier for cells, growth factors or other biofactors (Karen *et al.*, 2000; Molly, 2008).

Collagen and hydroxyapatite (HA) are the two major components of bone, seperately about 30% and 60% (Xu, 2011). Collagen is widely found in bone (Type I), cartilage (Type II) and blood vessel (Type III). It has excellent biocompatible biodegradable properties. It is also easily resorbed by the body and allows attachment to cells. Hydroxyapatite, with similar to chemical composition and morphology of bone apatite, can provide a good adhesion to the local tissue due to its surface chemistry and has been shown to enhance osteoblast proliferation and differentiation (Xie *et al.*, 2004). It also has high mechanical stiffness (Young's modulus) and a hard brittle surface. As such, collagen-hydroxyapatite composite has been inspiring interests of scientists and engineers (Du, 1978; Kikuch, 2001; Yu, 2004). However, there exist many disadvantages of collagen-hydroxyapatite composite that it can not meet requirements for artificial bone: (a) the discrepancy between degradation rate and osteogenesis progress; (b) the low surface activity; (c) the disagreement with nature human tissue about mechanical strength.

In order to overcome problems above, microcrystalline cellulose is introduced to collagen-hydroxyapatite composite and the structure and properties of the composite are investigated.

MATERIALS AND METHODS

Preparation of Collagen-Hydroxyapatite-Microcrystalline Cellulose (CHA-MCC) Composite

The CHA-MCC composites were synthesized by biomimetic mineralization method, sonication dispersion, dehydrothermal treatment (DHT), blending, freeze-drying and cold isostatic compaction technique. Briefy, collagen was dispersed in a 0.5 mol/L acetic acid aqueous solution. H_3PO_4 aqueous solution, $Ca(OH)_2$ aqueous solution were successively and gradually added into the reaction vessel with stirring frequently and starting materials for collagen-hydroxyapatite (Col-HA) composites were mixed at an 35/65 initial weight ratio by stoichiometry. The reaction temperature controlled using an oil bath and the pH of reaction solution controlled through NaOH aqueous solution by pH meter was set as 37°C and 7, respectively. Precipitates thus were obtained at the respective preparation temperature (37°C) for 48 h and were subsequently filtrated. After centrifugation, precipitates were freezed drying at −50°C under vacuum (GT2-Type-8, LYOTECH). The lyophilized samples were stabilized using dehydrothermal treatment (DHT). For DHT cross-linking, samples were placed in a vacuum oven at a temperature of 130°C for 24 h. The collagen-hydroxyapatite composites obtained were mixed in microcrystalline cellulose suspension with a weight ratio from 1/1 to 7/1. The mixture were ultrasonic dispersion for 48 h. Subsequently, the mixture were freezed dried at −50°C under vacuum (GT2-Type-8, LYOTECH). The product were cylindrically shaped upon a uniaxial pressure and consolidated further under an isostatic pressure of 200 MPa during 6 h.

Characterisation

FTIR spectra of CHA-MCC composites were obtained at room temperature using Bruker VERTEX 70 FTIR measurement (VERTEX 70, GER) in the range of 4000-400cm^{-1} using KBr pellets. And samples were subjected to X-ray diffraction (XD-3X, CHN) using CuKα radiation generated at 36 kV and 20 mA, the range of diffraction angle was 10°-70° 2θ. Micromorphology of samples were characterized using a scanning electron microscope (JSM-7100F, Japan) with an accelerating voltage of 3 kV.

The Atomic Ratio of Calcium to Phosphorous (Ca/P)

The calcium and phosphorous ratio of composites were determined according to the o-cresolphthalein complexon (OCPC) method and the molybdenum blue method respectively, combined with UV-Vis spectrometer (UV-2550, Japan).

$$C_{sample}(g/ml) = C * \left(\frac{A_{sample}}{A} \right)$$
$$\frac{Ca}{P} ratio = \frac{C_{sample(Ca)}}{C_{sample(P)}} \quad (1)$$

where C represents the calcium or phosphorous content of the respective standard solutions ($CaCO_3$ and KH_2PO_4), C_{sample} represents the composites'.

Porosity and Density Measurement

The density and porosity of scaffolds were measured by liquid displacement method using ethanol. Samples with a known weight (W_o) was immersed in a graduated

cylinder in a known volume of ethanol (V_o) for 24 h. The total volume of ethanol in the cylinder and ethanol -impregnated scaffold was recorded (V). The volume of scaffold was $V-V_0$ and the ethanol-impregnated scaffold removed from the cylinder was weighed (W). Each sample was measured in triplicate.

$$Porosity=\frac{W-W_0}{\rho_{ethanol}}\times\frac{1}{V-V_0}\times 100\% \quad (2)$$

$$\rho=\frac{W}{V-Vo} \quad (3)$$

where $\rho_{ethanol}$ is 0.789g/cm^3.

Swelling Tests

Swelling measurements were run in simulated body fluid (SBF, pH=7.4), at 37.0±0.5˚C with three parallel measurements using two different methods.

The first method was determined by measuring periodically the weight of swollen samples (W_{ws}) with hanging over until no dripping. In this case, we assessed the swelling ability of the composite structure with its pore system.In the second method, for a period of time, the same kind of swollen samples were removed the excess water in the pores by centrifugation within filter paper. Then, they were pressed between new filter papers to remove the residue water and then were weighed (W_{wm}). In this way the ability of the composite itself to absorb water was assessed.

$$Swellingratio(\%)=\left(\frac{W_w-W_d}{W_d}\right)\times 100\% \quad (4)$$

where W_w represents W_{ws} or W_{wm}.

Mechanical Tests

Mechanical testes were carried out with a constant pressure testing machine, YWE-300. The tests were carried out at a crosshead speed of 10 N/s. Each experiment was repeated three same samples and the average was reported.

RESULTS AND DISCUSSION

As shown in the FTIR spectra in Fig.1 , the appearance of peaks at 1058cm^{-1} and 964cm^{-1} is attributed to stretching mode of $PO4^{3-}$. And the distinctive peak in the region of 500~600cm^{-1} of hydroxyapatite, 568cm^{-1} is also observed. And the band at 1340cm^{-1}, ascribed to the wagging vibration of covalent bond between carboxylic acid groups of collagen and calcium ions of hydroxyapatite. Another red-shift of the band corresponding to C-O band, from 1658cm^{-1} in collagen to 1647cm^{-1} in CHA, can be observed. There are chemical reaction between collagen and hydroxyapatite. Rhee and other researchers have proved that carboxylic acid groups and carbonyl groups of collagen is the other nucleation site (Zhang, 2004; Zhai *et al.*, 2005). The additional -OH^- at 3343cm^{-1}, the C-H symmetrical stretching of methylene at 2900cm^{-1} of MCC and the C-O stretching within ether of cellulose molecule at 1112cm^{-1}, 1165cm^{-1} appear in FTIR spectrum of CHA-MCC composite. The band at 1340cm^{-1} of CHA, the peaks of C-O stretching vibration and -OH bending vibration between 1032cm^{-1} and 1430cm^{-1} disappear. It implies that there is chemical reaction between CHA and MCC owing to the increasing number of -OH from MCC.

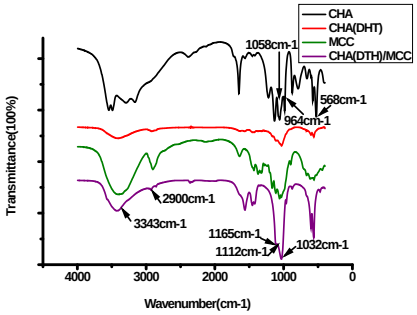

Figure 1. FTIR of collagen/hydroxyapatite/ microcrystalline cellulose composite

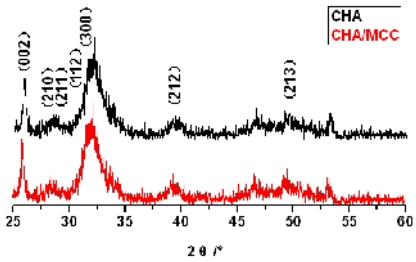

Figure 2. XRD of collagen/hydroxyapatite and collagen/hydroxyapatite/microcrystalline cellulose composite

XRD spectra of CHA and CHA/MCC composites are analyzed (Fig. 2). Diffraction patterns of samples show characteristic peaks of HA. The maxima peaks can be assigned to the HA included in the broad region of overlapping peaks corresponding to (002), (211), (112), (300) and (213), respectively. The peaks at 28.93° and 39.20° are also located between the positions expected for (210) peak and (210) peak of hydroxyapatite. The strong reflection intensity of (002) reflection indicates the occurrence of preferably orientated growth of apatite nanocrystals on organic collagen. X-ray diffraction indicates HA is poorly crystalline , which is similar to nature bone.

Futhermore, the average Ca/P ratio is 1.112±0.012, belonging to the range of stoichiometric hydroxyapatite (Ca/P=1.57±0.603) (Mathers and Czernuszka, 1991). A slight lower Ca/P ratio obtained in this study is probably due to the insolubility of some $CaCl_2$ in calcium solution through ionic interaction with negative charged groups. Besides, in reaction solution, the concentration of OH^- may be not enough to induce the complete reaction between calcium ion and phosphonium ion.

Figure 3 presents the morphology of CHA and CHA/MCC composites. These SEM photomicrographs show that the surface of composites are compact, microcrystalline cellulose disperses homogeneously within composites and combines with collagen-hydroxyapatite powder durably. By using ultrasonic dispersion, there are no agglomeration of microcrystalline cellulose or collagen-hydroxyapatite powder and delamination after isostatic compaction. And the composites form an irregular and interconnected porous network, with diameters on a nanoscale, about 150±50nm. The porosities observed in these composites suggest that they might be osteoinductive while their compositions should allow their eventual resorption.

All CHA/MCC composites with different ratios are found to be highly porous, retaining porosities above 70%. The relative density of the scaffolds was then calculated, as shown in table 1, approximately 0.7g/cm^3. Both porosity and density of composites meet needs of artificial bone, based on these of spongy bone (1.8-2g/cm^3, 5-30%) and compact bone (0.14-1.2g/cm^3, 30-90%) (Masoud *et al.*, 2010).

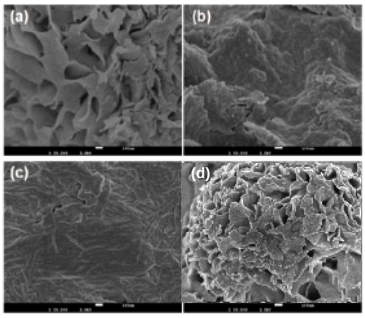

Figure 3. SEM micrographs of collagen/hydroxyapatite/microcrystalline cellulose composite: (a) CHA, (b) CHA(DHT), (c-d) CHA/MCC(1:1) (a-c: surface, d: fracture surface)

Table 1. Porosity of collagen/hydroxyapatite/microcrystalline cellulose composite, spongy and compact bone

Sample	Ratio of weight (collagen/hydroxyapatite to microcrystalline cellulose)	ρ(g/cm^3)	Porosity(%)
CHA/MCC	1:1	0.661±0.002	78±0.69
CHA/MCC	2:1	0.669±0.013	79±0.45
CHA/MCC	3:1	0.674±0.004	77±1.02
CHA/MCC	5:1	0.662±0.009	78±0.92
CHA/MCC	7:1	0.656±0.005	72±0.34

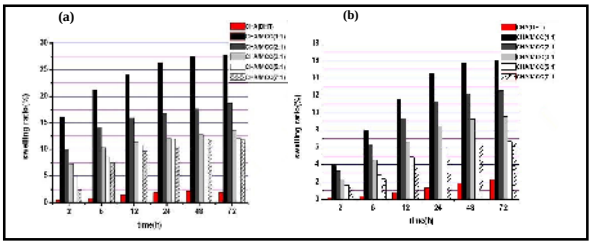

Figure 4. Swelling ratio of collagen/hydroxyapatite (DHT) composite and collagen/hydroxyapatite/microcrystalline cellulose composite(different weight ratios of collagen/hydroxyapatite to microcrystalline cellulose)

Figure 4(a) and 4(b) showed that the swelling properties were associated with the swelling ability of the composite structure with its pore system and the ability of the composite itself to absorb water. It is obvious that swelling equilibrium is reached at approximately 24 h. Swelling ratios of CHA/MCC materials are much higher than CHA, caused by the presence of MCC. The results clearly revealed that MCC has an important effect on the swelling behavior of CHA/MCC materials and makes a control of it.

The CHA composite cross-linked by DHT has a higher compressive strength compared with CHA samples. The increase content of MCC in composite gives rise to an increase in the stiffness of the material. The composite with a CHA/MCC weight ratio of 1/1 shows the highest compressive strength. And the compressive strength of all samples meet the requirement of artificial bone, being considerably higher than cancellous bone (1-20MPa) and near to compact bone (100-200MPa) (Gibson, 1985).

Table 2. Compressive strength of collagen/hydroxyapatite, collagen/hydroxyapatite (DHT) and collagen/hydroxyapatite/microcrystalline cellulose composite

Sample	Ratio of weight (collagen/hydroxyapatite to microcrystalline cellulose)	Compressive strength / MPa
CHA/MCC	1:1	99.05±1.74
CHA/MCC	2:1	73.56±2.05
CHA/MCC	3:1	58.94±1.49
CHA/MCC	5:1	50.23±1.92
CHA/MCC	7:1	48.92±1.85
CHA	—	36.50±1.37
CHA(DHT)	—	49.19±0.91

CONCLUSIONS

A novel composite with collagen-hydroxyapatite and microcrystalline cellulose (MCC) was fabricated by biomimetic mineralization, sonication dispersion, dehydrothermal treatment (DHT). Hydroxyapatite in composite is poorl crystalline, which is similar to nature bone. And material has an irregular and interconnected porous microstructure, with diameters on a nanoscale, about 150±50nm. retaining porosities above 70% and density approximately 0.7g/cm^3. CHA-MCC material shows high hydrophilicity, good swelling property and superior mechanical property. The rate of hydrophilicity, swelling and mechanical properties of the material can be modified by DHT and the microcrystalline cellulose concentration.

Acknowledgements

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A NOVEL BONE SCAFFOLD MATERIAL BASED ON COLLAGEN/HYDROXYAPATITE/GELATIN COMPOSITE

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A novel bone scaffold with collagen-hydroxyapatite (CHA) and gelatin (Gel) was fabricated using biomimetic mineralization method, combineed with sonication dispersion, crosslinking, blending and lyophilization. The structure and properties of the scaffold were investigated. By the introduction of gelatin into collagen-hydroxyapatite, the scaffold is easy to be shaped with three-dimensional porous microstructure. Both gelatin and crosslinking affect the mechnical properties. The novel collagen-hydroxyapatite-gelatin composite could be a candidate of scaffold materials for bone tissue engineering

Keywords: collagen, hydroxyapatite, gelatin

INTRODUCTION

Nowadays, there is a large demand in biomedical bone materials because of various bone defects or damage caused by bone fractures, osteoporosis, osteoarthritis and so on. Bone tissue engineering rise in response to the proper time and conditions. This field means that a suitable material is either uesd to induce bone remodeling from surrounding tissue, or as a carrier or template to implant bone cells or other growth factors for the healing of bone morbidity. Therefore, bone tissue engineering materials must be non-toxic, low-immunogenic, biocompatible, biodegradable, and have a corresponding matching of the mechanical properties. Materials commonly used are metal materials, ceramic materials, natural or synthetic polymer materials and composite materials, such as titanium, calcium phosphate, bioactive glasses, collagen (Col), gelatin (Gel), polylactic acid (PLA), polycaprolactone (PCL) and many other materials (Du *et al.*, 1998; Yamaguchi *et al.*, 2001; Liao *et al.*, 2004; Masoud *et al.*, 2010).

Collagen and hydroxyapatite thought as organic and inorganic constituents of natural bone is one of the most worthy of the topics studied, the biocompatible, biodegradable, osteoinductive characteristics of which have drawn the attention and favor of researchers (Kikuchi *et al.*, 2008; Lickorish *et al.*, 2004; Tsai *et al.*, 2008). However, poor plasticity, difficult molding and the mechanical strength of the collagen/hydroxyapatite composite materials having significant differences with natural bone are challenging issues limiting its clinical application. For the preparation of a more ideal bone repair material, the laboratory conducted a series of studies, this paper presents the fabrication of novel collagen/hydroxyapatite/gelatin composite materials. The materials are composed of a natural matrix made of collagen-hydroxyapatite embedding gelatin, to furnish the right mechanical properties to implant position, and collagen as well as hydroxyapatite could act as chemo-attractor for cells and tissue. Different concentrations of the components are made. And as biological cross-linkers, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide

hydrochloricde (EDC)/N-hydroxysuccinimide (NHS) was chosen to modify materials comparable to materials without crosslinking.

MATERIALS AND METHODS

Preparation of Collagen-Hydroxyapatite-Gelatin (CHA-Gel) Composite

CHA powder was prepared by biomimetic method, which has been reported previously (He *et al.*, 2013). Briefy, collagen was dispersed in a 0.5 mol/L acetic acid aqueous solution. H_3PO_4 aqueous solution, $Ca(OH)_2$ aqueous solution were successively and gradually added into the reaction vessel with stirring frequently and starting materials for collagen-hydroxyapatite (Col-HA) composites were mixed at an 35/65 initial weight ratio by stoichiometry. The reaction temperature controlled using an oil bath and the pH of reaction solution controlled through NaOH aqueous solution by pH meter was set as 37°C and 7, respectively. Precipitates thus were obtained at the respective preparation temperature (37°C) for 48 h and were subsequently filtrated. After centrifugation, precipitates were freezed drying at −50°C under vacuum (GT2-Type-8, LYOTECH).

The CHA-Gel composite was prepared by blending, solvent-casting technique, biological crosslinking treatment and lyophilization method. CHA powder was added into gelatin solution and ultrasonically mixed (final composition Gel to CHA 10%, 15%, and 20% (wt/wt %)). Then the mixture were poured into a self-made polyethylene mold and dried in air at room temperature. This was kept in a ethanol solution containing 50 mM EDS and 25 mM NHS at at room temperature for 4 h to complete the crosslinking. Subsequently, the mixture was washed with 0.1mM Na_2HPO_4 solution and finally lyophilize at −50°C under vacuum for 24 h (GT2-Type-8, LYOTECH). The composite prepared in this way is denoted as CHA-Gel composite.

Characterisation

FTIR spectra of CHA-Gel composites were obtained at room temperature using Bruker VERTEX 70 FTIR measurement (VERTEX 70, GER) in the range of 4000-400cm^{-1} using KBr pellets. And samples were subjected to X-ray diffraction (XD-3X, CHN) using CuKα radiation generated at 30 kV and 20 mA, the range of diffraction angle was 10°-90° 2θ. Fracture surface of samples were sputter coated with gold in a vacuum evaporator, and photographed using a scanning electron microscope (Quanta200, USA) using an accelerating voltage of 3 kV.

Mechanical Tests

Mechanical testes were carried out with a tensile tester (UTM2203, China). The tests were carried out at a crosshead speed of 1mm/min. Dumbbell specimens with size 4mm ×20mm were tested for tensile property according to China National Standard (GB/T 1040-3). Five same samples of each experiment with the average reported.

RESULTS AND DISCUSSION

In Figure 1(A), the main absorption bands of collagen, hydroxyapatite and gelatin appear. The main absorption bands corresponding to collagen are: amide A at 3424 cm^{-1},

amide B at 2931 cm^{-1}, amide I at 1700-1600 cm^{-1}, amide II at 1550-1500 cm^{-1} and amide III at 1300-1200 cm^{-1}. The main absorption bands of hydroxyapatite are: ν_1 mode of PO_4^{3-} at 964 cm^{-1}, ν_3 mode of PO_4^{3-}at 1096 cm^{-1} and 1035 cm^{-1} for the asymmetric HA, ν_4 mode of PO_4^{3-} at 601 cm^{-1} and 562 cm^{-1}, ν_2 mode of PO_4^{3-} at 470 cm^{-1}, and ν_5 mode of PO_4^{3-} at 420 cm^{-1}. The main absorption bands corresponding to gealtin are: N-H stretching vibration at 3500-3200 cm^{-1}, -CH vibration at 2845-2760 cm^{-1}, -C=O vibration at 1631 cm^{-1} and $-OH^-$absorption peaks at 1390-1450 cm^{-1}.

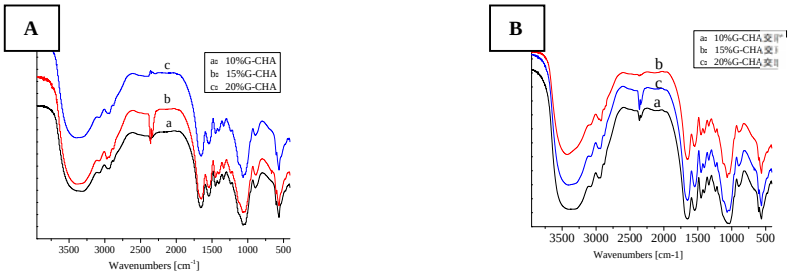

Figure 1. FTIR spectra of collagen/hydroxyapatite/gelatin composites (A) uncrosslinked composites and (B) crosslinked composites

For CHA-Gel composite, the peaks' position of the starting CHA and Gel does not have red-shift change in the composite. This means that CHA and Gel still keeps their original structure in the composite. Comparison of composite using the cross-linkage agent EDC/NHS (Figure 1(B)), it is confirmed that the organic-inorganic bonding between CHA and Gel from the strong amide bands shows the frequency change: amide I band in the range of 1700-1600 cm^{-1} and amide II band in the range of 1550-1500 cm^{-1}. By effects of EDC/NHS, Gel molecules were chemical modified and affected the lattice interaction between organic molecules and HA interfaces. The -COO groups of Gel make a covalent bond with Ca^{2+} sites in the interfacial surface of HA.

The results of XRD patterns revealed that HA within CHA-Gel composite matched with the JCPDS PDF database pattern of hydroxyapatite, but reflected low crystallinity (Figure 2(A)). The reaction was not carried out at higher temperature. Consequently, the HA crystals formed were the immature fine crystallites. The crystallinity of composites was lower with higher Gel content, possibly due to a higher proportion of the amorphous Gel phase and the small size of the HA crystals. Comparison of patterns after crosslinking, some new peaks and steeper peak development indicate that the introduction of EDC/NHS may leads to reaction within compound and the compound is related with the interaction among Col, Gel and HA molecule. As commonly stated in literatures, the co-use of EDC and NHS greatly improved the molecular structure between Col and HA, or Gel and HA (Ofner III and Bubnis 1996; Wissink et al. 2001).

The morphology and microstructure of CHA-Gel composites are examined using SEM. And section micrographs of the synthesized CHA-Gel composites of different ratios without or with crosslinked are shown in Figure 3. It shows that CHA-Gel composite has a typical lamellar-like structure and is interconnected by micropores inside.

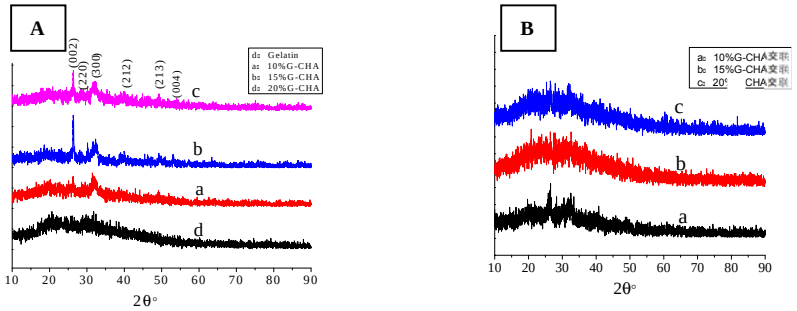

Figure 2. XRD spectra of collagen/hydroxyapatite/gelatin composites (A) uncrosslinked composites and (B) crosslinked composites

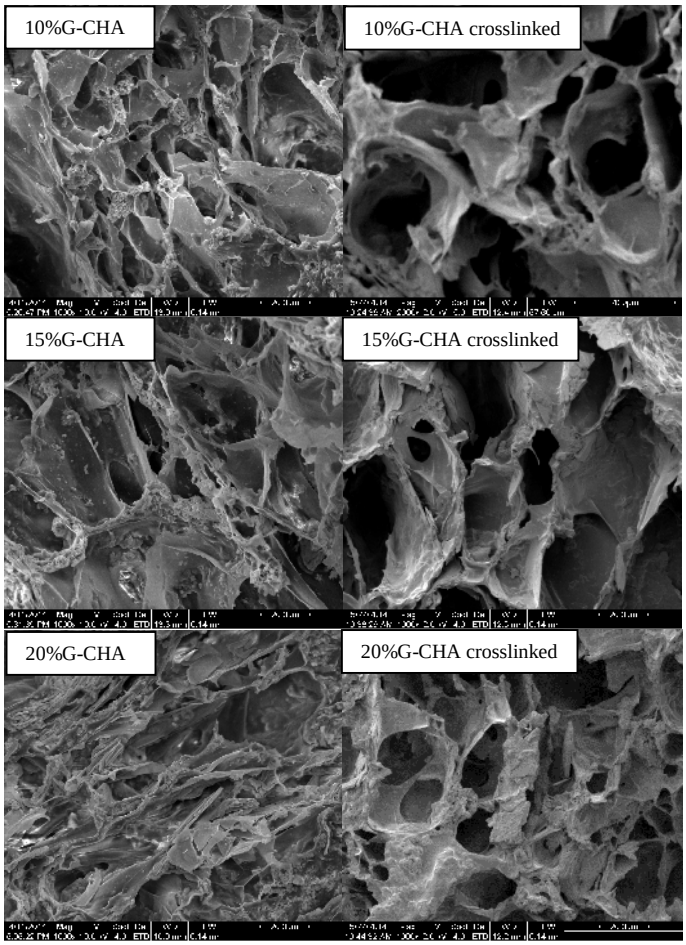

Figure 3. SEM micrographs of collagen/hydroxyapatite/gelatin composites (section)

The micropores in materials could greatly enlarge the surface contact area for protein adsorption. Hence this will enhance protein absorption on pore surface and inside pore to facilitate bone formation. With increasing content of gelatin, the pores of CHA-Gel composite appear to be partly fine adhesion beyond composite layers along their interfaces to each other.

On the other hand, interconnected porosity depends on cross-linking of composites. It is showed that the microstructure of CHA-Gel composites before and after cross-linking were also compared in Fig. 3. In composites crosslinked, the pores obtained are more homogeneous. Moreover, CHA-Gel composites crosslinked show a increased degree of interconnected porosity and a lower density with respect to their corresponding uncrosslinked counterparts. The high porosity and low density of these composite are also benefit to increasing the number of cells adhering to the scaffolds on implantation, and shortening the time for bone tissue formation (Zhang *et al.*, 2003).

Figure 4 reports the tensile strength and elongatian at break of uncrosslinked and crosslinked CHA-Gel composites. Generally, with increasing gelatin form 10 to 20wt%, the tensile strength and elongatian at break of composites decrease. The addition of gelatin into composite should have incresse the tensile strength. However, lyophilization of materials makes gelation in dry condition lose its elasticity. The tensile strength decreases sharply from 90.13±1.58MPa to 61.74±1.76MPa and the differences among elongation at break are not significant. After crosslinking, the trend of elongatian of crosslinked CHA-Gel composites at break also drops . However, the tensile strength enhance with increasing the percentage of gelatin. Such a phenomenon illustrates that the crosslinking causes the interaction within composites, to a certain extent, it promotes bongding between gelatin and CHA, or between CHA-Gel and crosslinker.

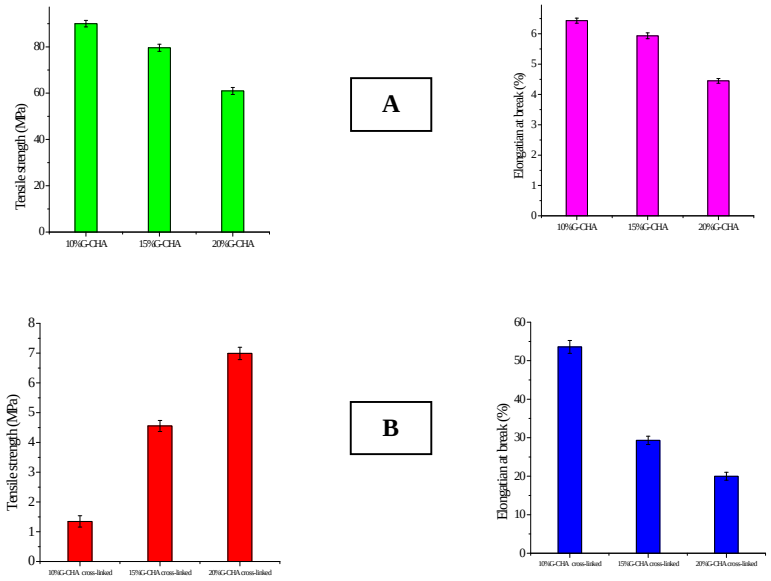

Figure 4. Mechanical properties of collagen/hydroxyapatite/gelatin composites (A) uncrosslinked composites and (B) crosslinked composites

For both crosslinked and uncrosslinked CHA-Gel composites, the mechanical response is also affected by sponge porosity degree together with pore size and pore orientation. As reported in SEM analysis above, with respect to their corresponding uncrosslinked counterparts, crosslinking makes materials' pore more homogeneous, the sizes of pores more bigger, porosity degree higher and the density lower. The microstructure of CHA-Gel composites also give primary information on their mechanical property.

CONCLUSIONS

The CHA-Gel composites were prepared by layer solvent casting combined with lyophilizationand crosslinkingas a biomedical material. The gelatin produced a more homogeneous porous structure at millimeter scale with high porosity degree and low density. The modification of EDC/NHS crosslingker has significant effects on the mechanical property of composites. With crosslinking, the elongation at break increases sharply. However, the tensile strength decreased. .

Acknowledgements

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THE INFLUENCE OF MARINE ALGAE AND NATURAL PLANT OILS ON COLLAGEN-BASED CREAM PROPERTIES

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The aim of this paper was to investigate the effect of various marine algae, natural plant oils and Laurus nobilis L. essential oil on stability, rheological and antimicrobial properties of collagen-based cosmetic cream. For this purpose, 3 types of algae [*Spirulina platensis (Cyanophyta), Haematococcus pluvialis (Chlorophyta) and Laminaria dictyota (Phaeophyta)],* 3 types of natural plant oils (olive, nut and laurel) and one essential oil were incorporated in an comercial collagen-based cosmetic cream which was used as a control. The rheological assessment was carried out by analyzing pseudoplastic flow and thixotropic behaviour, stability was performed by centrifugation during time and microbiological tests were carried out against germs, yeasts and molds, *S. aureus*, coliforms and *E. coli* and *P. aeruginosa*. This work showed that all the tested collagen creams showed a pseudoplastic flow and a thixotropic behaviour which promotes the flow formulation and a corresponding application on the skin and they were stable. Among the all formulations, the best properties were given using the following ingredients: Spirulina platensis, olive oil and laurel essential oil.

Keywords: cream, algae, natural plant oil

INTRODUCTION

Cosmetic industry focuses its attention on natural ingredients for cosmetics uses in order to limitate the toxic solvents (Chaudhari *et al.*, 2011; Conde *et al.*, 2014). The algae found in marine environments exhibit anticoagulant, antiviral, antioxidative, anticancer, antiinflammatory and immunomodulatory actions, and could have potential for the development of nutraceutical, pharmaceutical and cosmetic products (Senevirathne and Kim, 2013). The dominating species of marine micro and macroalgae in commercial production includes *Haematococcus pluvialis*, *Padina pavonica*, *Sargassum* sp., *Coralina* sp., *Chlorella vulgaris*, *Spirulina platensis* and *Laminaria* sp. There are many studies carried out in the *in vitro* conditions, which evidence antimicrobial activity of essential oils (Kunicka-Styczyn´ska *et al.*, 2013). Therefore, it appears that these natural compounds can successfully be used in the cosmetic industry as a preservative. Creams are semisolid emulsions which are widely used as a means of altering the physical properties of the skin (particularly the hydration state). The structure of these creams has been the subject of considerable study optimising their physical properties depenting on the ingredients (Peramal *et al.*, 1997). For the semisolid systems generally, for the creams particularly, the rheological characteristics knowledge represents an important aspect in their formulation process because the flow parameters can determine adequate consistency, extrusion capacity from the recipient, quality and stability control during

storage, ease of application, adhesion on the skin (Ghica *et al.*, 2012b; Gilbert *et al.*, 2013; Savary *et al.*, 2013). The aim of this research was to study the influence of some algae, natural plant oils and essential oils on a known comercial cream (Reference CPNP number 1388019) in order to obtain new cream formulations.

MATERIALS AND METHODS

Materials

Three types of algae, Spirulina platensis (SP), Haematococcus pluvialis (HP) and Laminaria dictyota (LD), were obtained as powder.

Spirulina platensis is a microalga whose composition is suitable for use as food supplement and can be used to combat malnutrition (Fox, 1996). It's composition includes high levels of protein (64-74%), polyunsaturated fatty acids and vitamins (Cohen, 1997), and antioxidant compounds (Collar *et al.*, 2007). This microalgae is classified as GRAS (Generally Recognized as Safe) by FDA (Food and Drug Administration), which ensures its use as food without risk to health.

Haematococcus pluvialis is a freshwater species of Chlorophyta from the family Haematococcaceae. This species is well known for its high content of the strong antioxidant astaxanthin, which is important in aquaculture, and cosmetics (Lorentz and Cysewski, 2000).

Laminaria digitata is a large brown alga in the family Laminariaceae. L. digitata was traditionally used as a fertiliser and spread on the land. It is not only used as an organic fertiliser but also for the extraction of alginic acid, the manufacture of toothpastes and cosmetics, and in the food industry for binding, thickening and moulding (URL, 1).

Leaves of Laurel (Laurus nobilis L.) were collected from Amanos Mountain (Anatolian region) in blooming period and dried at room temperature. Essential oils were obtained from dried leaves. These samples were subjected to hydro distillation for 3 h using a Clevenger type apparatus. Essential oils obtained were dried over anhydrous sodium sulphate and stored at – 20ºC until GC-MS analysis. Yield of obtained oils was 3.8% and main component of laurel essential oil was 1,8 cineol (eucalyptol) with 62% ratio.

"The cream with collagen and vitamins" with Reference CPNP number 1388019 were obtained by the currently used technology in Collagen Department of Division Leather and Footwear Research Institute and was used as reference collagen cream in this study.

Preparation of Creams

Starting from reference collagen cream, 10 new formulations of creams were prepared having the composition presented in Table 1.

Flow Behaviour Evaluation

The rheological properties of the tested creams were conducted with a rotational viscometer Multi-visc Rheometer-Fungilab equipped with standard spindle TR 8 and ultrathermostat ThermoHaake P5 maintained at 33^{o}C, keeping the same experimental conditions as previously described (Ghica *et al.*, 2012b). The viscometer allows, for each rotational speed or shear rate specific to the spindle used, the determination of the shear stress and the viscosity of the creams. For each formulation the ascending and descending rheograms were recorded. The rheological measurements were performed in triplicate and the average results were reported. The Power law model, expressed as

viscosity as a function of shear rate (eq. 1), was applied for the assessment of creams flow behaviour:

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1)$$

where m and n are parameters correlated with the tested creams formulation factors and determined through the linearization of eq. (1) by double logarithmic method (Ghica *et al.*, 2012a).

Table 1. Composition of algae-based creams

New cream formulation	Reference cream (g)	Algae type	Solvent
C1	92.5	-	Water
C2	92.5	SP	Olive oil
C3	92.5	SP	Nut oil
C4	92.5	SP	Laurel oil
C5	92.5	SP	Water
C6	92.5	HP	Olive oil
C7	92.5	HP	Nut oil
C8	92.5	HP	Laurel oil
C9	92.5	HP	Water
C10	92.5	LD	Nut oil

The ratio between algae and solvent was 2:5. All the samples were treated with laurel essential oils 4 % (v/v).

Stability Determination by Centrifugation

5 g sample of every creams were put into vials and were centrifugated at 4500 rot/min during 30 min for 3 times using centrifuge (Hettich Micro 200 mode). The sample was considered stable if it was not separated into two phases.

Microbiological Analysis

Standard plate count method was used for the determination of total aerobic microbial count (TAMC) on Plate Count Agar culture medium, at 30 ± 1°C. The selective enumeration of yeasts and molds was carried out on Chloramphenicol Yeast Glucose Agar medium at 25 ± 1°C after 5 days of incubation. *Staphilococcus aureus* was analysed on Baird Parker Agar medium. Coliforms and *Escherichia coli* were determined using Violet Red Bile Agar (VRBL) medium. The detection of *Pseudomonas aeruginosa* was made on Cetrimide Agar medium. Reference strains were used for all these analyses.

RESULTS AND DISCUSSION

The flow profiles for the creams analyzed at 33°C recorded as viscosity versus shear rate are presented in Figure 1a-d. The rheological profiles illustrated in Figure 1a-d indicated for all tested collagen creams a typical non-newtonian pseudoplastic behaviour, the viscosity decreasing for the shear stress increase. This behaviour facilitates the creams flow. Similar flow patterns were obtained for the creams with the same type of algae (Figure 1a-b). Figure 1c shows that the reference collagen cream is more viscous than cream with HP and SP. From Figure 1d it can be noticed that the presence of LD in cream formulation induced a significant increase of viscosity.

The quantification of rheological behaviour of tested creams was realized by means of the Power law model (eq. 1). The values of the parameters m and n as well as of the correlation coefficient characteristics to this model are listed in Table 2.

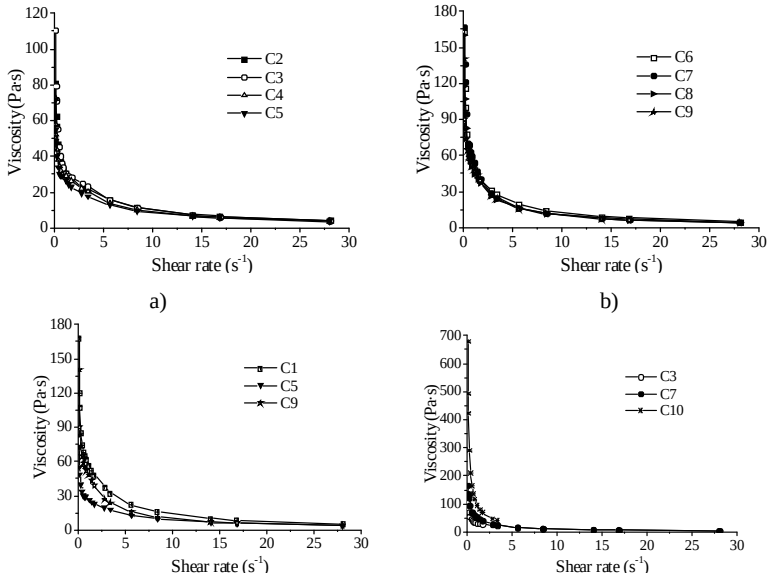

Figure 1. The rheograms recorded at 33°C for: a) Creams with SP and different types of oils; b) Creams with HP and different oils; c) reference cream and creams with different algae in water; d) creams with different algae and nut oil

Table 2. The values of the m and n parameters and the correlation coefficients specific to the Power law model applied to collagen creams

Cream	m	n	R
C1	52.564	0.440	0.9905
C2	29.879	0.381	0.9926
C3	31.802	0.474	0.9939
C4	27.780	0.374	0.9908
C5	25.263	0.397	0.9924
C6	46.176	0.477	0.9931
C7	50.168	0.487	0.9950
C8	44.089	0.505	0.9979
C9	42.834	0.438	0.9819
C10	110.210	0.730	0.9982

Analyzing the data presented in Table 2 it can be observed that the values recorded for parameter m (associated with the viscosity obtained for the shear rate of $1 \cdot s^{-1}$) (Ghica et al., 2012a) decreased by adding the SP in the reference collagen cream composition by 1.65-2.08 times and 1.04-1.22 times by adding HP respectivelly, while the presence of LD algae determines an increase of about 2.09 times.

The creams were also tested in terms of thixotropic behaviour. In Figure 2 the ascending and descending flow curves for the creams C1, C4, C8 and C10 are shown for exemplification.

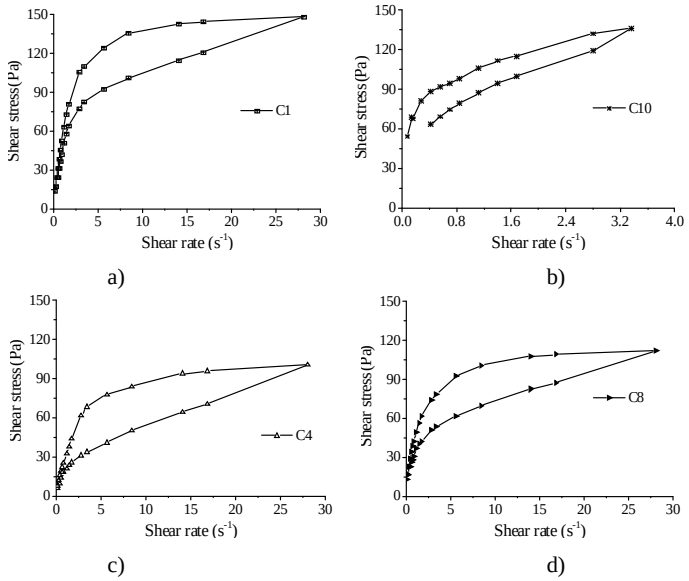

Figure 2. Flow curves (up/down) obtained at 33°C for collagen creams: C1; b) C10; c) C4; d) C8

For the same shear rate, the point on the descending curve corresponds to lower shear stress compared to the ascending curves, obtaining the hysteresis thixotropy area (Ghica et al., 2012b). All the formulation presented a thixotropic behaviour, which cause easily to display on skin.

All the creams were studied by stability point of view and all of the were very stable. Although, we noticed some differences between them as following: the best stability was observed for samples 6 and 1 and lower stability was for samples 7 and 9.

The microbiological analysis showed that *S. aureus*, coliforms and *E. coli* and *P. aeruginosa*, yeasts and molds were absent for all the samples. Some of samples contain aerobic microorganisms as we presented in the Table 3.

Table 3. Total aerobic microbial count (TAMC), (CFU g-1) developed by creams at 30°C

Cream	TAMC	Cream with LO	TAMC
C1	0	C1*	0
C2	0	C2*	0
C3	0	C3*	0
C4	0	C4*	0
C5	45	C5*	40
C6	15	C6*	0
C7	145	C7*	10
C8	120	C8*	90
C9	95	C9*	30
C10	200	C10*	10

As we can notice from Table 3, the ingredients influence the microbiological properties of creams. Comparing the microbiological activity of algae into creams we can see that *S. platensis* is the most efficient one comparing with *H. pluvialis* and *L. digitata*. *L. digitata* is not recommended to be added in cream as such because developed germs 2 times more than admitted limit. It could be added only with essesntial oil. From natural plant oil the most efficient were olive oil followed by laurel and than nut oil. The laurel essential oil decreased significantly the microbial contamination level for all the samples.

CONCLUSIONS

All the tested collagen creams showed a pseudoplastic flow and a thixotropic behaviour which promotes the flow formulation and a corresponding application on the skin. All the tested creams were stable. Among the all formulations, the best properties were given using the following ingredients: *Spirulina platensis*, olive oil and laurel essential oil.

Acknowledgements

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CONCEPTION AND ELABORATION OF BIOGELS TO DELIVER ANTI-BIOFILM AGENTS

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Over the past 15 years, the impact of biofilms on persistent infections and their potential role on chronic wounds have been extensively documented. However, up to now, no efficient system to deliver anti-biofilm agents has been described. The aim of this study is to conceive a "smart" dressing against biofilms in chronic wounds. We developed an innovative biogel system containing various anti-biofilm agents to improve eradication of biofilm pathogenic bacteria. The anti-biofilm strategy consisted in preventing bacterial colonization, disrupting the biofilm and eradicating pathogen bacteria (< 10^3 CFU/mL). Combination of PHMB (54 mg/mL), an antiseptic agent and EDTA (10mM) a cation chelating agent, eradicated *P. aeruginosa* and *S. aureus* biofilms. These anti-biofilm agents were entrapped in gelatin gels which have the capacity to deliver molecules with controlled release. This combination in the biogel affected *in vitro* biofilms depending on the gelatin and antiseptic concentrations. The use of ephemeral gels, where the gelatin network hydrolysis by enzymes was programmed and timed-controlled, permitted to stimulate the agent release and enhanced our results on various biofilms. Chronic wounds are a common and expensive problem in public health. Gelatin biogels have a great potential to entrap and deliver antibiofilm agents over a longer period and at smaller concentrations than the current wound care treatments.

Keywords: biogel, anti-biofilms, drug delivery.

INTRODUCTION

Many pathologies and age may deregulate healing, leading to chronic wounds which do not heal within 6 weeks. The curing duration is depending on the weakness of the patient (Menke, 2007). Moreover, chronic wounds are very sensitive to bacterial colonization which can evolve to infection.

In recent years, the presence of biofilms on persistent infections and their potential negative role on wound healing have been extensively studied (Steven, 2004). Biofilms are structured communities of microorganisms surrounded by a polymeric matrix; they are found on a wide range of biotic and abiotic surfaces. The biofilm extracellular polymeric matrix contains different types of exopolymers such as polysaccharides, proteins, DNA and lipids (Flemming, 2010). Biofilms seem to be associated to a lengthened inflammatory phase (Singh and Barbul, 2008). The main clinical problem of biofilm-associated infections is the treatment failure due to the high resistance level to antibiotics and other antimicrobial drugs. Nowadays, the current health care strategies against biofilm infected wounds are still poorly developed (Ammons, 2010).

As presented here, we conceived and elaborated a biogel system containing various anti-biofilm agents (fig.1) to improve eradication of biofilm pathogenic bacteria and prevent wounds against a pathogenic bacterial colonization.

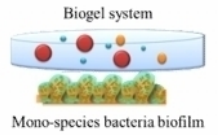

Figure 1. Anti-biofilm strategy

The originality of our system consisted in the combination of two different types of molecules: an antiseptic usually applied in chronic wound care and a chelating agent active against MMPs which are over-expressed in chronic wounds. Several compounds were tested. We selected respectively PHMB (PolyHexaMéthylène Biguanide) which is one of the active compounds of Prontosan®, an antiseptic solution with anti-biofilm properties commercialized by B. Braun and EDTA, a divalent cation chelator known to have activity against biofilms for a large range of bacteria (Banin, 2006).

EXPERIMENTAL METHODS

Materials

Gelatin was provided by Rousselot (103-10-51). It is extracted from ox bone through an alkaline process (pI 4.7). The enzymes used in this study are a serine protease, Esperase (P5860 – Sigma) and a Tranglutaminase from microbial origin, produced by Ajinomoto. PHMB is a solution provided by Pareva.

Both strains, *Pseudomonas aeruginosa* CIP 103 467 and Staphylococcus aureus CIP 4.83 were provided by Institut Pasteur, Paris. *Pseudomonas aeruginosa* was grown in LB broth (Lennox) and *Staphylococcus aureus* in TS bacto™ soy broth (Lennox) both at 37°C.

Preparation of Gelatin Gel

Gelatin powder was solubilized in Tris –HCl buffer 50mM pH 7.4 at 40°C for 30min. The solution was composed of 5 or 7% of gelatin (W/V) and 1.5 U/mL of transglutaminase (final concentration). Various molecules can be added into the solution so as to be finally entrapped in the gel. Gelation was performed at 40°C. The obtained gel was irreversible with temperature.

Rheology

Rheology measurements were performed using a Rheostress Anton Paar MCR301, operating in the oscillatory mode with a deformation strain of 1% and a frequency of 1 Hz. A 50 mm plate/plate geometry was used. The storage modulus G' and loss modulus G'' were recorded as a function of time. Temperature (37°C) was controlled using a Peltier device.

Biofilm Formation and Bacteria Assay

Bacteria were suspended overnight at 37°C and re-suspended in culture medium at 0.001 DO_{595nm}. The 24-well plates containing 14 mm-diameter glass slides were inoculated with the bacterial suspension (1mL/well) and incubated at 37°. The culture medium was changed every 12h. After 24 hours of biofilm growth, the wells were rinsed twice with saline solution ([NaCl] = 9 g. L-1). The solutions or gel to be tested were then brought into contact with the biofilm for 24h at 37°C. The viable biofilm bacteria were quantified on agar after glass slide sonication to detach the biofilm. The control was a medium solution diluted in saline solution with the same ratio as the one used for the treatment.

RESULTS AND DISCUSSION

Gelatin Gel Properties

Gel Formation

A gel is a soft matter composed of a liquid phase entrapped in a polymer network. Gelatin has the capacity to form a hydrogel which aqueous phase represents 95% of the gel mass. A 5% (W/V) gelatin solution may spontaneously turn to a gel, but only below 30°C. At the same protein concentration, a chemical gel may be formed at 37°C by the action of transglutaminase, an enzyme, which forms covalent bonds between lateral chains of the protein.

Rheological measurements (fig.2, white symbols) evidenced a viscoelastic behavior; the gel reached an elastic modulus, G', of 800 Pa after 5h. The gelatin solution turned to a gel only 9 min after the transglutaminase addition (G' ≥G'').

Viscoelastic Properties

The gelatin gel has the capacity to entrap molecules in its aqueous phase. The aim of this study was to conceive a drug delivery system from this gel. However, encapsulation of antiseptic agents decreased the mechanical properties of the gel (fig. 2). So, the formulation was adapted by increasing the gelatin concentration to 7% (W/V) to obtain a handeable gel (G'/G''>10).

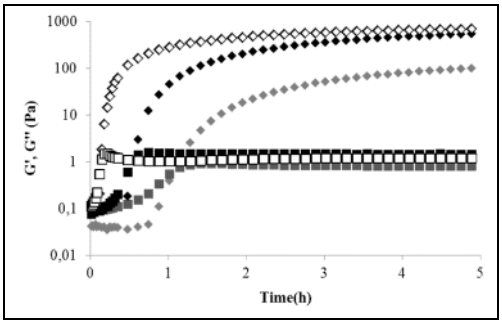

Figure 2. Rheological properties of a chemical gelatin gel over time. The gel was tested with (grey and black symbols) and without (white symbols) 4 mg/mL of PHMB for a gelatin concentration of 5% (white and grey symbols) or 7% (black symbols). G' is represented by diamonds and G'' by squares.

The results highlighted the influence of PHMB on the mechanical properties of the gel. After 5h (fig.2, compare white and grey symbols), the 5% gelatin gel entrapping PHMB was 7 times less elastic than the control gel. Furthermore, gelation was delayed as the gel time (time where G'=G'') was increased 7 times. Using a more concentrated gelatin solution allowed to reduce the gel time and obtain satisfactory elastic properties. The addition of EDTA at the used concentration (20 mM) did not disturb the gel formation and properties (data not shown).

Diffusion of Molecules from the Gel

Influence of Molecules Properties

Molecules entrapped into a gelatin gel diffuse to the external environment according to their and gel properties. To visualize and predict diffusion, some molecules were used with different charges and weights. The anti-biofilm strategy consisted in delivering a treatment on a wound potentially contaminated by a biofilm. This biofilm can be assimilated to a soft gel. So, our experimental diffusion model was a gelatin gel entrapping molecules layed on a soft alginate gel. The diffusion from one gel to the other was studied over time.

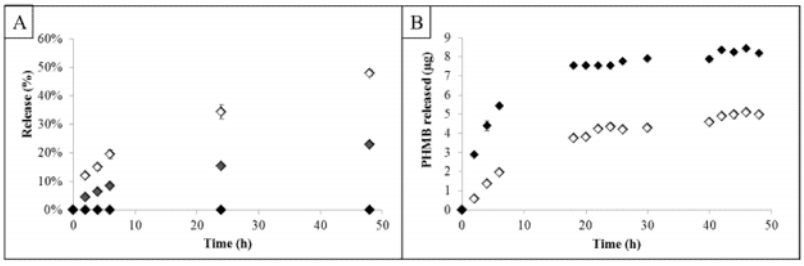

Figure 3. Diffusion kinetics of molecules from a 7% gelatin gel over time: [A] Diffusion kinetics from a gelatin gel to a 1% alginate gel of molecules of various molecular weights: 300Da methylene blue (white symbols), 70kDa dextran (grey symbols) and 2,000kDa blue dextran (black symbols). [B] Diffusion kinetics of PHMB at 4 mg/mL (white symbols) or at 8 mg/mL (black symbols) (98 and 196µg respectively per gel) from a gelatin gel to a solution.

The diffusion kinetics of molecules is theoretically dictated by diffusion laws: the release depends on the molecule size and charge as well as on gel network structure (data not shown). This was verified with our two gels; the smaller the molecule was, the faster it diffused from one gel to another (fig.3[A]). But, when the molecule size was larger than the mesh size of the gel network, the molecule was blocked into the gel, (see blue dextran behavior of fig.3[A]).

The antiseptic chosen in the project is PHMB, a small size molecule. Its diffusion from the gel should be fast. Nonetheless, only 4% of the initial PHMB concentration included in the gel diffused in the surrounding solution within 24h (fig.3[B]). The drug seemed to be retained in the gel. This can be explained by the positive charge of PHMB at physiological pH, which is opposite to the gelatin network charge. Thus it diffusion to a gel or a biofilm could be limiting.

Effect of Anti-Biofilm Agents

PHMB and EDTA Effects on Biofilm

Some studies suggest that combining EDTA with an antibiotic can enhance its activities against bacteria [Lambert (2004)]. The effect of combined PHMB and EDTA was thus tested on biofilms of *P. aeruginosa* and *S. aureus*, two pathogenic bacteria frequently encountered in chronic wounds. In a first step the activity of antiseptic

molecules in solution was measured. The number of viable bacteria was calculated after 24h of treatment on the biofilm.

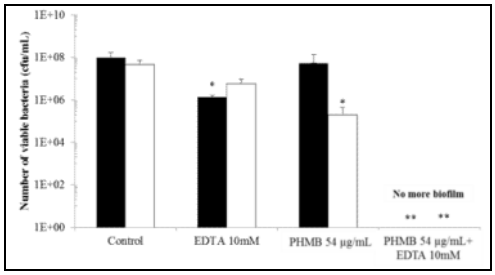

Figure 4. Anti-biofilm effect of EDTA and PHMB on biofilm of *P. aeruginosa* (black bars) and *S. aureus* (white bars)

From these results (fig.4), it was obvious that the addition of EDTA potentialized the effect of PHMB. EDTA was more efficient on *P. aeruginosa* biofilm than on *S. aureus* with a decrease of the biomass higher than 2 logarithms. PHMB alone showed no effect on *P. aeruginosa* biofilm and a reduced effect on *S. aureus* biofilm while, at the same concentration, it was totally bactericidal on planctonik bacteria of both strains (data not shown). However, a very high anti-biofilm effect (> 99.999%) was obtained with PHMB combined to EDTA, showing a synergistic effect of the two compounds. Then, the active compounds were entrapped into a gel.

Anti-Biofilm Effect of the Gel

Entrapment of PHMB with EDTA in a cross-linked 7% gelatin gel was possible (data not shown). Even if the elastic properties are weak when a high antiseptic concentration is used (G' = 100 and 50 Pa, after 5h of gelation, for respectively 4 and 8 mg/mL of PHMB), the gels were still handleable. To test the anti-biofilm effect, these gels were brought into contact with the biofilm during 24h.

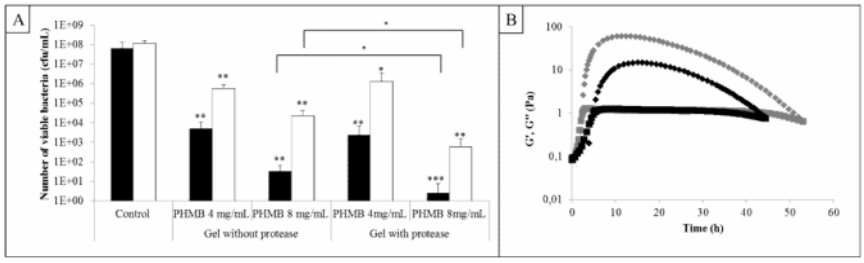

Figure 5. Anti-biofilm effect of the 7% gelatin gel containing 10mM EDTA and PHMB with or without protease: [A] Biomass evaluation after 24h of treatment entrapped in a gelatin gel containing or not a protease. *P. aeruginosa* is represented by black bars and *S. aureus* by white bars; [B] Rheological properties of a gelatin gel containing 4 mg/mL (grey symbols) or 8 mg/ml (black symbols) of PHMB, EDTA and a protease. G' is represented by diamonds and G'' by squares.

The treatment entrapment in a gel decreased molecule efficiency on the biofilm (compare fig.4 with fig.5 [A]). Indeed, the PHBM concentration had to be increased from 54µg/mL to 4 and 8 mg/mL to induce a significant biomass reduction highlighting a high sensitivity of *P. aeruginosa* biofilm to active molecules. This sensitivity was 60 and 400 times higher than that of *S. aureus* for respectively 4 and 8 mg/mL (fig.5[A]). However, the biofilms were not totally eradicated in these conditions.

The aim of the project was to reach a biomass reduction of 5 logarithms; limit not reached with the treatment on the *S. aureus* biofilm. As shown on figure 3[B], the gelatin network disturbed active molecule diffusion. To improve the results, the gel formulation had to be optimized. An innovative drug delivery system, developed in ERRMECe laboratory (Klak, 2012), allows a gelatin gel formation followed by a controlled-time resolubilization, leading to enhanced molecular release; this ephemeral gel is named Enzgel. This gel is based on the action of two antagonistic enzymes, one generating and the other cleaving covalent bonds, respectively a transglutaminase and a protease. Thus, a protease, Esperase, was added to the gelatin solution containing the treatment; a gel was first formed which later re-solubilized (fig 5[B]). The protease decreased the elastic properties and increased gelation time; but the gel was still handleable (G'/G'' >10). The network hydrolysis was complete after 51h and 41h for respectively 4 and 8 mg/mL of entrapped PHMB. This system promoted molecule diffusion from the gel to the surrounding medium allowing their anti-biofilm activity to express (fig.5[A]). For 8 mg/mL of PHMB, the biomass was reduced by 5 logarithms for *S. aureus* biofilm and by 7 logarithms for *P. aeruginosa* biofilm, an improvement of 40 and 11 times compared to the gel without protease. The efficiency rates fixed in the project were thus fulfilled.

CONCLUSION

In the United States, 8 million people are affected by chronic wounds which lead to a cost of 20 billion dollars per year. The presence of biofilm may extend the healing delay. Our strategy to limit or eradicate biofilms was based on two major points: destabilize the biofilm and its matrix; eliminate pathogenic bacteria by the use of an antiseptic.

The use of EDTA enhanced the PHMB effect on biofilm. The great potential of gelatin gel to entrap and deliver agents over a longer period was used. The use of an ephemeral gel, where gelatin network hydrolysis was programmed and time-controlled, allowed stimulating the molecule release and enhance treatment activity against biofilms. Finally an efficient system, able to control the bacteria level in various biofilms was conceived and elaborated.

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COLLAGEN-DOXYCYCLINE SPONGIOUS FORMS FOR INFECTED TISSUES TREATMENT

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The purpose of the present work was to develop and characterize some spongious forms based on collagen and doxycycline, uncross-linked and cross-linked with glutaraldehyde, obtained by lyophilization. The prepared sponges were analyzed by FT-IR spectroscopy, water up-take and optical microscopy. The doxycycline release from collagen spongious forms was investigated and the kinetic mechanism was determined. The results of this paper indicated that the drug delivery is influenced by cross-linking degree and composition of spongious forms.

Keywords: collagen, doxycycline, drug delivery.

INTRODUCTION

Tissue infections can appear for different reasons, the most common being bacteria and medications (Christian *et al.*, 2007). The best way to effectively treat such an infection could be with antibiotics and pain medication (King, 2011).

Tissue regeneration approaches require a biocompatible material such as a scaffold to support cell proliferations as well as to deliver drugs needed for a proper recuperation. Many researchers study other alternatives such as natural polymers: chitosan, alginate or collagen, combined with ceramics for improved properties of obtained composite material (Oliveira *et al.*, 2010).

Among natural polymers, collagen is the most abundant protein in mammals and it is commonly extracted from bovine tissue. Collagen scaffolds are used in tissue regeneration, either in sponges, thin sheets (membrane) or gel / hydrogel forms (Albu *et al.*, 2011). Collagen has the proper properties for tissue regeneration such as pore structure, permeability, hydrophilicity and it is stable *in vivo*. Collagen scaffolds are also ideal for cells deposition, such as osteoblasts and fibroblasts and once inserted, growth is able to continue as normally as in the tissue (Trandafir *et al.*, 2007).

Collagen presents better biocompatibility and biodegradability when it is compared with other polymers and doxycycline is an antibiotic with both positive and negative spectra. The collagen-doxycycline spongious samples we prepared by lyopilization can be used for treatment or regeneration and prophylaxy of tissues that are damaged after an infection.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 1.72% (w/w) was extracted from calf hide using technology currently available at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu, 2011). Doxycycline hyclate (DH) was purchased from Sigma-Aldrich, China. Sodium hydroxide and hydrochloric acid were of analytical grade. Type I collagenase obtained from *Clostridium histolyticum* was purchased from Sigma-Aldrich, Germany and glutaraldehyde (GA) from Merck (Germany).

Preparation of Collagen Scaffolds

The concentration of each collagen gel was adjusted at 1% and 7.3 pH using 1M sodium hydroxide (the pH of the physiological medium). 0.2% doxycycline hyclate was added to half of collagen gel (w/v), then the collagen gels were cross-linked with 0.25% glutaraldehyde (reported to collagen dry substance) as Table 1 presents.

Table 1. Composition and name of collagen gels

Code of gels	Col, %	DH, %	GA, %
Coll	1	0	0
Coll-R	1	0	0.25
Coll-DH	1	0.2	0
Coll-DH-R	1	0.2	0.25

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer using the lyophilization program presented in Figure 1.

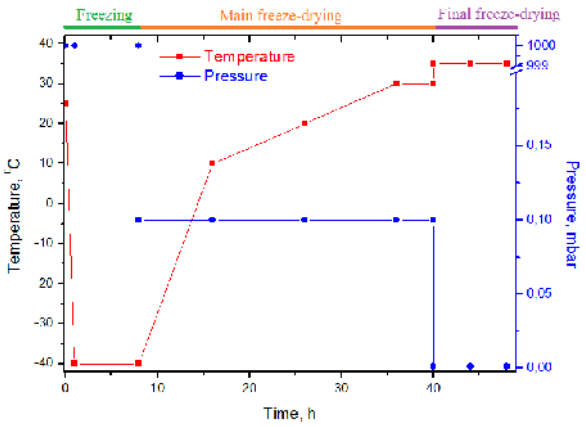

Figure 1. Graph chart of freeze-drying process

The resulting matrices were named as shown in Table 1.

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Water Absorption

In order to determine the water absorption, the scaffolds were first immersed in water at 36°C. At scheduled time intervals, the samples were withdrawn and weighed. The water absorption was calculated using the following equation:

$$\% \text{ Water up-take} = (Wt - Wd)/Wd \text{ g/g} \quad (1)$$

where Wt denotes the weight of the swollen samples at immersion time t, and Wd denotes the weight of the dry samples. All the samples were studied in triplicate.

Optical Microscopy Study

All images were captured with a Leica Stereomicroscope model S8AP0, 20-160x magnification capacity. For better evaluation of the samples, a 20x magnification and incident external cold light were used.

Doxycycline Hyclate *In Vitro* Release Kinetics Study

Doxycycline hyclate *in vitro* release evaluation from collagen sponges was conducted using a transdermal sandwich device adapted to a dissolution apparatus as previously described in our studies (Ghica *et al.*, 2013; Barbaresso *et al.*, 2014). In brief, doxycycline-collagen matrices un- and cross-linked with glutaraldehyde were fixed in the sandwich device and then immersed into the receiving medium (phosphate buffer of 7.4 pH, maintained at 37°C) from the release vessel. At different periods of time, samples of 5 mL were extracted from the release medium and replaced with an equal volume of fresh phosphate buffer solution, prewarmed at 37°C. The absorbances of the collected solutions were spectrophotometrically assessed at 347 nm, using a Perkin-Elmer UV-Vis spectrophotometer and the released doxycycline amount was evaluated based on the calibration curve, previously determined (Albu *et al.*, 2009). The Power law model was applied for the drug release kinetics investigation from collagen sponges (2).

$$\frac{m_t}{m_\infty} = k \cdot t^n \quad (2)$$

where m_t/m_∞ represents the fractional release of drug at time t, k - the kinetic constant, n - the release exponent characteristic for the drug transport mechanism (Ghica *et al.*, 2013).

RESULTS AND DISCUSSION

After lyophilization the 3D porous collagen sponges based on collagen and doxycycline, cross-linked and uncross-linked, were obtained, with the appearance presented in Figure 2.

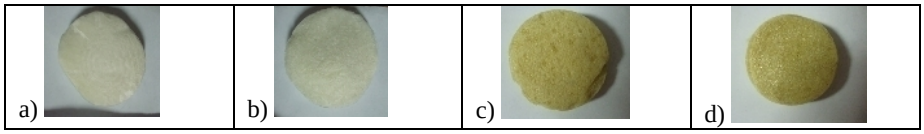

Figure 2. Collagen spongious forms: a) Coll; b) Coll-R; c) Coll-DH; d) Coll-DH-R

The samples from Table 1 were analysed by FT-IR spectroscopy, water absorbtion, optical microscopy and the samples with doxycycline were studied *in vitro* to establish the mechanism of drug release.

From the FT-IR spectra (Figure 3a) the typical bands from collagen can be observed: amide A, B, I, II and III (Albu, 2011).

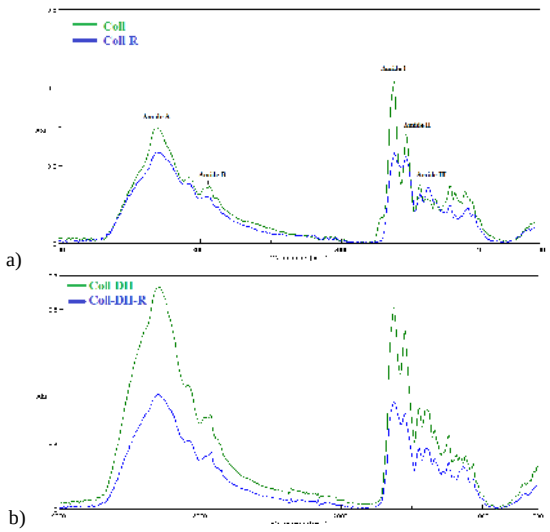

Figure 3. ATR-FTIR spectra of a) Coll and Coll-R; b) Coll-DH and Coll-DH-R

It is noticed that amide A shifts to lower wave number (from 3308 to 3305 cm^{-1}) to probably forming of hydrogen bonding. The amide B shift from 2937 to 2959 cm^{-1} when glutaraldehyde was added which indicates the cross-linking reaction. Moreover, this cross-linking is given by the ratio between areas of Coll and Coll-R. The lower area indicates the sample was cross-linked. There were no notable differences between wavenumbers of amides I, II and III, only between their intensities. Nevertheless, the triple helix of collagen kept its integrity, the value of A_{III}/A_{1451} being 1 for reference sample – Coll.

Comparing cross-linked and un-cross-linked reference collagen samples with ones with doxycycline we can notice that collagen kept his structure and the doxycycline presence are given by the following peaks: 1134, 1066, 1033 cm^{-1}.

The obtained sponges absorb an increased amount of fluid and swell, thus the drug will diffuse more easily.

The water up-take for all the studied samples is presented as kinetics during 72 hours in Figure 4.

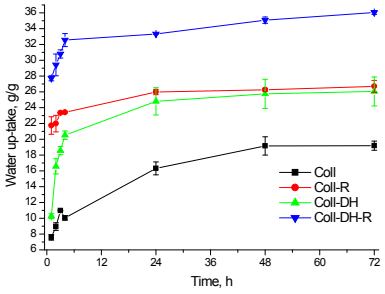

Figure 4. Kinetics of water up-take during 72 hours for spongious forms

Figure 4 presents the water up-take during 72 hours for the studied samples, swelling ratio after 4 hours for the designed sponges. The un-cross-linked samples absorbed a lower amount water than the cross-linked ones due to the porous structure formed during cross-linking. The spongious form with drug up-took a higher amount of water, doxycycline making them more hydrophilic. Thus, the spongious form Coll-DH-R which contains both DH and GA up-take about double amount of water compared with the reference one, Coll.

Optical image for a representative sample Coll-DH-R is shown in Figure 5.

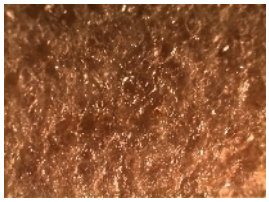

Figure 5. The optical microscopy image for Coll-DH-R

Figure 5 presents a denser structure with interconnected pores for Coll-DH-R.

The release kinetic profiles of doxycycline from un- and cross-linked collagen matrices were recorded as a function of time and presented in Figure 6. The released drug percent after 12 hours of experiment was about 1.3 times smaller in the case of cross-linked sponge due to the presence of cross-linking agent (Table 2).

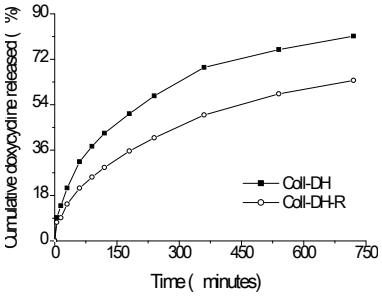

Figure 6. Cumulative release profiles of doxycycline from un- and cross-linked collagen sponges as a function of time

For the investigation of the kinetic mechanism the Power law equation was applied (eq. 2). The parameters specific to this model, the determination coefficient $(R)^2$ and the cumulative released drug percent are listed in Table 2.

Table 2. Values for determination coefficients and kinetic parameters specific to Power law model; released drug percent

Collagen sponges	Correlation coefficient	Kinetic constant ($1/min^n$)	Release exponent	Released percent (%)
Coll-DH	0.9904	0.059	0.405	81.18
Coll-DH-R	0.9966	0.032	0.455	63.61

The values obtained for the release exponent indicated for both matrices an anomalous drug diffusion mechanism, in accordance with our previous studies (Albu *et al.*, 2008).

CONCLUSIONS

Porous forms based on collagen and doxycycline with and without glutaraldehyde were prepared by lyophilization. The FT-IR analysis showed that collagen kept its structure in all the samples. The spongious form showed a porous structure with interconnected pores. The hydrophilicity was lowest for collagen reference spongious forms and highest for the sample which contains doxycycline and cross-linking agent, this form being a promising support for tissue regeneration.

Acknowledgements

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COLLAGEN POLYDISPERSIONS WITH SPECIFIC PROPERTIES FOR SEEDS TREATMENT

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Collagen polydispersions obtained by associated enzymatic and chemical processes for high-yield extraction of collagen from leather waste, under mild reaction conditions, are fit for application in agriculture. The present study highlights the specific properties of collagen polydispersions for cereal seed treatment. Collagen polydispersions were characterized by chemical and instrumental analyses: gravimetric, volumetric, potentiometry, gas chromatography and HPLC, IR spectroscopy, tensiometer methods, Dynamic Light Scattering (DLS). Analytical investigation has shown that the collagen polydispersions have bioactive properties due to the content of free amino acids, with a total of approximately 10 g/100 ml solution and very small sized particles composition, in the 1-10 nm range, able to penetrate the seed coating; the larger sized particles, situated in 100-1000 nm and 1000-10000 nm ranges, ensure the bioactive deposit in the film matrix on the surface; the wetting ability of collagen polydispersions, which is lower than that of water, ensures film matrix formation on seed surface, with long term releasing ability, leading to seed nutrition and stimulating germination. The synergy of collagen hydrophilicity, its known biodegradability, bio-active potential and film-forming properties recommend collagen polydispersions for applications in mixtures for seed treatment.

Keywords: collagen, chromium-tanned waste, seed treatment.

INTRODUCTION

Development of concepts for collagen-based biomaterials began many years ago and new elements are constantly revealed (Ramshaw *et al.*, 2001; Trandafir *et al.*, 2007). In present, the materials based on protein extracted from collagen and collagen matrix materials, mainly used in medical, pharmaceutical and cosmetics fields, are made using primary collagen resources (Santos *et al.*, 2013; Jayathilakan *et al.*, 2012). In order to use proteins in agriculture, secondary collagen resources such as by-products from natural leather processing industry were identified. Most of the research in recent years has focused on extracting collagen from leather waste (Jian *et al.*, 2008; Zainescu *et al.*, 2011) and using these extracts in crop fertilization (Lacatus *et al.*, 2009; Gaidau *et al.*, 2009), as an alternative for the synthesis amino acids used in fertilizer formulas (Chitu *et al.*, 2010; Light *et al.*, 2005) or as an amendment to agricultural soils (Zainescu *et al.*, 2010), but the aim has been to recover collagen from untanned hide waste. However, the largest amount of leather waste resulting from tanneries is chrome tanned leather waste (over 20% of the preserved hides that are processed), which is on the one hand, an environmental problem, and on the other hand, an untapped resource of protein. More recent research focuses on the exploitation of bio-active properties of these collagen extracts in seed treatment to increase germination potential and protect against pests (Gaidau *et al.*, 2013; Lomate *et al.*, 2011).

This paper is focused on presented the collagen extracts recovered, from chromium(III) tanned leather waste, a systematically avoided resource, due to the psychological impact generated by the term “chrome”. Only a small segment of the population knows that modern leather industry, which applies the best practices, neither

uses nor directly discharges hexavalent chromium, generator of extreme toxic effects. Our own previous research (Niculescu *et al.*, 2009; Gaidau *et al.*, 2010) has shown that combined processes for extracting collagen from these by-products may have a very high efficiency in separation of chromium, so that its content in collagen polydispersions would be within the strict limits allowed for drinking water (max. 50 ppb). In this research the collagen polydispersions are obtained by associated chemical-enzymatic processes with high yields extraction, under mild reaction conditions. The biological potential and film-forming properties of collagen polydispersions, recommend this as a valuable bioactive additive in mixtures for seeds treatment.

EXPERIMENTAL

Experimental Techniques

The collagen hydrolysates were obtained from tanned leather by-products, by chemical-enzymatic processing, at atmospheric pressure and temperature ≤ 80°C, for a total duration of max. 6 hours, followed by processing the resulting product be decanting and filtering, mixing batches and conditioning by adjusting pH at max. 7.

Methods of Analysis

The collagen hydrolysates obtained were analysed using: STAS 8574-92, Finished hides and fur finished hides; SR ISO 5397-96 for total nitrogen determination; SR EN ISO 4045-02 for pH determination; SR EN ISO 13903-05 for determination of amino acids content; Sorensen method to determine aminic nitrogen; STAS 8602-90 for chrome oxide determination; chromatography, with a gas chromatograph coupled with a mass spectrometer, AGILENT 7000 GC/MS TRIPLE QUAD Gas Chromatograph, to identify dipeptide, tripeptide and amino acid sequences and HPLC (Thermo Electron, Finningen Surveier) was used for the qualitative and quantitative determination of aminoacids (according to SR EN ISO 13903); IR spectral analysis by FT/IR-4200 (Jasco) with ATR device equipped; Dynamic Light Scattering (DLS), to determine particle size and distribution with ZetaSizer device Nano ZS (Malvern, UK); the CAM 200 optical system from KSV Instruments was used to measure contact angles under static conditions.

RESULTS AND DISCUSSIONS

The intermediary and final collagen hydrolysates, described in Table 1, were assessed by chemical and instrumental analyses, to establish chemical and physical characteristics and bioactive properties induced by the free amino acid content.

Table 1. Description of collagen hydrolysates samples

Sample code	L1C	L2C	MC	CMC
Description	Collagen hydrolysate from lot no. 1	Collagen hydrolysate from lot no. 2	Mixture of collagen hydrolysates from lots no. 1 and no. 2	Conditioned mixture of collagen hydrolysates, at pH = 7

Results of analyses performed in order to establish the chemical composition of collagen hydrolysates are presented in Table 2.

Table 2. Physical-chemical characteristics for collagen hydrolysates

No.	Characteristics, MU	L1C	L2C	MC	CMC
1	Dry substance, %	8.00	8.16	8.24	8.77
2	Total ash, %	8.50	8.95	7.89	7.87
3	Organic substance, %	91.50	91.05	92.11	92.13
4	Total nitrogen, %	16.13	15.69	15.78	15.17
5	Amino nitrogen, %	1.12	1.20	1.04	1.04
6	Average molecular weights, Da	10800	8800	12900	12900
7	Protein substance, %	90.63	88,11	88.71	85.18
8	pH	9.38	9.35	8.88	6.90

Aminic nitrogen content over 1%, corresponding to an average molecular mass below 13000 Da, indicates the possibility of a very high polydispersion of protein fragments. The presence of dipeptide, tripeptide and free amino acid fragments in collagen hydrolysates was investigated by chromatography, using a gas chromatograph coupled with a mass spectrometer.

Figure 1 presents the chromatogram of a collagen polydispersion (L1C), with molecular masses of components marked on each peak. The chromatogram reflects the fact that dipeptide and tripeptide fragments were separated, as well as fragments with mass values close to the mass of amino acids, in accordance with the results of previous research and confirming the reproducibility of the framework model for developing collagen hydrolysates (Niculescu *et al.*, 2009).

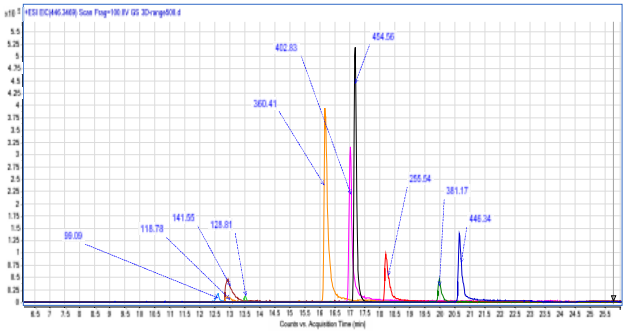

Figure 1. Chromatography of collagen hydrolysates

The IR-ATR spectroscopy method was used for the structural analysis of intermediary collagen hydrolysates and the experimental batch of collagen hydrolysate obtained by their combination. Corrections of ATR, CO_2 and H_20, baseline, were performed for each sample.

Figure 2 comparatively presents IR spectra of collagen hydrolysate batches collected from processes of hydrolytical disintegration of leather waste (collagen hydrolysates LC.1 and LC.2), of the final batch (MC.3) developed by combining batches of extracted hydrolysate and experimental batch obtained after pH correction (CMC.4).

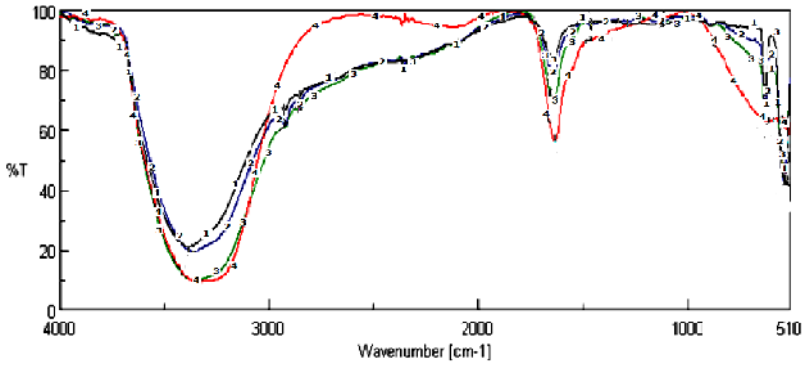

Figure 2. IR spectral analysis of collagen polydispersions: 1–L1C, 2–L2C, 3–MC, 4–CMC

Noteworthy is the presence of wave numbers in the 3100-2600 cm^{-1} and 1660-1610 cm^{-1} spectral ranges, specific to free amino acids, as well as in 1400-1465 cm^{-1} and 1264-1450 cm^{-1} spectral ranges, characteristics to proline amino acids, aspartic acid and glutamic acid (Balaban *et al.*, 1983; Barth, 2000). The spectral analysis provides information in accordance with chromatographic analysis, which signalled the dipeptide, tripeptide and amino acid content of collagen hydrolysates, extracted from semi-processed hide fragments.

Using the high-performance liquid chromatography method, it was established that collagen hydrolysates obtained by hydrolytical disintegration of residual semi-processed hide fragments have a total amino acid content of approximately 10 g/100 ml solution, with an average concentration of amino acids, presented in Figure 3.

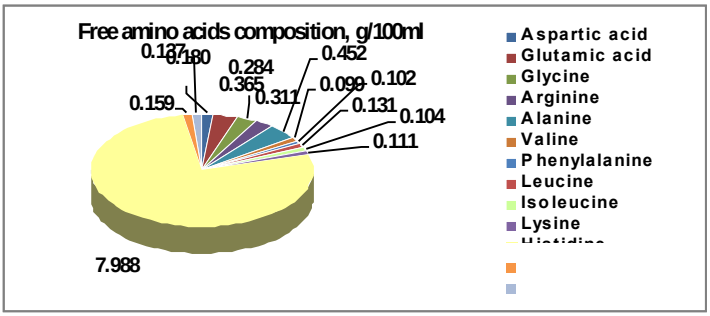

Figure 3. Average concentration of free amino acid in collagen hydrolysates

Particle size and distribution in the representative batches of collagen hydrolysates, L1C and L2C, obtained in this study, were analysed by DLS (Dynamic Light Scattering). Results of analyses are presented in table 3.

Table 3. Distribution of particle size in collagen hydrolysates

No.	Sample	Distribution of particle size, %		
		1-10 nm	100-1000 nm	1000-5000 nm
1	L1C	88.0	7.3	4.7
2	L2C	93.7	4.2	1.1

Another important feature of surface treatment products is the contact angle. The contact angle of a liquid drop and a solid surface is a sensitive indicator of changes in surface energy and in the chemical and supramolecular surface structure. Knowing the contact angle allows us to estimate the type of interaction between the surface and the liquid. In this regard, we determined contact angles of collagen hydrolysates in relation to glass, as inert control surface, and contact angles of collagen hydrolysates in relation to the surface of cereal seeds.

As seen in Figure 4, the contact angle varies in the 24°-44° range, relative to glass, but has much higher values and a broader range, 65°-105°, relative to the seed surface.

The contact angle of collagen hydrolysates in relation to seeds is found to be at least double compared to the contact angle in relation to glass, a consequence of surface roughness, on the one hand, and of collagen hydrolysate characteristics, on the other hand, important in this regard being the slight decrease of contact angle upon addition of acetic acid, for conditioned mixture of collagen hydrolysates, CMC compared with the mixture of collagen hydrolysates, MC.

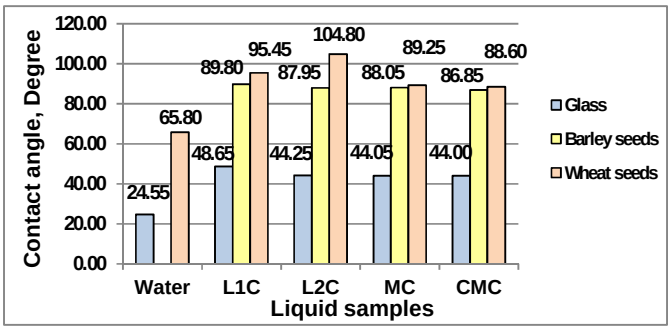

Figure 4. Comparative contact angles

It is noticed that particularly the wetting ability of collagen hydrolysates is lower than that of water, and their hydrophobicity is therefore favourable to the film matrix formation on seed surface.

CONCLUSIONS

It was proved that the collagen hydrolysate extracted from solid wastes from tanneries through chemical-enzymatic hydrolysis under atmospheric pressure conditions, at temperatures in the range of ≤ 80°C, by stirring, for maximum 6 hours, has bioactive properties, due to the diversified content of free amino acids, including essential amino acids, with a total of approximately 10 g/100 ml solution.

It was proved that the collagen hydrolysates predominantly contain very small sized particles, in the 1-10 nm range, able to penetrate the seed coating, but they also contain larger sized particles, situated in 100-1000 nm and 1000-10000 nm ranges, which will ensure the bioactive deposit in the film matrix on the surface.

The wetting ability of collagen hydrolysates is lower than that of water, property associated to the contact angle and favourable to film matrix formation on seed surface.

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THE INFLUENCE OF SILVER NANOPARTICLES ON THE SURFACE MORPHOLOGY OF FILM-FORMING MATERIALS AND THEIR ANTIMICROBIAL EFFICIENCY

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The surface morphology was investigated, surface area, bacterial adhesion and hydrophilic/hydrophobic character of antimicrobial coating materials. The materials have a different surface morphology depending on the content of silver nanoparticles. The new antimicrobial materials show a low bacterial adherence, facts suggest that the release of Ag^+ ions by the new material could be the major cause for the low bacterial adhesion on these materials. In exchange, the surface morphology matters in the improvement of overall performance. The surface morphology was investigated by SPM and the hydrophilic nature of the material was determined by the Goniometer method. The structural parameters (surface area, pore size distribution, pore volume) were determined by BET analysis. It was demonstrated that our formulations of film-forming materials with nanosilver in their composition have antimicrobial activity at these bacteria: *Staphylococcus Aureus* and *Bacillus Cereus*.

Keywords: nanoparticles, antimicrobial, surface

INTRODUCTION

In recent years, nanomaterials have gained interest due to their unique properties (Alissawi, 2013). Composition and morphology control of NPs plays an essential part in their special applications (Liu *et al.*, 2010). Nanomaterials that can be used in antimicrobial systems must be biocompatible (Podsiadlo *et al.*, 2005). The term "nanocoating" is commonly used for filling the polymer matrix with dispersed nanoparticles, having the average size below 100nm (Liu *et al*., 2010). The mixture of polymers and NPs opened the way to obtain flexible materials that have spectacular antimicrobial properties, electrical, optical or mechanical. In addition, nanoparticles can act in the polymer matrix, and can change the orientation of the polymer and its morphology, sometimes leading to micro phase separation (Menno *et al*., 2011). Silver NPs have effective antimicrobial properties, compared with other salts or silver compounds, due to the extremely large specific surface, providing better contact with microorganisms (Nedelcu *et al*., 2014).

EXPERIMENTAL PART

Materials

All the chemical substances were of analytical grade. Ethyl glycol acetate provided by Polydis, dispersion agents type Pigment disperser A, S supplied by BASF, film-forming material MPAS provided by the ICAA, dioctyl sodium sulfosuccinate surfactant supplied by Sigma-Aldrich and silver nanoparticles presented in a previous paper (Pica *et al*., 2012).

Equipment

The SPM Ntegra Aura NT-MDT platform offers opportunities for investigating the topography in 3D imaging and physical properties of surface materials starting at the microscopic level up to the nanometer level. The structural parameters (surface area, pore size distribution, pore volume) were computed from N_2 sorption (adsorption and desorption) isotherms, obtained using Quantachrome NovaWin 1200e automated surface area analyzer. KSV CAM 101 apparatus equipped with live digital camera and special analysis software was used for static contact angle measurements performed on metallic samples.

Preparation of Antimicrobial Film Forming Materials

There have been 3 antimicrobial film-forming material formulations (denoted as AM1, AM2, AM3), using the method of synthesis in solution. This method assumes the existence of a solvent (water, glycol) in which the AgNPs inflates and the basis polymer of the film-forming material is dissolved. The entropy gained by removing the solvent molecules allows the polymer chains to diffuse between the nanoparticles of the nanosilver. After the solvents evaporation a dry film with antimicrobial properties is formed. The method of synthesis of film-forming materials was presented in a previous paper (Pica *et al*., 2012). For antiseptic reasons, 250 –550 ppm AgNPs with particles size of 40 nm were used. The preparation and characterization of these AgNPs was presented in a previous paper (Pica *et al*., 2012).

RESULTS AND DISCUSSION

Surface Topography of Antimicrobial Film-Forming Materials

The SPM Ntegra Aura NT-MDT platform offers opportunities for investigating the topography in 3D imaging and physical properties of surface materials starting at the microscopic level up to the nanometer level. Investigations can be carried out in ambient environment or in vacuum (up to 10^{-2} torr). Figure 1 are three -dimensional images (3D) antimicrobial film-forming materials deposited on glass plates. SPM obtained studies on thin films showed very rough surfaces, with a columnar structure and uneven shapes of “valleys and hills”.

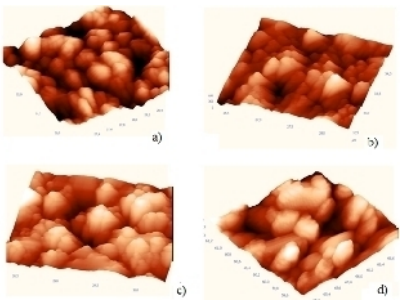

Figure 1. Surface topography of antimicrobial film-forming materials – images (3D), a) AM1; b) AM2; c) AM3; d) CM

Structures of film-forming materials are homogeneous and porous due to the tendency of nanoparticles, as development of "islands", whose surfaces are becoming increasingly large. The images of the antimicrobial film-forming materials do not show significant differences between them, but compared with conventional film-forming material (CM), these differences are significant. Was obtained an average roughness (AR) between 306-320 nm for antimicrobial coating materials doped with AgNPs; and an average roughness of 196 for conventional coating material (CM). The results achieved confirm the studies to this point, namely that the introduction of NPs into a polymer material increases the contact surface, the surface area, pore volume and surface roughness. Rough morphology is beneficial to the film in terms of durability and antimicrobial properties, which are significantly increased.

Table 1. Average roughness (AR) of antimicrobial film-forming

Sample	AgNPs content [ppm]	Roughness [nm]
AM 1	550	320
AM 2	450	312
AM 3	400	306
CM (without silver NPs)	0	196

Surface Area and Pore Size Analysis of AgNPs - Doped Antimicrobial Film-Forming Samples

The structural parameters (surface area, pore size distribution, pore volume) were computed from N_2 sorption (adsorption and desorption) isotherms, obtained using Quantachrome NovaWin 1200e automated surface area analyzer. The samples were first outgassed at 60°C for 180 min under vacuum to 0.3 Pa final pressure, and the isotherm were measured over the P/P0 (relative pressure range) from 0.05 to 0.995 (absorption) and 0.995 to 0.05 (desorption).

Table 2. Physisorption data for AgNPs and samples AM1 -AM 3

Sample	Surface area (S_{BET}), [m^2g^{-1}]	Pore volume, [cm^3g^{-1}]	C value from BET model
AM1	13.94	0.014	3.701
AM2	11.43	0.015	4.133
AM3	11.05	0.016	4.215
AgNPs	18.33	1.91 (monolayer)	0.456

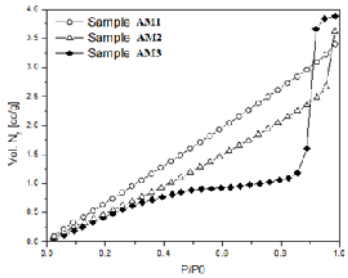

Figure 2. Nitrogen adsorption isotherm for antimicrobial film forming materials

The BET specific surface are (SBET) and the monolayer volume coverage were determined employing the Brunauer-Emmett-Teller (BET) equation.

The lack of a sharp "knee" point for the AgNPs-containing polymer samples indicates that the attractive adsorbate-adsorbent interactions are much weaker for N_2 molecules interacting with the polymer chains within the film-forming material sample, than with the Ag metal surface. The values of the C parameter of the BET equation are also reported in table 2. The C parameter is a measure of the strength of the interaction of the adsorbate (N_2) with the surface and the higher the C parameter, the sharper the 'knee point' in the early part of the isotherm at low P/Po.The value of C for nitrogen adsorption at 77 K on porous inorganic materials such as alumina or silica is typically in the range of 80–150 (Gregg and Sing, 1982). The C values shown in table 2 for the AgNPs samples are similar.

Contact Angle Measurements – Static Sessile Drop Method

Goniometer Method

The simplest way of measuring the contact angle is with a goniometer, which allows the user to measure the contact angle visually. The measurement of each contact angle was made for 60 seconds at 2 seconds frame interval. The contact angles reported were the mean of 2 determinations. Smaller contact angles correspond to increased wettability (hydrophilic surfaces, <90°), whereas higher values correspond to hydrophobic surfaces (>90°).

Table 3. Mean values for contact angles for the analyzed specimens

Sample	Contact angle (mean value), [°]	Comments
CM (without silver nanoparticles)	70.5	<90°, low hydrophilic
AM1	85.4	<90°, low hydrophilic
AM2	85.1	<90°, low hydrophilic
AM3	84.8	<90°, low hydrophilic

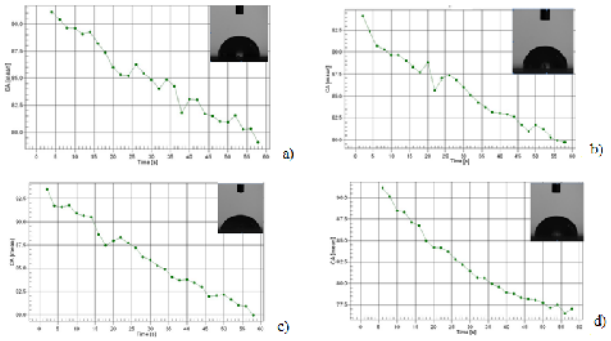

Figure 3. Variation of the contact angle in time for the: A – AM1 sample; B – AM2 sample; C – AM3 sample; D – CM sample

The samples were homogeneous in composition along the surface. Figure 3 shows the variation of the contact angle in time for the CM sample and AM1-AM3 samples using as reference liquid water. The samples AM1-AM3 show no significant differences in terms of contact angle values, and all of them have a higher value for the contact angle as compared to the CM (see table 3).

Antimicrobial Tests

Surface load of bacteria varies with the species of bacteria and is influenced by the growth environment, pH and ionic strength, age of bacteria, and surface structure of the bacteria. However, the relative contribution of bacterial surface load to bacterial adhesion was not clearly understood. For this reason we preferred to get a slightly hydrophilic antimicrobial material which retains a small amount of water. The bacteria are carried on the coating material by moisture (water). In return, moisture activates the silver nanoparticles which release silver ions. The resistance of the antimicrobial coatings to mass infection with the micro organisms was determined according to STAS 12719. This method is basically the direct contact between the AM antimicrobial coatings and the suspension of microorganisms. The AM1-AM3 film forming materials are applied on filter paper rings, dried and inserted into the culture dishes with micro organisms.

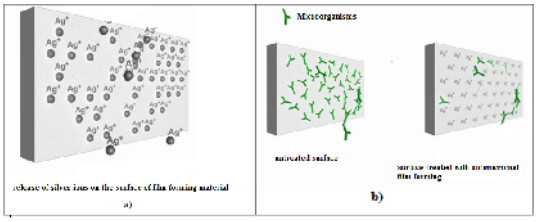

Figure 4. The mechanism by which a film-forming material exhibit antimicrobial effect: a) release of silver ions on the surface of film-forming material, b) the untreated surface is full of microbes and the surface treated with antimicrobial film-forming material destroys the microbes

Figure 4 shows schematically a surface treated with film-forming material that releases silver ions on the surface compared with untreated surface that is contaminated with germs. As you can see the surfaces treated with antimicrobial film-forming material, suppress microorganisms and won't let them grow. An active layer of film-forming material may be effective only if the active substance is released. Antimicrobial activity of a coating containing silver depends on the concentration of Ag^+ released. As shown in Figure 5, all the coating formulations (denoted as AM1- AM3), have antimicrobial activity, with a inhibition capacity between 87-99% (*Staphylococcus Aureus*) and 85- 98% (*Bacillus Cereus*) and the blank sample marked with CM (without silver nanoparticles) does not have antimicrobial activity. The nanosilver content positively influences the antimicrobial activity; the best results were obtained by the sample AM1, in which the AgNPs content is 550 ppm.

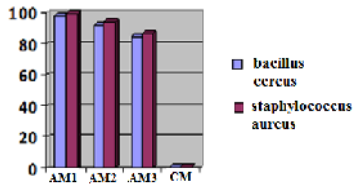

Figure 5. Growth inhibition of AM1- AM3 antimicrobial film-forming materials against *Staphylococcous Aureus* and *Bacillus Cereus*

CONCLUSIONS

The results achieved confirm the studies to this point, namely that the introduction of nanoparticles into a polymer material increases the contact surface, the surface area and pore volume and also the surface roughness and its hydrophilic character. The roughness morphology is beneficial for the particle in terms of sustainability and antimicrobials properties, which are significantly increased. It was shown that our formulations of film-forming materials with nanosilver in their composition have antimicrobial activity at these bacteria: Staphylococcus aureus, and Bacillus cereus.

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EXTRACTION OF COLLAGEN FROM FISH WASTE AND DETERMINATION OF ITS AMINO ACID COMPOSITION

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In this paper, the analysis of the most famous ways collagen-containing solid waste recycling of fish and leather industry was made. Their influence on the properties of the obtained hydrolysates was shown. The method for processing of collagen containing wastes from gutting mackerel (*Scomber*) is developed for the preparation of biopolymer materials for various purposes. The proposed method involves acid-enzymatic hydrolysis of waste in a solution of acetic acid in combination with the previous washing with alkali to remove soluble proteins. By means of ion-exchange chromatography using 339M automatic analyzer (Microtechna, the Czech Republic) have determined that the resulting hydrolyzate is balanced in amino acid composition and can be used to produce organic fertilizer and as growth promoter and as feed additive and after further modification as a component of biopolymers.

Keywords: collagen containing fish waste, acid-enzymatic hydrolysis, amino acid composition.

INTRODUCTION

Collagen is a general extracellular structural protein involved in the formation of connective tissues. Collagen occurs in genetically distinct forms identified as type I to type XIX. They vary considerably in amino acid composition and structure.

Industrial utilization of collagen is very wide. The main sources of industrial collagen are limited to those from pigskins and bovine hides and bones. Collagen is ductile and is used in different fields, such as leather and films, cosmetics, biomedical and pharmaceutical industries, and in food (Ratnasari *et al.*, 2013; Gaidau *et al.*, 2013; Bostaca and Crudu, 2013). A considerable proportion of collagen is consumed in the manufacture of food gelatins that have a number of functional properties as gel and mousse, thickening agent, emulsifier, stabilizer, protective colloid (Schrieber and Gareis, 2007).

The occurrence of bovine spongiform encephalopathy (BSE) and foot/mouth disease (FMD) along with religious constraints has resulted in an anxiety among users of collagen and collagen-derived products from land-based animals. In recent years, increasing attention has been paid to alternative collagen sources, such as fish skin, which comprise about 30% of the total fish weight available after fish fillet preparation (Gómez-Guillén *et al*., 2011).

Modern production of fish is accompanied by the formation of a large number of collagen wastes (bones, fins, skin, scales, viscera, etc.) that ranged from 30 to 70% by weight of the feedstock (Shahidi and Botta, 1994). Partial use of them, on one hand, leads to the loss of an important protein based product and other purposes, and to the other, to pollution

Several studies have focused on the characterization of different fish collagens. Most fish collagens consist of two α-chain variants, which are normally known as α1 and α2. In addition to differences in molecular types, fish collagens have been shown to vary widely in their amino acid composition. In particular, the physical properties of the

protein and the quantity of the amino acids proline and hydroxyproline vary significantly among fish species, and this is strictly correlated with the outside temperature of the animal's environment (Muyonga *et al*., 2004).

The greatest features of fish collagen are lower denaturation temperature and viscosity than collagens of land-vertebrated animals. These features distinguishing fish collagens from land-vertebrated collagens are very important for food processing. This led to a recent increasing interest in fish collagens (Kimura and Ohno, 1987; Leuenberger, 1991).

The shrinkage temperatures (T_s), values of fish-skin collagens range from about 35°C to 57°C, according to the mean temperature of the environment[1], while mammalian collagen has a T_s value of about 62°C. By an examination of the complete amino-acid analyses, fish-skin gelatin hydroxyproline and mammalian gelatin are 67 and 95 respectively (residues of amino acid per 1000 total residues); the lower the amount of hydroxyproline the lower the value of T_s (Rigby and Spikes, 1960). Interchain hydrogen bonding between hydroxyl groups of hydroxyproline and backbone carbonyl groups was, on this basis, suggested as an important stabilizing feature of the collagen structure.

The aim of this work is to develop a method to use collagen-containing materials after the production of mackerel (*Scomber*), and to determine a rational way of using the obtaining collagen-based materials.

MATERIAL AND METHODS

As an object of our investigation was used waste mackerel (offal) obtained after butchering of the fish. Typically, these wastes are not reused and disposed to the landfills. Wastes were preserved using salt (100% v/w salt). The chemical composition of the fish waste is presented in Table 1.

Table 1. Physical-chemical properties of the salted fish waste

Content in the waste, %	
– moisture	43.5
– mineral substances	38.6
– fatty substances	5.9
– total nitrogen content	7.9

Obtaining of Collagen Hydrolysates

Collagen hydrolysate is denatured and partly hydrolysed protein. Collagen hydrolysates display properties that depend on the raw material source and processing method. Collagen hydrolysates may be prepared through acid hydrolysis (mostly dilute H_2SO_4, HCl or H_3PO_4), alkaline hydrolysis (for example NaOH, KOH or $Ba(OH)_2$), enzymatic hydrolysis or microbial breakdown.

The use of enzymes in combination with acids or alkalis and high temperatures to obtain hydrolysates is the best method found to create collagen hydrolysates preventing breaking down of amino acids, carbohydrates and other nutrients contained in the waste (Nam *et al.*, 2008).

In this work alkali-enzymatic and acid-enzymatic methods were used. The level of hydrolysis was determined by total nitrogen content.

Alkali-enzymatic hydrolysis of fish waste was carried out for 6-8 hours at 40°C as follows: offals were washed with running water, crushed to the consistency of stuffing, loaded into the reactor, 50% of water was added; 1.6% v/w H_2O_2; 2% v/w NaOH. The enzymatic hydrolysis was then carried out for 4 hours, enzyme demand was 3%. The resulting mixture was adjusted to pH 6.8 with a solution of sodium carbonate. After separation of the layers of the hydrolysate, it was evaporated to the desired concentration.

The level of hydrolysis was monitored by total nitrogen content of the hydrolysis product, it was 12.2 g /l. The disadvantage of the obtained product was a dark brown color and an unpleasant "fishy" smell.

To intensify the process of hydrolysis, further treatment with hydrogen peroxide was used. The effectiveness of this treatment was confirmed in previous studies using waste from the leather industry (Plavan *et al*., 2013). Unfortunately, in the case of fish waste, such treatment was not effective. Although the total nitrogen content in the final product was 15.4 g /l. The unpleasant smell of the product was still present as result of the formation of peroxides via partial oxidation of the fats.

Relative high fat content in the collagen-containing waste of the fish industry affects negatively the properties of hydrolysates. Fat undergoes oxidation, which leads to a rapid breaking down of the resulting products, it is also the source of the unpleasent "fishy" smell. Therefore, it is necessary to carry out degreasing, the essence of which is to free the pores, capillaries and extracellular space of the fat contained.

The most rational way to degrease collagen-containing wastes from the fish industry is the use of enzyme pretreatments. Enzymes break down proteins and the structure of the tissue, thus as a result – the release of the fat.

For the acid-enzymatic hydrolysis, solutions with different concentrations of acetic acid and enzyme were used. The enzyme was Zime SB: activity 1500 U/g, the optimal pH 3.5-6.5. Consumption of enzyme was 1.3%. The degree of hydrolysis was determined by the total nitrogen content in the final product (Table 2).

Table 2. Characteristics of the methods and products of hydrolysis

Variant	Washing with alkali	Carrying hydrolysis				Content, g/l		
		alkali	enzyme	acid	H_2O_2	Nitrogen	Dry matter	Ash
1	-	+	+	-	-	12.2	218.9	124.4
2	-	+	+	-	+	15.4	215.3	120.2
3*	+	-	+	+	-	11.2	15.2	4.9
4**	+	-	+	+	-	14.3	22.3	9.0

*Alkali washing duration 24 hrs. Alkali-enzymatic hydrolysis duration 4 hrs, at 40°C.

** Alkali-enzymatic hydrolysis duration 4 hrs., at 40°C and 8 hrs. Room temperature (left overnight). Alkali washing duration decreased to 1,5 hrs. Alkali was dosed in 3-4 receptions after 30 min.

The Method of Ion-Exchange Liquid-Column Chromatography

To conduct qualitative and quantitative analyses of amino acid composition of the collagen-containing material of the resulting hydrolyzate, ion-exchange liquid-column chromatography with the 339 M automatic analyzer (Microtechna, the Czech Republic) was employed.

RESULTS AND DISCUSSION

Decreasing the alkali washing time leads to an increase in mineral content in the final product (Table 2). The increasing of the acid-enzymatic hydrolysis reaction speed has positive effect on the quality of the final product, indeed the content of total nitrogen increases.

As a result of hydrolytic decomposition of fish collagen, the number of basic amino acids increased due to breaking down of the peptide bonds (Table 3), arginine content increased to 9.59%. Hydrolysate content of the essential amino acid histidine in fish collagen is 1.37%. Very important amino acids for the nutrition of young animals are: isoleucine and leucine (1.72 and 5.04%), methionine (2.03%), threonine (3.92%), and phenylalanine (2.84%), the latest is higher in fish collagen hydrolysate than in hydrolysate from cattle skins.

Table 3. Amino acid composition of fish and cattle hide hydrolyzate (%)

Amino acid	Nativecattle hide collagen (Heidemann, 1993)	Cattle hide collagen hydrolyzate (Plavan *et al*., 2013)	Native fish collagen (mackerel) (Sun Young Lim, 2012)	Fish collagen hydrolyzate
Glycine	33.5	7.10	3.4	17.68
Proline	12.2	6.86	3.8	6.64
Alanine	12.0	6.76	5.4	7.79
Hydroxyproline	9.4	8.25	5.4	1.76
Glutamic acid	7.6	6.13	14.8	15.16
Arginine	5.2	7.11	5.6	9.59
Aspartic acid	4.6	4.21	10.3	7.66
Serine	3.1	2.35	4.3	5.92
Leucine	2.2	1.77	8.4	5.04
Lycine	2.5	5.94	10.5	4.73
Valine	1.7	4.05	6.1	2.63
Threonine	2.0	3.74	4.9	3.92
Isoleucine	1.1	1.55	5.0	1.72
Phenylalanine	1.1	2.19	4.6	2.84
Methionine	1.0	0.68	3.0	2.03
Histidine	0.3	1.02	6.6	1.37
Tyrosine	0.0	1.34	1.2	1.64
Total	100	100	100	100

The presence of reactive capable amino groups, gives us the ability to change the properties of hydrolysates (Zhongkai Zhang, 2006). The most reactive capable groups of proteins are those containing the amino acids serine (whose primary group is the -OH group), hydroxyproline (secondary -OH), threonine (secondary -OH), tyrosine (phenolic -OH), aspartic and glutamic acids containing the group –COOH and lysine and arginine containing alkaly groups (Mokrejs *et al*., 2010). Hydrolysates can be chemically modified (Bucevschi *et al.*, 1999) by the application of crosslinking reagents (in particular aldehydes, starch, enzymes).

Thus, the amino acid composition of the obtained hydrolysate is balanced. It can be used as an organic fertilizer and as growth promoter in animals food, after further modifications as a component of composite materials and biopolymers.

CONCLUSION

A method was developed for the disposal of collagen-containing mackerel (S*comber*) waste to get collagen-based biomaterials for various purposes. The method involves the acid-enzymatic hydrolysis of the waste in a solution of acetic acid and enzyme in conjunction with the previous washing with carried out with alkali to remove soluble proteins. Reducing the duration of alkali washing leads to an increase in mineral content in the final product. The increasing of the acid-enzymatic hydrolysis has positive effects on the quality of the final product and the content of total nitrogen increases. The amino acid composition of the resulting hydrolysate is balanced and it can be used to produce organic fertilizers and growth promoters in animals food, after further modifications as a component of composite materials and biopolymers.

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INVESTIGATION OF PHYSICOCHEMICAL AND BIOLOGICAL CHANGES IN THE COLLAGENS UNDER THE INFLUENCE OF THE PROKARYOTIC ORGANISMS-ACIDS SYSTEM

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Great attention is being paid nowadays to the ecological safety and rational technologies of leather production. New technologies of waste processing make it possible to get products that could find wide application in different industries. Products of collagen dissolution (PCD) are among them. To get a high quality product with specified properties it is necessary to research the characteristics of the modified collagen and to analyze the influence of proteins present both in leather tissue and combined whey. The objective of the research is to study the collagen-chemical and thermo-dynamic properties of modified collagen (PCD) produced on the basis of different nature organic acids and combined whey. In the paper physical and chemical properties of colloid system PCD-acid were investigated. Adsorption and dampening ability, structural, mechanical and rheological characteristics of modified collagen were also defined. There has been found out that the surface activity of the modified collagen produced on the basis of combined whey is the result of symbiotic influence of proteins present both in derma (collagen) and in the combined whey (casein). Research of the mechanism of collagen - acid interaction on properties of collagen dissolution products could make it possible to better understand collagen structure.

Keywords: modified collagen, prokaryotic organisms-acids system, combined whey

INTRODUCTION

The current stage of the human society development witnesses global use of natural resources. The characteristics feature of any resource cycle is not only the increasing amount of the resource being taken out but also environment contamination. Material resources are applied in production processes where some part of them is processed into final products while the other one forms waste.

Both liquid and solid waste is formed in great amount in leather and fur processing. In the course of processing one ton of raw material (hides and skins) using the traditional technology the amount of final material (leather) is 200 kg., i.e. leather industry produces a great deal of waste. Waste accumulation in great amount influences negatively the environment, its utilization costs much. Great attention is being paid nowadays to the ecological safety and rational technologies of leather production.

New technologies of waste processing make it possible to get products that could find wide application in different industries. Products of collagen dissolution are among them. The objective of the research is to study the collagen-chemical and thermo-dynamic properties of modified collagen (products of collagen dissolution – PCD) produced on the basis of different nature organic acids and combined whey.

EXPERIMENTAL AND RESULTS

The Study of the Influence of Solvents' Nature on the Quality of Produced Modified Collagen

To produce modified collagen (PCD) there have been applied collagen-containing hide waste being contoured and subjected to partial biological decay.

After being selected the specimens were subjected to soaking and sharpened liming made according to the traditional technology of hide treatment for the footwear upper. Modified collagen has been produced by alkali-salt technology.

Hides having been limed were washed in the running water (t = 8°C) for 24h. then cut pieces of the size 2x2 cm were salted for 4h at the temperature 18±2°C, LC = 3, Na_2SO_4 concentration – 40g/dm^3. After that the specimens were subjected to alkali-salt treatment in sodium hydroxide and sodium sulphate. The specimens were again salt-treated in the solution of sodium sulphate for 4h with LC = 5 and the temperature of 18±2°C.

To remove the alkali surplus the neutralization by the solution of ammonia sulphate for 3h with pH4 was used. The rest of alkali was controlled by the lack of pink colour of the cut treated by the indicator of phenolphthalein. Finally hide waste was thoroughly washed in the running water (t = 8±2°C) until there was no sulphate-ions (quality reaction with boron chloride is the lack of amorphous white sediment) in the washing water. Acid treatment of collagen-containing raw material was made in different organic acids with pH2 – 3: lactic acid (pH2.3), aminic acid (pH2.85), acetic acid (pH2.5), combined whey (mixture of curds and cheese whey) with pH3.04 and 269°T.

The time of destruction of acid-liable binds of collagen-containing raw material conducted with LC = 5 and the temperature of 20±2°C was different depending on the acid type and amounted for the system with: lactic acid – 144h or 6 days and nights, aminic acid – 480h or 20 days and nights, acetic acid – 48h or 2 days and nights, combined whey – 384h or 16 days and nights. To produce the monodisperse system of PCD the Braun type 4191 blender was applied for 2 min (v = 10 r/s). To remove the acid surplus from the colloid system-PCD-organic acid the dialysis was performed for 7 days and nights (with lactic acid), 9 days and nights (with aminic acid), 6 days and nights (with acetic acid), 7 days and nights (with combined whey). The temperature of the running water for all the treatment variants was 8±2°C.

Colloid systems produced with different acids looked differently: PCD – lactic acid was non-transparent; viscid-fluid yellowish system with the density of 1000 kg/m^3 and pH 6.44; PCD-aminic acid was non-transparent, viscid, grey-yellowish system with the density of 997 kg/m^3 and pH 6.90; PCD-acetic acid was grayish, non-transparent, viscid, non-homogenous system with the density of 1055 kg/m^3 and pH 7.5, and PCD-combined whey was non-transparent, viscid, beige-colour system with the density of 1013 kg/m^3 and pH 6.31.

The content of the amino nitrogen, dry sediment and protein concentration were defined in the PCD. Physical and chemical properties of PCDs produced with the help of organic acids are given in Table 1.

Digital ionometer U-135 was applied to measure pH, the density was measured by aerometer.

Table 1. Physical and Chemical properties of colloid system PCD-acid

Acid used for dissolving	pH	Density kg/m^3	Dry remnant, %	Amino Nitrogen, g/dm^3	Protein concentration, g/dm^3
Aminic	6,90	997	7,96	0,126	2,37
Lactic	6,44	995	22,20	0,196	2,74
Acetic	7,50	1055	4,70	0,390	4,78
Combined whey	6,31	1013	28,40	0,462	5,48

Minimum content of amino nitrogen, protein and dry remnant is characteristic for the system PCD-aminic acid, while the maximum one – for the system PCD-combined whey (Table 1). Such high content of protein in the colloid system PCD-combined whey could be explained probably by the presence of proteins from not only hide tissues but also from casein containing secondary waste.

The produced modified collagen for all the treatment variants contained only ammonia cation.

A very important characteristic of PCD is the molecular mass according to which it is possible to define the survival of the three-spiral collagen structure and identify it. Molecular mass was calculated on the equation of Mark-Khuvink, where $K=1{,}34\ 10^{-4}$, $\alpha=0{,}71$ at the temperature 22-25°C.

Molecular mass of PCD depended greatly upon the type of acid treatment. PCD on the basis of cultured milk compositions coefficient (CMC) had smaller molecular mass than PCD on the basis of pure acids. It might be connected with the following: applying CMC as an acid agent the disordering of collagen fibres is caused not only by the acidic but also ferment dissolution.

The greatest molecular mass of PCD was registered after being treated by SMC 4 ($72.4 – 74.6 \times 10^3$ c.u.). increasing the concentration of PCD the molecular mass for all the acidic treatments increased on 2000 conventional units.

The Study of Thermodynamic Properties of Modified Collagen

The most important characteristic of the inter-phase surface of the system bio-polymer-solvent is surface tension that characterizes the surplus of free surface energy per 1m^2 of the inter-phase surface as its presence is characteristic for disperse systems. Adhesion refers to surface phenomena (adsorption and dampening) and means molecular interaction of contacting surfaces of condensed phases of different nature. Adhesion provides certain connecting strength between two surfaces thanks to the molecular forces of different nature. Adhesion work characterizes the strength of adhesion connection. It corresponds to the possible connection break per the unit of area.

To study the thermo-dynamic properties there have been used modified collagen solutions (PCD), produced by dissolving collagen-containing raw material in lactic, aminic and acetic acids and combined whey.

Surface tension was defined by stalagmometric method. Solutions with different PCD concentration were applied to define surface tension and dampening angle. Protein concentration was defined by Yarosh method.

Analysing the influence of modified collagen concentration on the degree of surface tension it can be pointed out that the increase of PCD concentration in the system results

in the decrease of the degree of surface tension for all the studied variants. The maximum decrease of surface tension degree is for the colloid system PCD-combined whey. It could be connected with the symbiotic influence of proteins present both in derma (collagen) and combined whey (casein).

Adsorption process takes place due to the decrease of surface tension. Adsorption isotherm was built according to the surface tension isotherm using Gibb's equation. To calculate Gibb's quantity the tangent method that made it possible to define the surface activity was applied (Figure 1).

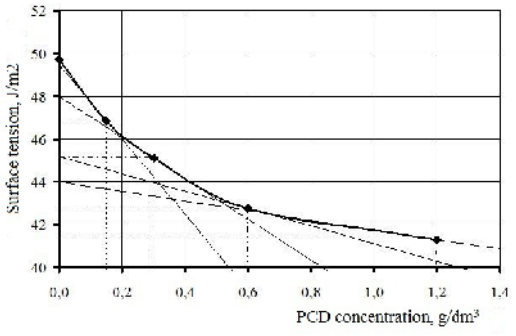

Figure 1. Isotherm of surface tension for colloid system PCD-combined whey

On the basis of experimental data has been found out that Gibb's quantity increased with the increase of PCD concentration in the system biopolymer-organic acid, while in the combined whey it did not change. That is why we believe the application of combined whey could provide greater disordering of collagen macromolecules.

Thus, according to the results achieved in the research it can be stated that adsorption ability of the organic acids under study increases in the line aminic acid – acetic acid – combined whey – lactic acid.

Study of PCD Influence upon Dampening Ability of Organic Acids (on the Example of Wax Film)

To study the dampening ability of the system PCD-acid there have been measured dampening angles Θ. Ten parallel measurements have been made for every concentration under study. The experimental data show that with the increase of PCD concentration in the organic acid solution the edge angle decreases which demonstrates the dampening ability. The maximum dampening effect is characteristic for the system PCD-lactic acid, and the least one – for the PCD – combined whey. This might be connected with the presence of basic hydroxide group in the molecule of lactic acid that contributed to the development of inter-phase interaction. Increase in the dampening ability of the colloid system PCD – aminic acid can be explained by the presence of aldehyde group in the molecule of the given acid.

Study of Adsorption and Dampening Ability, Structural, Mechanical and Rheological Characteristics of Modified Collagen

It has been defined that the increase of modified collagen concentration in the colloid system PCD – organic acid leads to the decrease of the quantity of surface tension for all the studied variants. The maximum decrease of surface tension was characteristic for the colloid system PCD – combined whey, which might be connected with the symbiotic influence of proteins present both in leather tissue and combined whey.

That is why there was applied the combined whey being a by-product consisting of lactic and curds whey as a reagent for the acidic hydrolysis of the collagen-containing raw material in further research. To study the influence of a specific type of modified collagen on some thermodynamic properties of the colloid system PCD – combined whey there has been made a number of solutions with the decreasing concentration of PCD, g/dm^3: 3.0; 2.0; 1.0; 0.5; 0.25; 0.17, and 0.05.

To estimate surface activity and dampening ability experimental data for the change in the quantity of surface tension and dampening angle has been achieved. A drop of the liquid under research was placed on the covered with wax glass and the dampening angle was measured. Experimentally there has been proved that lactic acid concentration in combined whey does not greatly influence the thermo-dynamic characteristics of the modified collagen produced.

Unique dependence is characteristics for all the variants of substances having surface properties, and namely, with the influence of PCD concentration in the colloid system PCD – combined whey the quantity of surface tension decreases for all the systems being studied. The maximum decrease of the surface tension quantity has been achieved for the colloid system modified collagen – combined whey with the lactic acid content of 20 g/dm^3 and made up 59,14 j/m^2. It might be connected with the fact that the collagen-containing product, was characterized by a great number of highly-molecular segments that were formed as a result of preserving in its structure peptide binds.

Structural and mechanical properties of modified collagen with respect to viscidity have been studied to confirm the results achieved. Viscidity was defined on the viscometer VPZh-2 (SS 10028-67) with the diameter of capillary d = 0.99 mm. Solutions density was measured by aerometer.

On the basis of the data it can be stated that putting modified collagen into the colloid system PCD – combined whey results in the viscidity decrease for the variant where the acidic hydrolysis was made applying combined whey with 20 g/dm^3 lactic acid.

CONCLUSIONS

On the basis of the results achieved the following conclusions could be made:

1) maximum decrease of surface tension and correspondingly, high dampening ability is provided by modified collagen produced while applying in acidic hydrolysis the combined whey with the lactic acid concentration of 30 g/dm^3;

2) there have been studied adsorption and dampening abilities, structural and mechanical, rheological characteristics of modified collagen;

3) there has been found out that the surface activity of the modified collagen produced on the basis of combined whey is the result of symbiotic influence of proteins present both in derma (collagen) and in the combined whey (casein).

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MATHEMATICAL MODELS OF COLLAGEN STRUCTURE DISORDERING BY CULTURED MILK COMPOSITIONS

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A lot of enterprises face nowadays the problem of utilizing protein-containing waste. In recent decades based on the results of research aimed to study connective tissue there has been offered a way to utilize protein waste, i.e. to produce on their basis products of collagen dissolution (PCD). The process of PCD manufacturing is a complex of chemical and technological processes based on breaking both alkali- and acid-labile bonds. To decrease protein losses in breaking acid-labile bonds it is recommended to use cultured milk compositions (CMC). CMC are a symbiosis of acid-tolerant microorganisms, as well as organic acid and enzymes produced by them. Objective of the study was to obtain the models of changing physical and chemical properties of PCD in reference with the conditions of the process of disordering its structure. The disordering the collagen structure is a complex multi-stage process. Mathematical modelling has been chosen as the basic method of solving the problem. The feature of the research is the use of cultured milk compositions as acid agent. Research data could make it possible to develop the process and increase the quality of products of collagen dissolution at the cost of preserving in the collagen structure a considerable amount of polypeptide groups.

Key words: collagen, mathematical model, cultured milk compositions

INTRODUCTION

In recent decades based on the results of deep research aimed to study such a multifunctional system as connective tissue there has been offered a way to utilize protein waste, i.e. to produce on their basis products of collagen dissolution (PCD).

The process of PCD manufacturing is a complex of chemical and technological processes based on breaking both alkali- and acid-labile bonds. To speed up intermolecular horizontal bonds breaking in the structure of mature collagen there have been traditionally applied gas limes or alkali-salt solutions. As a result, the number of hydrolysed peptide bonds in collagen can increase that leads to the decrease of molecular mass of polypeptide chains. Besides breaking alkali-labile bonds in PCD manufacturing it is necessary to provide the breaking of acid-labile ones, the latter is achieved by applying acid of different chemical nature. However, acid-labile bonds are broken mainly at increased temperatures that cause additional protein losses because of its transfer to the treating solution.

To decrease the protein losses some researchers offer to apply enzymes (Pat. 2094999 RF). However, in this case it is impossible to achieve a high degree of substrate hydrolysis because of enzymes inactivation. In this connection hydrolysis is done in the regime of partial enzyme input that increases the amount of the latter and, consequently, the cost of the final product, i.e. the efficiency of enzymatic process decreases considerably.

To decrease protein losses in breaking acid-labile bonds it is recommended to use cultured milk compositions (CMC) developed by the authors (Pat. 2399678 RF). CMC are a symbiosis of acid-tolerant microorganisms, as well as organic acid and enzymes produced by them. Acid-tolerant microorganisms in CMC provide acid-liable bonds breaking.

On the basis of the above it could be supposed that in case of breaking intermolecular acid-liable bonds of CMC it might be possible to get PCD with high molecular masses and good colloid and chemical properties at the cost of preserving a considerable amount of poly-peptide groups in PCD.

MATERIALS AND METHODS

PCD have been manufactured by raw materials treatment in CMC after preliminary alkali-salt treatment. Not standard leather raw materials (GOST – State standard 28425-90. "Leather raw materials. Technical conditions") has been applied. After rehydration, fatting, ashing and deashing made according to the accepted technology of leather production by chroming the treatment has been conducted according to the scheme on Figure 1.

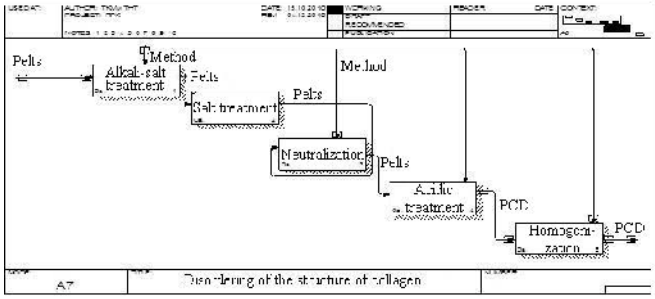

Figure 1. Raw material treatment to manufacture PCD

Acidic treatment of collagen-containing raw material has been made by CMC produced by cultivating symbiosis of kefir fungi in pasteurised quark whey (Pat. 2399678 RF).

Figure 1 shows that disordering the collagen structure to get a PCD is a complex multi-stage process; its results depend on a number of factors. Taking this into account as well as the peculiarities of the modelling object, mathematical modelling has been chosen as the basic method of solving the problem.

RESULTS AND DISCUSSION

The process of collagen structure disordering to produce PCD is a practical problem and it requires the application of simple mathematical means.

The treatment temperature and titratable acidity have been chosen as input variables. The treatment temperature is to be optimal as its decreasing could result in inhibiting metabolic processes in microorganisms while temperature increase leads to their death. The quality of the product produced depends on the degree of acid-liable bonds destruction due to organic acids and enzymes available in CMC. Decreasing the titratable acidity up to 200°T and less does not give positive results due to the insufficient amount of lactic acid in CMC. The treatment temperature influences upon the microorganisms life and productivity and, consequently, upon the degree of defibering. The degree of destruction of acid-liable bonds depends upon the value of titratable acidity.

The weight fractions of ash and fatty substances have been used as output variables. The weight fraction of ash is the index characterizing the PCD composition as well as the presence of additional admixtures that can decrease the quality of the final product. The weight fraction of substances being extracted by organic solutions can characterise the changes in the product composition during treatment as a result of extraction being caused by symbiosis action of microorganisms within temperature range. Initial availability of fatty substances in the leavens of pH chlorine-potassium extract characterises the presence of H^+-ions in the media under study; it is very important for acid treatment of collagen-containing raw material. Gelatine output is the criteria that characterises the degree of collagen structure disordering, emission of low-molecular proteins in the course of changing the condition of PCD production.

To begin with, there has been made an experiment to define the factor space. The experiment results have shown that dependence of such physical and chemical indexes as weight fraction of ash, fat substances, pH chlorine-potassium extract and gelatine output upon the process temperature and titratable acidity is not linear. Besides, there has been found out that output variables in case of input variables being limited can change linearly. With the account of that the plan of full factor experiment has been chosen to build the mathematical model. The number of experiments for two input variables ($k = 2$) equals $N = 2k = 4$.

The coefficients of the regression equation have been defined by the method of least squares according to the known technique. The expanded matrix of experiment planning in the coded (X_1, X_2) and natural (Z_1, Z_2) variables as well as the experiment results to define the weight fraction of mineral substances (Y_1), weight fraction of fat substances (Y_2), pH chlorine-potassium extract (Y_3), gelatine output (Y_4) are given in Table 1. All the research has been done in the laboratory.

There has been defined the succession of experiments with the help of the random numbers table (randomising) to compensate the experiment systematic errors. The mathematical model achieved looks like

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 \quad (1)$$

The statistical analysis of the value of model's coefficients estimation and the accuracy control has been made according to the regression analysis formulas.

Regression equation coefficients has been considered significant in case $t_{calculated} > t_{tabled}$, where $t_{calculated}$ and t_{tabled} are calculated and tabled values of the Student criterion for the equation coefficients (defined on the significance level equal to 0,05, the number of freedom degree $f = 4$). If the calculated Student criterion is less than the tabled one, then the regression equations describe the experimental data adequately.

The models achieved are valid only for the chosen range of input variables changes, i.e. for the dissolution temperature 4 – 24°C and the titratable acidity of CMC 143 – 331°T.

To define the optimal parameters of the collagen dissolution process the coded variable equation was changed into the natural values equation (normalized model). There have been found equations describing the dependence of gelatine melting out, weight fraction of the substances being extracted by organic solutions and mineral substances as well as pH chlorine-potassium extract upon the process conditions.

While defining the gelatine output the regression equation is as follows:

$$Y = 79{,}6652 + 16{,}8063X_1 + 12{,}3547X_2 - 10{,}372X_1X_2 \quad (2)$$

This equation has been used to make the parameters of the collagen dissolution process optimal. The equation analysis shows:

a) the coefficient b_1 has got the highest value (absolute value), so it has been the process temperature that has got the greatest influence upon the gelatine output in the range under research;

b) the coefficient b_{12} has proved to be significant, so in the range of two input variables under research the joint influence of the temperature and titratable acidity of the composition has a considerable influence on the output variable (though according to the absolute quantity the value is not large).

While defining the gelatine output the normalised equation after transformations looks as follows:

$$Y = 4{,}2947Z_1 + 0{,}2957Z_2 - 0{,}0114Z_1Z_2 - 11{,}4995 \quad (3)$$

Figure 2 shows the model of the dependence of gelatine output weight fraction upon process parameters.

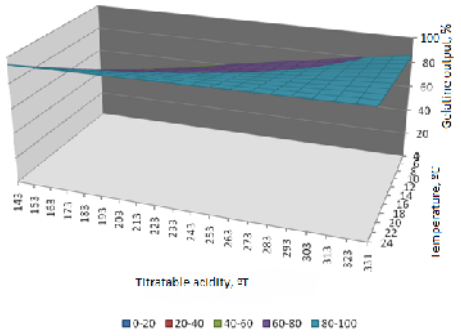

Figure 2. Model of dependence of gelatine output weight fraction upon process parameters

The gelatine output change is proportional to the temperature and titratable acidity increase. While choosing optimal parameters for collagen acidic dissolution besides gelatine melting out parameter an additional estimation criterion to define the quality of the product is necessary. Theoretical values of gelatine output dependence upon titratable acidity have been calculated according to the equation.

Analysing the model, it is necessary to point out that gelatine melting out grows considerably with the titratable acidity of the composition and temperature increase.

While defining the weight fraction of mineral substances the regression equation is as follows:

$$Y = 3{,}6864 - 0{,}6508X_1 + 0{,}6938X_2 \quad (4)$$

The equation analysis shows that:

a) the coefficient b2 has got the greatest value (according to the absolute quantity), so the titratable acidity of the cultured milk composition has a considerable influence on the weight fraction of the mineral substances in the range under research;

b) the coefficient b12 has turned out to be not significant, so in the range of two input variables under research the joint influence of the temperature and titratable

acidity of the composition has not got a significant influence on the output variable. While defining the weight fraction of the mineral substances after transformation the normalized equation can be written as follows:

$$Y = 2{,}85 - 0{,}07Z_1 + 0{,}01Z_2 \quad (5)$$

The resulting equation was applied to make the parameters of collagen dissolution process optimal. Figure 3 demonstrates the model characterising the dependence of the weight fraction of mineral substances upon the parameters of the process.

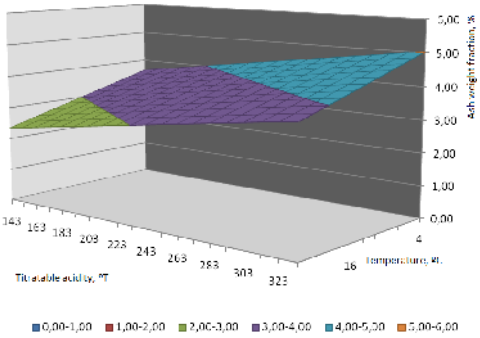

Figure 3. Model of dependence of weight fraction of mineral substances of PCD upon the process parameters

The change of the weight fraction of mineral substances is proportional to the temperature and titratable acidity increase. This might be connected with dependence of the metabolic activity of microorganisms and consequently the degree of collagen structure disordering upon the parameters mentioned above. The authors suppose that while choosing the optimal parameters of collagen acidic dissolution this very model should be applied with the account of the PCD properties which are to be achieved.

CONCLUSIONS

The analysis of models and their graphical interpretation has demonstrated that besides the gelatine output indexes, the ones of the weight fraction of substances being extracted by organic solvents as well as mineral substances pH chlorine-potassium extracts it is necessary to apply an addition estimation criterion to define the quality of the product achieved, for example, the microbiological analysis of CMC or a deeper research into the structure of PCD/PCD films which could help to produce high qualitative characteristics of the product.

There have been built according to the research results the models of changing physical and chemical properties of PCD in reference with the conditions of the process of disordering its structure. Thus, according to the authors the optimal process parameters to produce PCD is the temperature of 24±2°C, titratable acidity of 250 – 300°T and applying of CMC based on the jeaven of kefir fungi cultivated on pasteurised whey.

These data could make it possible to develop the process and increase the quality of products of collagen dissolution at the cost of preserving in the collagen structure a considerable amount of polypeptide groups.

Acknowledgement

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IONIZING RADIATION EFFECTS ON BIOGEL USED FOR SERUM PROTEIN ELECTROPHORESIS

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Irradiation with high energy electron beams allows direct sterilization of the plates with agarose gel packed in sealed aluminium foil bags and used for human serum protein electrophoresis. Human blood serum contains a large amount of protein (6.5 to 8.5 g/dL) and a greater diversity but, in terms of medical interest, the following six show better importance: albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin. The effects of electron beam irradiation upon this biogel and on the process of the proteic fraction separation were investigated at different absorbed doses in order to establish the optimum level. Avoiding dysfunction coming especially from changing of serum, dye or fixing solution or from mechanical defects (cracked gels on the support film) resulted in obtaining satisfactory results, as proved by tests made at 12 months after the agarose gel film making and irradiation. Microbiological decontamination of the agarose gel films irradiated in the dose range 3-12 kGy could be maintained for a period of a year. Also, agarose gel film can be microbiological decontaminated by irradiation with EB in the range 7-11 kGy without undergoing major changes in the electrophoretic properties for a storage period that can be appreciated to 12 months. There are two dose ranges where the irradiated films change more significant the separation of protein fractions: a low dose range between 4 and 6 kGy, and a range of doses between 12 and 20 kGy.

Keywords: electron beam, electrophoresis, agarose gel, proteic fractions

INTRODUCTION

Serum protein electrophoresis, using gels as a separation, migration and attachment medium is a fast and accurate method to obtain, by specific laboratory tests, extremely important information in diagnosing and monitoring patients treatment for a large number of clinical diseases in human medicine: multiple myeloma, chronic inflammation, autoimmune diseases, liver cirrhosis, chronic hepatitis, monoclonal gammapthies, Waldenström's macroglobulinemia, etc. (O'Connell *et al.*, 2005).

Gel serum protein electrophoresis with migration and separation in electric field, called "gel electrophoresis" is used as an analytical method because it allows clear separation of proteins from a protein blend, in well defined areas for each constituents, while maintaining intact their structural and functional properties. The advantage of this method is that separation zones or bands obtained by electrophoresis can be attached, viewed and stored for long periods, allowing the researcher or clinician to quickly assess the number, concentration and nature of a mixture of proteins and the degree to which that protein mixture differs from a standard protein mixture, e.g. a mixture of serum proteins in healthy patient or a monitored patient during treatment (Jacoby and Cole, 2000).

Proteins make up 6-8% of the human blood and they are macromolecular substances of polypeptide nature, fulfilling the basic functions, specific to living organisms. Serum is blood plasma without fibrinogen and other clotting factors. Medical and

biochemical research laboratories are constantly facing the problem of separation of serum proteins without disrupting their bio-physical and chemical properties. The separation's accuracy of protein fractions, on which depends the diagnosis and treatment of patients is mainly caused by the chemical and biochemical characteristics of the gel used as a medium for migration. Therefore, we aimed to achieve an electrophoresis kit with an improved gel, with features that allow rapid and accurate diagnosis of diseases investigated by electrophoresis of proteins isolated from human blood, simultaneously ensuring a long period of conservation of the kit.

Although very suitable for electrophoresis (Hoffman *et al.*, 2000; Ravel, 1995; Nauck, 1995), agarose-based gels is a favorable environment for microorganisms. This alters micro electrophoretic properties of gel, especially the resolution in electrophoretic separation of protein fractions. As a result, the development of germs of various microorganisms, but especially fungi on agarose films reduces accuracy in interpreting the results and the correct diagnosis and treatment of patients investigated on proteinographs. Also, agarose gels are easy to degrade under the action of light, dehydrate under heat action. The first important required measure is encapsulation of gel films in special cassettes to keep them away from contamination and degradation under the action of heat, humidity and light. Classical methodology applied to avoid contamination with microorganisms of the biogel films is their processing under special conditions of instruments and rooms sterility, which greatly increases the product cost.

This paper shows the solution of electron beam (EB) irradiation for decontaminating microbiological films of agarose gels, and its net benefits compared with other methods. EB sterilization (Burns *et al.*, 1996; Mehnert, 1996; Mondelaers, 1998), is able to keep the agarose gels advantages (speed, flexibility, lack of toxicity) and to eliminate the drawbacks (low conservation time) for this type of biogel. Irradiation with high energy electron beam allows direct sterilization of agarose films ready embedded in casettes and aluminized foil which protects them from moisture and light.

Also, the effects of the EB absorbed dose on the process of the protein fraction separation with irradiated agarose gel, have been investigated. Therefore, we aimed to achieve an electrophoresis kit with an improved gel, with features that allow rapid and accurate diagnosis of diseases investigated by electrophoresis of proteins isolated from human blood, simultaneously ensuring a long period of conservation of the kit. There is not any previous work in the literature examining the use of EB irradiation to the sterilization of the agarose gel put on plastic plates used for electrophoresis.

EXPERIMENTAL

The experimental setup with EB irradiation of agarose gel film is shown in Fig. 1, and mainly consists of the ALIN-10 electron linear accelerator of 6.23 MeV and 164 W maximum output power, built in Romania, National Institute for Lasers, Plasma and Radiation Physics, and the mobile platform on which aluminum bags containing plastic boxes with agarose gel plates are placed. EB irradiation was carried out in a very precise manner, regarding both electrical measurements like the absorbed dose rate D*, the absorbed dose D, the kinetic energy E_{EB} and geometric measurements of the distance H between the EB output window and the mobile surface on which irradiated samples are positioned, and the cross-section S of the EB field at the distance H. Dose distribution cannot be homogeneous across all cross section S of the EB field. In our case, we have imposed that the maximum non-uniformity to not exceed 15%. A post acceleration beam focusing and bending is utilized for ALIN-10 accelerator to project

EB at right angles to the accelerating structure. This allows a selection and a precise measurement of electron energy used for irradiation of biological systems.

An equation derived from the area through-put equation is a product line speed equation wherein the Linear Processing Coefficient, K, is commonly used in the low-energy EB area in relating average beam current to line speed (Cleland, 1984):

$$D\ (kGy) \cdot V_{platform}\ (cm/min) = K \cdot I_{EB}\ (\mu A) \quad (1)$$

wherein K is typically ~10 to 30 depending on the electron energy, the EB field width, window thickness and air gap between the window and product. Experimental measurement results made to determine the relationship between D, $V_{platform}$ and I_{EB} have shown that the relationship (1) can be used in our irradiation experiments, too.

The EB irradiation was applied on sealed bags made from aluminum foils, each bag containing a plastic box (0.123 m x 0.1 m x 0.005 m) with agarose gel on a plastic plate (0.1 m x 0.085 m x 0.001 m). In the experimental studies was used 1% agarose gel put on the plastic plates. The used migration solution is Tris-barbital tampon pH 8.6. Electrophoresis of serum proteins on agarose gel was performed by the applied a voltage of 100 V during 20 minutes.

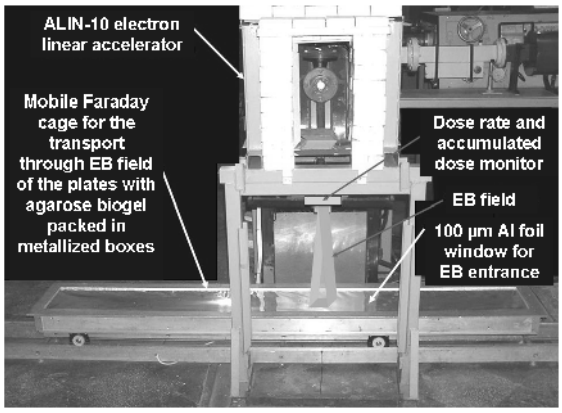

Figure 1. Frontal photo of the mobile Faraday cage containing boxes with agarose gel foils placed in sealed bags made from aluminum foils

RESULTS AND DISCUSSION

In order to evaluate the effects on the absorbed dose of parameters variations in the irradiation process, namely the EB average current and the platform speed from the relationship (1), were determined and plotted for an average factor K = 11.5, the characteristics showing the variation of dose D according to 7 different values, between 3.5 µA and 6.5 µA for I_{EB} and 15 different values, between 3.3 cm/min and 28.3 cm/min for $V_{platform}$. Also, we operated at large values of I_{EB} to benefit of the reducing of the effects of its variations on the dose and the possibility to use high speeds of the platform with samples and thus to obtain short times and high processing productivity. By irradiation on the conveyor belt of the packages containing foils coated with agarose, these, due to their continuous movement through the EB field, are exposed to

more uniform dose distribution compared with a static irradiation. It was calculated the useful penetration for E_{EB} = 6.23 MeV and the maximum number of packages stacked during irradiation. This information is important for processing productivity. Packages with agarose film cassettes were distributed on the mobile platform in 11 groups of 8 pieces placed one above the other.

In order to establish the optimum absorbed dose level for the sterilization of the plates with agarose gel, we studied the effect of different doses, from 4 kGy to 20 kGy. Regular tests were conducted over a period of 12 months, and were intended to identify the optimal dose of radiation for the microbiological decontamination while maintaining, and possibly improving, the electrophoresis properties of agarose biogel deposited on the support foils. Investigation was focused on changes that EB irradiation produced to agarose gel, compared with non-irradiated samples, in the electrophoresis of protein fractions. Human blood serum contains a large amount of protein (6.5 to 8.5 g/dL) and a greater diversity but, in terms of medical interest, the following six show better importance: albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin.

The results obtained from samples of a patient with normal proteic fraction values are presented in Fig. 2 and of a patient with acute inflammatory process are presented in Fig. 3. Relative protein concentrations within each fraction were determined as the optical absorbance percentage that is the ratio between the absolute quantity of each fraction (g/dl) and the total serum protein multiplied by 100. We mention that the values for each point on the characteristics of Figs. 2 and 3 are the result of the mediation of 10 different measurements made on each of the 10 positions on the same sheet, with the same serum from the same patient.

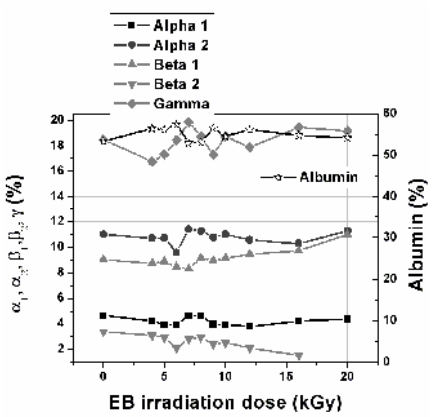

Figure 2. The results for serum protein electrophoresis obtained with agarose plates irradiated at different EB doses from a patient with normal proteic fraction values

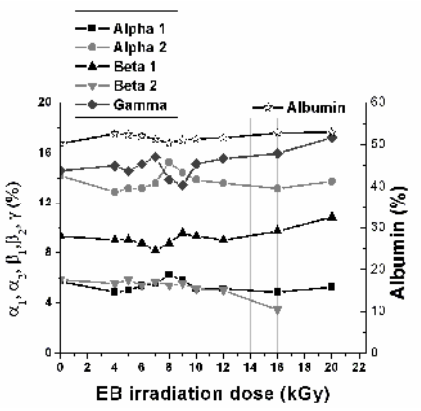

Figure 3. The results for serum protein electrophoresis obtained with agarose plates irradiated at different EB doses from a patient who had an acute inflammatory process

The analysis of experimental results led to the following conclusions:

a) Agarose films irradiated in the range 2-12 kGy differentially affects protein fractions: the smallest variations in relation to control values shows albumin protein fractions (+1.3% to -3.8%), alpha 1 (+7.4% and -4.5%) and beta 1 (0% to -4.9%) and

the highest variations are for the following protein fractions: alpha 2 (+13% to 0%), beta 2 (+21% with -0%) and gamma (+13% to 0%), beta 2 (+21% with -0%) and gamma globulin (+7.5% to -10.6%).

b) Separation of protein fractions alpha 2, beta 2 and gamma globulin shows more significant variations on agarose films irradiated with doses between 3kGy and 7kGy compared with values obtained from non-irradiated control films. However, the values obtained on foils irradiated with 8-9 kGy tend to return to the values obtained for the non-irradiated control films. It seems that certain doses of irradiation could improve the electrophoretic separation of protein fractions.

c) For each protein fraction there is an optimum doses range for which the separation of fraction is not substantially affected or tends to be very close to the values obtained for non-irradiated films. These ranges are: 8 - 12 kGy for albumin, 6 - 9 kGy for alpha 1; 8 - 9 kGy for alpha 2; 2 - 12 kGy for beta 1; 6 - 8 kGy for beta 2 and 11 - 12 kGy for gamma globulin.

d) The porous structure and the regularity and size of biogel's pores are essential in the process of migration, separation and attachment of protein bands obtained by electrophoresis. EB irradiation may affect this process, depending on the administered dose of radiation and the microscopic structure of agarose gel.

e) The irradiated and unirradiated agarose gel plates were microbiological tested on the following medium types: sabouraud medium for the fungi; gelose-blood for the gram-positive germs and CLED medium for the gram-negative germs. The microbiological results showed that above 5 kGy the irradiated plates are sterile.

Fig. 4 and Fig. 5 present a summary of the results obtained by investigating for a period of 12 months of a batch of 11 groups of 4 packages with agarose gel cassettes, at 11 different values of irradiation doses, in the range 2 – 12 kGy.

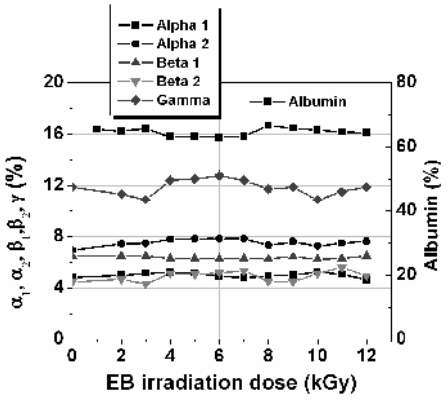

Figure 4. The synthesis graphic of the variations of protein fractions depending on the EB absorbed dose, determined at 4 days after irradiation

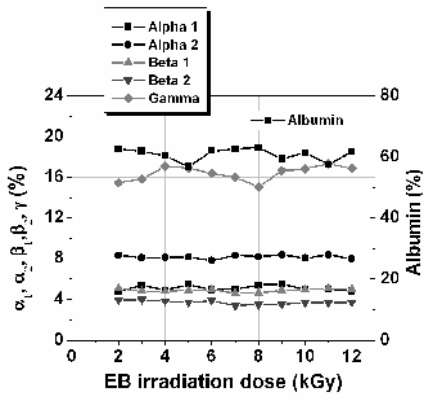

Figure 5. Variations of protein fractions depending on the EB absorbed dose, determined at 12 months after irradiation

Fig. 4 shows the synthesis graphic of the variations of protein fractions alpha 1, alpha 2, beta 1, beta 2, albumin and gamma depending on the EB irradiation dose, determined at 4 days after irradiation. Fig. 5 shows the synthesis graphic of protein

fractions variations determined after a year from irradiation that was made in the same day.

It was found that after a year, on the surface of the films irradiated with doses of 3-12 kGy, there was no development of molds that normally appears on the agarose gel and affects the most accurate electrophoretic separation of protein fractions. Otherwise, we found the non-irradiated control foils covered with mold. Also, in the 7-11 kGy range, we observed that agarose gel film can be decontaminated without major changes of microbiological electrophoretic properties. All irradiated films tested over 12 months gave, for all protein fractions, values showing less than 10% maximum deviations compared with the average values of each fraction. The most net and reproducible electrophoretic separations were observed at the irradiated films in the 7-11 kGy dose range. The described experimental setup with EB irradiation was used for electrophoresis kits treatment that were sent to medical clinics and hospitals.

CONCLUSIONS

Avoiding dysfunction coming especially from changing of serum, dye or fixing solution or from mechanical defects (cracked gels on the support film) resulted in obtaining satisfactory results, as proved by tests made at 12 months after the agarose gel film making and irradiation. Microbiological decontamination of the agarose gel films irradiated in the dose range 3-12 kGy could be maintained for a period of a year. Also, agarose gel film can be microbiological decontaminated by irradiation with EB in the range 7-11 kGy without undergoing major changes in the electrophoretic properties for a storage period that can be appreciated to 12 months. In addition, the separation of protein fractions in the 7-9 kGy range on the irradiated films, are better delineated than those of the same patient done on the unirradiated foil control.

The experimental setup presented in this paper ensure a sufficient productivity for a monthly service of a medium medical tests laboratory.

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SWELLING AND DRUG RELEASE OF POLY(VINYL ALCOHOL)/GELATIN COMPOSITE HYDROGEL

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Poly (vinyl alcohol)/gelatin (PVA/Gel) composite hydrogel was prepared by freezing-thawing and morphology of the hydrogel was characterized by scanning electron microscope (SEM). The swelling behavior in different pH buffer solutions was studied. With salicylic acid as model drug, the drug releasing process of the hydrogel was investigated. It was found that the polyvinyl alcohol/gelatin composite hydrogel has porous structure. The swelling rate and equilibrium swelling degree increases with the increase of gelatin content in it. The PVA/Gel hydrogel behaves sensitive to the temperature and pH. At the pH of 7.4, the drug release is the fast.

Keywords: poly (vinyl alcohol), gelatin, composite hydrogel

INTRODUCTION

Hydrogel is a kind of functional polymer materials with moderate crosslinking and three-dimensional network. It is insoluble in water, but can significantly swell in water by absorbing a lot of water. So it is good in water holding. The swelling is the most basic and important performance of hydrogel. It has found applications in many fields including biomedical materials and bio-engineering (Gan and Nong, 2010).

Polyvinyl alcohol hydrogel (PVA) has been widely used, mostly because of its biocompatibility, mechanical properties, film forming, non-toxic, and no side-effects. Its application has extended to medicine, food, environmental protection etc (Yoshida *et al.*, 1991; Woerly, 1997). However, the mechanical properties of PVA hydrogel is poor at room temperature, and it is not easy to control the biodegradation, resulting in less commercial value (You *et al.*, 2007). Prepared by the hydrolysis of collagen, gelatin is good in biocompatibility and biodegradability. With gelatin as raw material to prepare hydrogel, the utilization rate of the medicine may be greatly improved with a decreased side-effect and prolonged drug duration. However, pure gelatin is easily soluble in water, hard and brittle when dried. It is poor in mechanical properties too. When the two materials are blended to prepare hydrogel, it is expected to yield hydrogel with the combined advantages, biological activity, and different required swelling degree. Few studys are reported on the drug releasing hydrogels with PVA and gelatin.

The PVA/gelatin composite hydrogel was prepared by freezing-thawing. The swelling behavior at the pHs of both simulated gastric acid and normal human blood was studied, as well as the drug releasing process of the composite hydrogel with salicylic acid as the model drug.

MATERIALS AND METHODS

Main Materials and Reagents

Polyvinyl alcohol(PVA), analytical reagent, was from Tianjin Fengchuan Chemical Reagent Co., Ltd., China; Gelatin and Ortho-hydroxybenzoic acid were all analytical reagent and made by Tianjin Kemi'ou Chemical Reagent Co., Ltd., China.

Preparation of Pva/ Gelatin Hydrogels

PVA was dissolved in deionized water at 90°C and cooled to 60°C for subsequent use. Gelatin was dissolved in distilled water at 60°C to obtain gelatin solution. Both the solutions of PVA and gelatin were mixed at 60°C for 2h. After being ultrasonic de-gased, the mixture was poured in a self-made mold and frozen at -20°C for 22 hours, and then, completely thawed at room temperature. That is a freeze-thaw cycle. The process was repeated four times to obtain the PVA/gelatin hydrogels. According to the amounts of gelatin in the hydrogels, the samples were labeled as PVA/G5, PVA/G10, PVA/G15, PVA/G20, indicating the mass fraction of gelatin in the hydrogel of 5%, 10%, 15% and 20%, respectively.

Morphological Characterization of PVA/Gelatin Hydrogels

After being freeze-dried, a loose porous hydrogel was prepared. The dry hydrogel samples were gold sprayed and the surface morphology was observed by SEM.

Swelling Behavior of PVA/Gelatin Hydrogels

After being vacuum dried, the hydrogels were weighed, noted as m_0. The samples were then soaked in deionized water at 27°C and 37°C, respectively. The swelling ratio was calculated by equation (1):

$$\text{Swelling rate}=\frac{m_t-m_0}{m_0}\times 100\% \quad (1)$$

where m_t is the weight of the hydrogel sample at the soaking moment of t, and m_0 is the weight of the dry sample.

Swelling Kinetics of PVA/ Gelatin Hydrogels

In order to know the diffusion behavior of water molecules in the hydrogel, equation (2) and equation (3) were used (Rathna and Chatterji 2001):

$$F=\frac{m_t-m_0}{m_e-m_0}=kt^n \quad (2)$$

$$\ln F=\ln k+n\ln t \quad (3)$$

where m_e is the equibrium weight of the hydrogel, k is the swelling parameters, and n is the swelling index. When n is not more than 0.5, the swelling is Fick's diffusion process, and when is between 0.5 and 1.0, the swelling is non-Fick's diffusion process.

Drug Loading Behavior of PVA/ Gelatin Hydrogels

After the temperature of the PVA/gelatin solution was lowered from 60°C to 40°C, 4wt% salicylic acid solution was added and the mixture was stirred for at 40°C for 1h. After being ultrasonic degased, the mixture was poured in a self-made mold, followed with four freeze-thaw cycles, yielding the drug-loaded PVA/ gelatin hydrogels.

The Drug Releasing Curve of the Composite Hydrogel

At 37°C, hydrogel samples were added in 150mL buffering solution at pH 1.0 and stirred at 100rpm. 5mL solution was picked out and another 5mL same buffering solution was added. After 30min, the absorbance at 290nm was determined with UV-VIS spectrophotometer. Equation (4) was used to obtain the cumulative drug releasing amount at time *t*.

$$Q = 150C_t + \sum_{i=1}^{t-1} 5C_i \tag{4}$$

where *Q* is the cumulative drug releasing amount, *Ct* is the drug concentration obtained at the moment of *t*. The drug releasing curve of samples at pH 7.4, 37°C was obtained in the same way.

RESULTS AND DISCUSSION

After being dried, the PVA/gelatin hydrogels appear milky white and loose. The images observed with SEM were as shown in Figure 1.

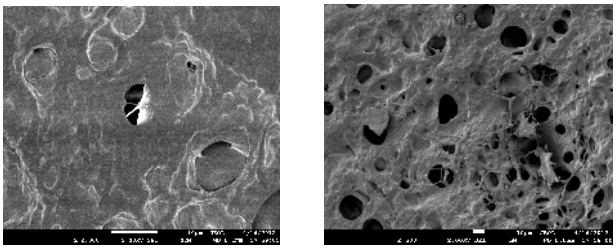

Figure 1. SEM images of PVA (left) and PVA/gelatin (right) hydrogel

In Figure 1, with the introduction of gelatin, the porous of the hydrogel became more abundant, and the structure became looser. There are plenty of such hydrophilic groups as amino and carboxyl in gelatin. When gelatin is blended with PVA, more water will be absorbed. When being frozen and vacuum dried, the water in the hydrogel will escape from the hydrogel while gelatin and PVA were still in a frozen state, resulting in the pore or holes in the samples. Besides, the difference in contraction coefficients of PVA and gelatin may be another reason for the porous structure.

In Figure 2, for the hydrogels with different gelatin contents, the swelling behavior is different. With increasing the gelatin content from 5% to 20%, both the swelling rate and the equilibrium swelling degree of the hydrogel increase. The molecular chain of pure PVA is well-structured, a crystalline polymer. Even after a freeze-thawing, the compact aggregation structure is kept. When gelatin is introduced in the system, gelatin will diffuse in the network of PVA to form a semi-interpenetrating network (Lin et al. 2010). New hydrogen bonding between, gelatin-PVA will be formed, and the original structure of pure PVA will be destroyed. As a result, the structure changed looser, making it easier for water to diffuse in swelling process. Besides, gelatin is a hydrophilic polymer, and the introduction of gelatin will increase the water absorption. So the swelling rate and equilibrium swelling degree are increased.

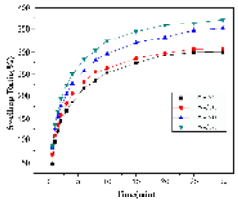

Figure 2. Swelling curves of PVA/ gelatin hydrogel at 37°C, pH1.0

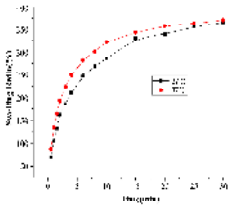

Figure 3. Swelling curves of PVA/G20 hydrogel at different temperatures

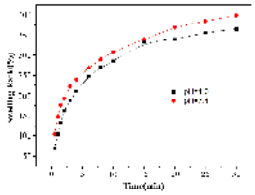

Figure 4. Swelling curves of PVA/G20 hydrogels at 37°C

Figure 3 shows the swelling curves of PVA/G20 hydrogel at pH 1.0 and different temperatures. At higher temperatures, it exhibited higher equilibrium swelling degree and greater swelling rate, indicating temperature sensitivity. With increasing the temperature, the molecules move faster, making the water diffusion easier. On the other hand, increasing the temperature will accelerate the movement of side groups in the system, also help increasing the binding and transferring of water molecules.

Figure 4 is the swelling curves of PVA/G20 hydrogels at different pHs. The swelling rate and equilibrium swelling degree at pH 7.4 are higher than those at pH 1.0. As a hydrolysate of collagen, gelatin contains plenty of carboxyl and amino groups to behave amphoteric electrolyte. At higher pH, carboxyl will dissociate to show $-COO^-$, and amino is in the form of $-NH_2$. At a lower pH, amino $-NH^{3+}$ will be formed, and the carboxyl group is in the form of -COOH. The isoelectric point of gelatin is about 6. At different pHs, the interaction force between the molecules is different. As a result, the aggregation structure and swelling degree of the hydrogel are different.

According to the swelling kinetics equation, the corresponding *lnF* and *lnT* were calculated, and *lnF-lnt* curves were obtained as shown in Figure 5 and Figure 6. The swelling index *n* was got by the linear-fitting the lines in the figures.

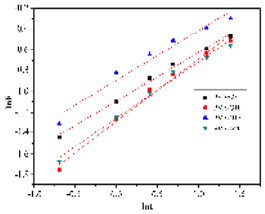

Figure 5. lnF-lnt of PVA/ gelatin hydrogels with various gelatin content at pH 1.0, 37°C

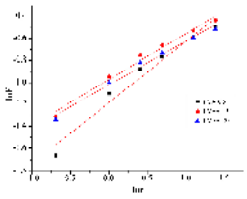

Figure 6. lnF-lnt of the PVA/ gelatin hydrogels with various gelatin content at pH 7.4, 37°C

Table 1. Swelling kinetics parameters of the hydrogel

	pH=1.0		pH=7.4	
	N	R2	N	R2
PVA/G5	0.4792	0.9924	0.3973	0.9596
PVA/G10	0.5476	0.9868	——	——
PVA/G15	0.5571	0.9670	0.5182	0.9813
PVA/G20	0.6042	0.9859	0.5302	0.9846

The swelling index *n* was shown in Table 1. At the gelatin content in the hydrogel of 5%, the *n* is less than 0.5, indicating a Fick's diffusion (Lin *et al.*, 2010). The water absorption is mainly by the hydrophilic interaction, and the water diffusion into the hydrogel is freely. The gelatin in the hydrogel is very little, and not much groups may be dissociated. So the interaction between the molecules in the hydrogel does not affect the water diffusion greatly. At the gelatin content of more than 10%, the *n* is between 0.5-1.0, showing a non-Fickian diffusion. With the addition of gelatin in the system, crosslinking sites are increased, and the chain segments between crosslinking sites turn smaller, which may slow down the relaxation rate of the macromolecules. Therefore, with increasing the gelatin content in the hydrogels, both the water diffusion and the relaxation behaviors in the swelling process may be affected. On the other hand, from the *n*, we know the at a higher gelatin content, there is less difference between the water diffusion rate and molecular chain relaxation rate (Nugent and Higginbotham, 2007).

The *n* at the pH of 1.0 is higher than that at 7.4. As an amphoteric electrolyte, at acidic condition, gelatin is positive-charged, which will react with PVA to slow down the relaxation rate, affecting the water diffusion. At pH 7.4, near the isoelectric point of gelatin, less reaction between gelatin and PVA takes place, the relaxation is less affected, yielding a decreased *n*.

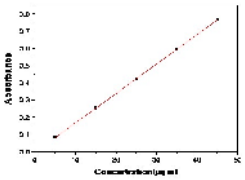

Figure 7. Standard curve of salicylic acid at pH 1.0

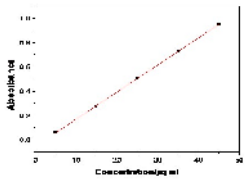

Figure 8. Standard curve of salicylic acid at pH 7.4

The standard curves of hydrochloric acid at the pH of 1.0 and 7.4 are shown in Figure 7 and Figure 8, indicating a linear relationship between the UV absorbance at 290nm and the drug concentration. So we may measure the salicylic acid content of a solution with unknown salicylic acid concentration by it.

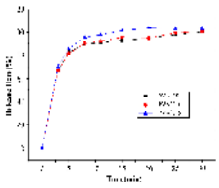

Figure 9. Drug releasing curves of the hydrogel with various gelatin content at pH1.0, 37°C

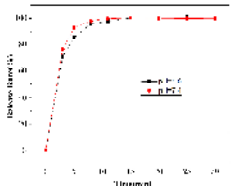

Figure 10. Drug releasing curves of PVA/G15 at different pHs, 37°C

The drug releasing curves of the hydrogels with different gelatin contents at pH 1.0 were shown in Figure 9. The drug-loaded hydrogels exhibited excellent drug releasing. The drug releasing curve may be roughly divided into three such stages as burst release, stable release and complete release. With increasing the gelatin content in the hydrogels, the drug releasing rate increases slightly, which agrees with the results of swelling rate. There are carboxyl and phenolic hydroxyl groups on salicylic acid, and gelatin contains plenty of amino, hydroxyl. With the addition of gelatin, the combining sites of drug on hydrogel is more, and much more pores are provided as shown in the SEM images in Figure 1. So with increasing the gelatin content, the drug releasing capacity is increased. In the present study, the pore affects the drug releasing more. Figure 10 shows the drug releasing curves of PVA/G15 hydrogel at indifferent pHs. The drug releasing of the hydrogel at different pHs is similar, although a rapid drug releasing rate is shown at pH 7.4, indicating a pH sensitive hydrogel, which will affect the drug releasing to some degree.

CONCLUSIONS

PVA/gelatin hydrogel with rich pores was prepared by freezing-thawing. The swelling rate and equilibrium swelling degree increase with increasing the gelatin content. At the same temperature, the swelling rate and equilibrium swelling degree at pH 7.4 are higher than those at pH 1.0. At the same pH, the swelling rate and equilibrium swelling degree at pH 7.4 are higher than those at pH 1.0. At the gelatin content less than 5%, the water diffusion in the hydrogel behaves Fick diffusion. The PVA/Gel hydrogel is sensitive to temperature and pH.

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THE EFFECTS OF IRON AND LIGHT INTENSITY ON BIOMASS AND PIGMENT SYNTHESIS OF *Heamotococcus pluvialis* UNDER LABORATORY CONDITIONS

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In the present study the effects of iron and light intensity on biomass and pigment synthesis of *Heamotococcus pluvialis* (Chlorophyta) was studied under laboratory conditions. In the culture medium composed different light intensity (50, 200 ve 475 µmol photon $m^{-2}sn^{-1}$) and by suplementing Fe+EDTA, the species *H. pluvialis* which subjected to 11 hours light and 13 hours darkness photoperiod, growing parameters was determined. The experiments were applied in 2 processes and during the experiment period optical density, chlorophyl-a (mg L^{-1}), dry weight(g/mL^{-1}) and astaxsanthin amount was followed. The results are; while astaxanthin was raising, chlorophyl-a was reduced; raising light intensity and Fe+EDTA supplement make raise the amount of astaxsanthin. The highist astaxanthin value (% 0.768) obtained from the study is provided by 200µmol photon $m^{-2}sn^{-1}$ light intensity with Fe+EDTA supplement ($p<0.05$).

Keywords: Haematococcus pluvialis, astaxanthin, light intensity, Fe, growth

INTRODUCTION

Photosynthetic cells are of great importance as primary producers of various organic matters and for their ability to regenerate atmosphere. Many algal biotechnologists have studied the application of the photosynthetic machinery of algal cells to the production of new bioactive compounds and to environmental processes over the last several decades. Microalgae have vast potential as sources for valuable pharmaceuticals, pigments, vitamins, proteins, fatty acids, sterols, polysaccharides and other biologically active compounds, or potential health benefits (Metting and Pyne, 1986; Richmond, 2004). A red ketocarotenoid pigment, astaxanthin (*3,3'-dihydroxy-β,β-carotene-4,4'-dione*), has received an increasing interest from the cosmetics, the food and the feed industries. *Haematococcus pluvialis* is the richest source of natural astaxanthin and is now cultivated at industrial scale. Astaxanthin is a strong coloring agent and a potent antioxidant – its strong antioxidant activity points to its potential to target several health conditions.

A quality of products from *H. pluvialis* has over 70% contents of diester form and over 80% contents of 3S, 3S' form in total astaxanthin. Therefore contents of astaxanthin in total carotenoids are show to over 95% and contents of astaxanthin per cell are show to ~5.0%. But disadvantages of production by microalgae are low cell density by light limitation and low salt medium (Borowitzka, 1988; Orosa *et al.*, 2001). Astaxanthin disperses towards the periphery of *Haematococcus* cells under light induction, and moves back towards the center after illumination is discontinued (Yong and Lee, 1991). No major quantitative or qualitative changes occur during this migration. Red cysts are more resistant to photoinhibition than green cysts, strongly indicating a photoprotective role for astaxanthin. The specific rate of astaxanthin accumulation is a function of the photon flux density *Haematococcus* cultures are exposed (Lee and Soh, 1991). Continuous illumination is most favorable for astaxanthin formation, and carotenoid content is correlated proportionally to light quantity (Kobayashi *et al.*, 1992).

Light intensity showed significant effect on cell growth and level of astaxanthin accumulation in the cells. The optimum photon flux density corresponding to the maximum level of algal biomass production was 60 $\mu E \cdot m^{-2} \cdot s^{-1}$. High light intensity

caused relatively large quantities of astaxanthin to be accumulated in the cells of Haematococcus (Ramirez *et al.*, 2001). Metal ion, oxidative stress and salt stress stimulate the formation of astaxanthin in this alga. The ferrous form of iron is known to give rise to free radical formation via the Fenton reaction, free radicals may play a role in astaxanthin formation and active oxygen species (O_2, H_2O_2, peroxy radical) also enhance the formation of astaxanthin. Factors affecting the bio-accumulation of such pigments were fully understood. Carotenoids accumulation by microalgae depend on both nutritional status (Cero'n *et al.*, 2005; Tittel, 2005) and environmental conditions, such as high light intensity (Bhosale, 2004), type of light (Janhke, 1999). In all cases, the induced algal cells must be used by the log phase growth to avoid the dry weight failure. Also, shifting of photosynthetic metabolism to carotenoids accumulation by lipid biosynthesis should be considered (El-Shafey *et al.*, 1999).

Iron is an essential element for phytoplankton owing to its importance in numerous metabolic processes. Bioavailability of iron depends upon every aspect of Fe chemistry (solubility, complexation, thermodynamics, kinetics of ligand exchange) in addition to phytoplankton uptake mechanisms and kinetics. There is still no conclusive agreement on describing and quantifying "bioavailable iron" (Wells *et al.*, 1995). Some of the operationally defined iron forms may have strong correlations with bioavailability of iron to phytoplankton (Wells and Mayer, 1991). The importance of the different factors inducing the astaxanthin is well known but not completely understood. There are a lot of known factors that affect the astaxanthin accumulation in *H. pluvialis*: nutrient limitation or supplement (Fabregas *et al.*, 2003), oxygen stress (Kobayashi *et al.*, 1992), high light intensity (Park and Lee, 2001), and blue light (Fabregas *et al.*, 2003; Lababpour *et al.*, 2004). Among these, the effect of light is undoubtedly the most important factor in the astaxanthin accumulation (Bubrick, 1991). In order to produce high level of astaxanthin from *H. pluvialis*, a proper design of photobioreactor and illumination by effective light sources are required. The quality of light, such as wavelength and/or emission spectra of light also affects the performance of algae cultivations (Lee, 1999) as well as astaxanthin production.

In order to produce astaxanthin, the process involves two stages which are green stage which involves cultivation of green algae in order to increase its mass. The second stage is referred to carotegenesis stage. In this stage, green alga undergoes transformation to red algae after being exposed to some certain stress conditions *H. pluvialis* with the maximum mass followed by the second stage to produce astaxanthin (Kobayashi *et al.*, 1992) under stress induction. Two stages production process have been proposed due to different culture conditions needed for production of green algae and astaxanthin accumulation. Algal growth related to productivity of astaxanthin. Therefore, this study focused on optimization productivities of *H. pluvialis* biomass so that can enhance productivities of astaxanthin by screening the effect of different light intensity and Fe+EDTA.

MATERIALS AND METHODS

Strain and Culture Conditions

Haematococcus is an ubiquitous green algae classified as: *Chlorophyta* (Phylum), *Chlorophyceae* (Class), *Volvocales* (Order), *Haematococcaceae* (Family), *Haematococcus* (Genus), *pluvialis* (Species).

The unicellular green algae *H. pluvialis* (34/12) was purchased from the Culture Collection of Algae at England and was cultivated photoautotrophically in the modified Bold's Basal Medium (MBBM), whose composition consisted of 246.5 mg·L^{-1} of $NaNO_3$, 24.99 mg·L^{-1} of $CaCl_2 \cdot 2H_2O$, 73.95 mg·L^{-1} of $MgSO_4 \cdot 7H_2O$, 4.98 mg·L^{-1} of $FeSO_4 \cdot 7H_2O$, 74.9 mg·L^{-1} of K_2HPO_4, 175.57 mg·L^{-1} of KH_2PO_4, 25.13 mg·L^{-1} of NaCl, 49.68 mg·L^{-1} of $C_{10}H_{16}N_2O_8$ (EDTA), 1.57 mg·L^{-1} of $CuSO_4 \cdot 5H_2O$, 1.19 mg·L^{-1} of $Na_2MoO_4 \cdot 2H_2O$, 11.13 mg·L^{-1} of H_3BO_3, 1.44 mg·L^{-1} of $MnCl_2 \cdot 4H_2O$, 8.83 mg·L^{-1} of $ZnSO_4 \cdot 7H_2O$, 0.49 mg·L^{-1} of $Co(NO_3)_2 \cdot 6H_2O$, 6.06 mg·L^{-1} of MoO_3, 30.86 mg·L^{-1} of KOH, and 0.98 mg·L^{-1} of H_2SO_4 in distilled water.

Experimental Plan

To examine the effects of Fe+EDTA and different light intensities on the test organism (*H. pluvialis*), $FeSO_4 \cdot 7H_2O$ and EDTA (C10H16N2O8) were added to the growth medium (Modified Bold Basal Medium). Each test was carried out in 2000 ml polyetilene bottles. The initial pH value of the solution was adjusted between 7 by adding nitric acid (0.1M) or sodium hydroxide (0.1 M). The experiments were conducted at 23°C. During the experimental period, optical density, dry weight (g L^{-1}), chlorophyll *a* (mg L^{-1}) and astaxanthin concentration (%) were measured every day as shown in Table 1.

Table 1. Experimental Design

Experiments	Light intensity (μmol photon m^{-2} sn^{-1})	Groups	Parameters
I	30 (Control) 50 (A) 200 (B) 475 (C)	I: Control, A, B, C + added Fe+EDTA II : Control, A, B, C	Optic Density (O.D) Chlorophyll-a (mg L^{-1}) Dry weight(gL^{-1})
II	30 (Control) 200 (B)	I: Control, B + added Fe+EDTA II: Control, B	Dry weight(gL^{-1}) Astaxsanthin (%)

Pigments Extraction Method (Chlorophyll, Astaxanthin)

One milliliter of culture sample was centrifuged at 3000 rpm for 10 min to pellet cell material. Pipetting the supernatant and collect in the tubes. Adding 1 mL of acetone to the centrifuge tube, vortex for 30 sec. Homogenizer for 3 min with 12.000 rpm for broking the cell walls. The sample was stored at 4°C refrigerator for 20 min to extract pigments. These steps were repeated until the color of cell debris became white or colorless. Gently mix and centrifuge at 3,000 rpm for 10 min. to pellet remainder. Measurement of O.D at 475, 663, 645, 750 and 850 nm in spectrophotometer.

Chlorophyll concentration and astaxanthin concentration were analyzed by a spectrophotometer (model HP8453B, Hewlett Packard, Waldbronn, Germany). Chlorophyll concentration was calculated by eq. 1.

chlorophyll a = (12,7 X A663) – (2,69 X A645)
chlorophyll b = (22,9 X A645) – (4,64 X A663) (1)

Astaxanthin concentration was calculated by a calibration curve obtained by synthetic astaxanthin (product number A9335, Sigma Chemical Co., St Louis, MO, USA) as a standard. For astaxanthin concentration less than 10 mg/L, the following calibration was used (eq. 2).

astaxanthin concentration (mg / L) = 0.0045 X OD475 (2)

Optical Density and Dry Weight

Optical density values were obtained according to the procedure reported by Boussiba and Vonshak (1992). The samples were measured at 680 nm absorbents value with spectrophotometer. The sample containing 10 ml algal suspension was filtered through a filters 47 mm (diameter) (Whatman GF/C) that was dried in a microwave oven (105°C in 8 min) and weighed prior to filtration. The filter was put in a glass Petri dish in the oven under the above conditions. After cooling the fitler in a dessicator (20 min.), it was weighed again (Boussiba and Richmond, 1979).

Statistical Analysis

Data were analyzed statistically using one way analysis of variance (ANOVA). When significant treatment effects were detected, Duncan's multiple range test was used to identify specific differences among treatment means at a probability level of 5%.

RESULTS AND DISCUSSION

Experiment I

The results obtained for different light intensities and Fe+EDTA in cultures grown for 15 days are shown in Figure 1. As seen in Figure 1 and Figure 2, the results of this study proved that dry weight of the cells value of *H. pluvialis* was effected by light intensities and iron added and when the results were compared, difference was recorded between groups ($p>0.05$). The highest dry weight values were recorded in Group I (4.43 gL^{-1}).

These results suggest that the amounts of dry weight was strongly effected by the amounts and the chemical speciation of iron (Fe+EDTA). Fe+EDTA and light intensity observed during dry weight increase must have had a strong impact on the iron forms and bioavailability in *H. pluvialis* cells.

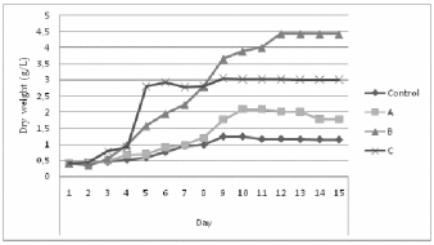

Figure 1. Dry weight values (g/L) of Group I

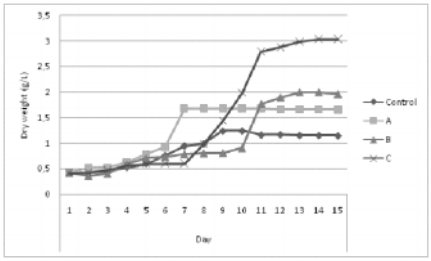

Figure 2. Dry weight values (g/L) of Group II

The maximum optical density value (0.23) was observed for the cultures supplemented with Fe(III) + EDTA and in 200 µmol foton $m^{-2}sn^{-1}$ light intensity . Analysis of variance of OD between groups showed that all groups were significantly different from each other and from the control group ($P<0.05$). (Figure 3, 4).

Dissolved Fe are constantly complexed by strong organic ligands in the aquatic systems. Strong Fe-binding ligands complex >99.9% of total dissolved Fe and can

dramatically increase the solubility of Fe in oxic waters, allowing greatly elevated dissolved Fe concentrations (Buck and Bruland, 2007). Furthermore, these strong Fe ligand complexes in natural waters appear to be largely bioavailable to marine phytoplankton (Maldonado and Price, 1999).

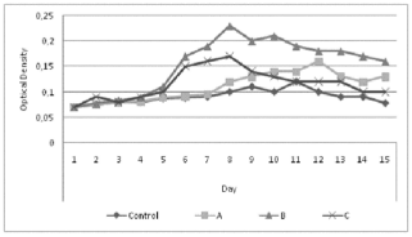

Figure 3. Optical Density Values of Group I

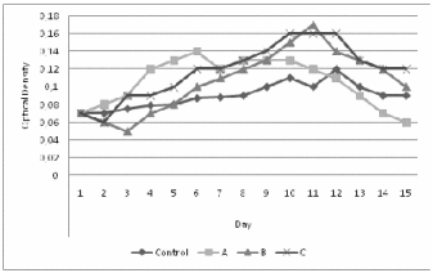

Figure 4. Optical Density Values of Group II

The highest chl *a* values (1.28, 1.57, 5.33 and 2.99 mg L^{-1}) were recorded in Group I. Statistical differences between all studied groups were significant (P<0.05) (Figure 5, 6). Martin and Fitzwater (1988) pointed out that an increase in chl *a* amount can be observed in Fe added culture on the third day of the experiment.

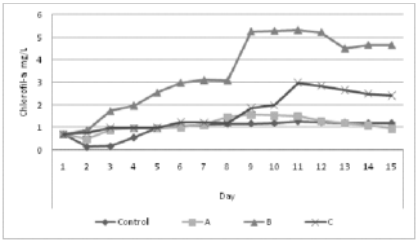

Figure 5. Chlorophyl-a values of Gruop I

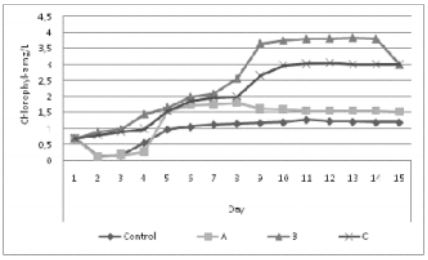

Figure 6. Chlorophyl-a values of Gruop II

Experiment II

The maximum dry weight value respectively 0.19, 0.25 and 0.27g/L was observed for the cultures group. Analysis of variance of dry weight value between groups showed that all groups were significantly different from each other and from the control group (P<0.05).

The maximum astaxanthin amounts respectively % 0.545 (Control), % 0.968 (Group I)) ve % 0.721 (Group II) was observed. Similarly, light induces stimulation of the phytoene synthase and phytoene desaturase in *Chlamydomonas reinhardtii*. An obligate photoautotroph, *Spirulina platensis*, was also reported to disply increased carotenoid levels under strong illumination (Liu, 1984) and in most cases, light causes a quantitative improvement in carotenoid content in microorganisms (Bhosale, 2004). Other hand, a significant enhancement in Chl a concentration was observed in relation to Fe addition.

CONCLUSIONS

Findings of this study indicate that Fe+EDTA and light intensity had relatively higher effect on the growth of *H.pluvialis*. Accordingly, these treatments (added Fe+EDTA and high light intensity) can be proposed for use in optimization of *H.pluvialis* cultures.

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III.
SYSTEMS AND TECHNOLOGIES

MATHEMATICAL MODELING OF PLASMA PARAMETERS PROCESSES FOR MULTIFUNCTIONAL TEXTILE

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This paper presents the mathematical modeling for plasma processes treatments used for textile materials functionalization. The subject fabric on this work was made by cotton. In this research it was started from initial known textile structural parameters and some parameters for oxygen plasma treatment. The goals of plasma treatment were to increase activation and cleaning textile material for adherence preparation of the colloidal silver. The main objective was to obtain a textile surface with reduced microbial charge for medical destination. For obtaining the optimal results are required plasma treatment parameters optimization. This optimization was done starting from analyzing the plasma processes parameters variation. This variation and experimental results was formed the start point for developing mathematical modeling presented in this papers.This variation and experimental results was formed the start point for developing mathematical modeling presented in this papers.

Keywords: mathematical, textile, plasma

INTRODUCTION

Plasma polymerization is a technique for obtain functional textile materials for any domain and for high performance clothing (Kilic *et al.*, 2009; Gulrajani *et al.*, 2011). By using air plasma treatments on cotton textile surfaces can occur surface modification (Bhat *et al.*, 2011) that conduct to improve the hydrophobicity and decrease contact angle (Karahan and Özdoğan, 2009). This surface functionalization can help in textile finishing and coating process by obtain the activated surface without using any else chemicals that are requiring wet process (Rauscher *et al.*, 2010). There are in situ experiments by using plasma nanotechnology (Haji *et al.*, 2013) and involving chemical modification of cotton fabrics by natural chitosan followed by incorporating silver nanoparticles in the fabrics (Thomas *et al.*, 2011).

EXPERIMENTAL PART

The experiment consisted in analyzing the cotton knit samples before and after 10 minutes plasma treatment. It is known from others experiments, developed in Multitexfunction Crosstexnet project, that for samples with 100% cotton composition the high values for resistances, pilling effect are obtained after 10 minutes oxygen plasma treatment, after this moment till 90 minutes experiment the natural polymer cellulose from cotton is supposed to accelerated depolymerization process.

The goal of plasma treatment was to activate the textile surface in order to make easier and durable chemical submission (colloidal silver, chitosan) by using foulard method, for obtaining medical bandages with antimicrobial properties.

For 20 samples analyzed were tested the tear and abrasion resistance to obtain the maximal pilling effect and tear force before and after 10 minute oxygen plasma treatment (Table 1).

Table 1. Experimental data

Sample No.	Pilling effect experimental 10 minutes plasma treatment	Pilling effect fitting approximate	String density experimental 10 minutes plasma treatment	Spring density fitting approximate
1	3.29	3.52	13.5	13.7808
2	3.325	3.51	13.6	23.6577
3	3.36	3.56	13.1	17.3394
4	3.395	3.54	13.6	13.7705
5	3.43	3.58	13.7	13.5635
6	3.465	3.57	13.2	14.002
7	3.5	3.59	13.8	13.8909
8	3.535	3.54	13.5	13.5229
9	3.57	3.56	13.5	13.4299
10	3.605	3.54	13.7	13.5618
11	3.64	3.29	13.8	13.5183
12	3.675	3.58	13.5	13.3027
13	3.71	3.68	12.9	14.0918
14	3.745	3.59	13.4	17.7966
15	3.78	3.44	13.9	25.2781
16	3.815	3.68	13.8	33.8494
17	3.85	3.69	13.5	35.3076
18	3.885	3.44	13.5	18.1836
19	3.92	3.89	13.8	-20.2942
20	3.955	3.98	13.2	-44.6882

RESULTS AND DISCUSSIONS

For experimental data from Table 1 was obtained an approximated mathematical model for pilling effect addicted to strings density for knitted samples. The approximated data from Table 1 for string density and pilling effect are obtained by using the next polynomial mathematical model (1).

We made the following notations for pilling effect and string density:

x- string density

f(x)- pilling effect

$$f(x) \quad 1.483 \quad x^9 * 4.812 \quad x^8 * \quad 6.937 \quad x^7 * \quad 5.832 \quad *x^6 \quad 3.15 \quad *x^5 - \\ 1.134 \quad x^4 * \quad 2.72 \quad x^3 * \quad 4.192 \quad *x^2 \quad 3.768 \quad *x - 1.504 \tag{1}$$

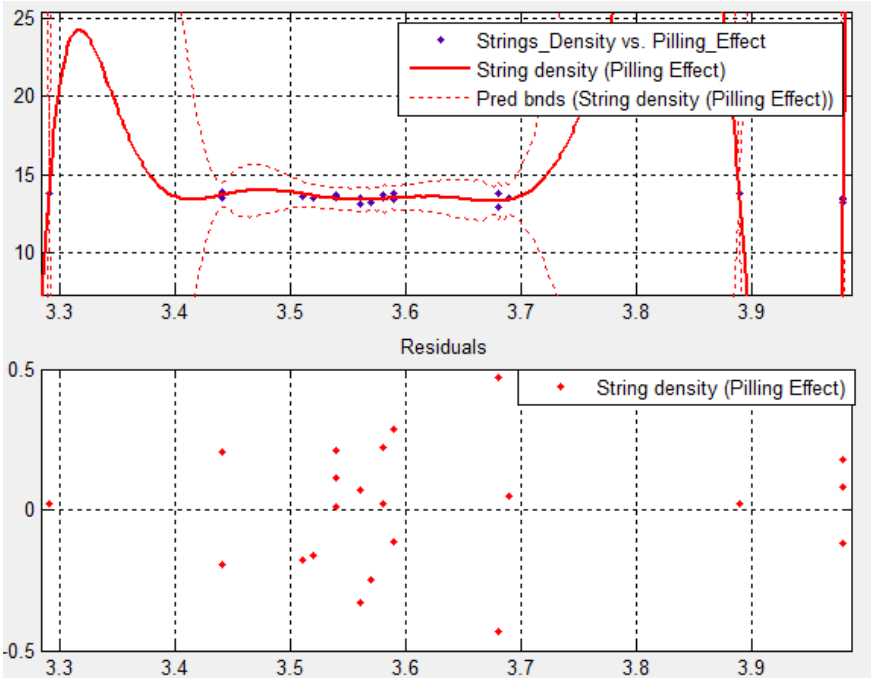

Figure 1. String density dependent on pilling effect model aproximation

In figure 1 is presented the string density addicted to pilling effect prediction and model approximation.

In figure 2 is presented the fitting string density dependent on pilling effect for dataset obtained by approximation.

The string density modification after 10 minutes plasma treatment can be computed by using cubic interpolation and explained in function of tear force and pilling effect modification (figure 3).

For contour data analyze (figure 4) we can see that the high values for pilling effect and tear force, after 10 minutes plasma treatment, are 199 N and approximate 3.7 values for pilling effect. By using plasma treatment the pilling effect can be reduced and this can conduct to increasing the textile surface elongation and indirectly decreasing the strings density.

For higher value of pilling effect we have elongation increasing. This means that elongation and electrostatic tension reduction is depending on pilling effect improvement after plasma treatment. String density is not influenced by tear force increasing, but can be indirectly proportional with pilling effect.

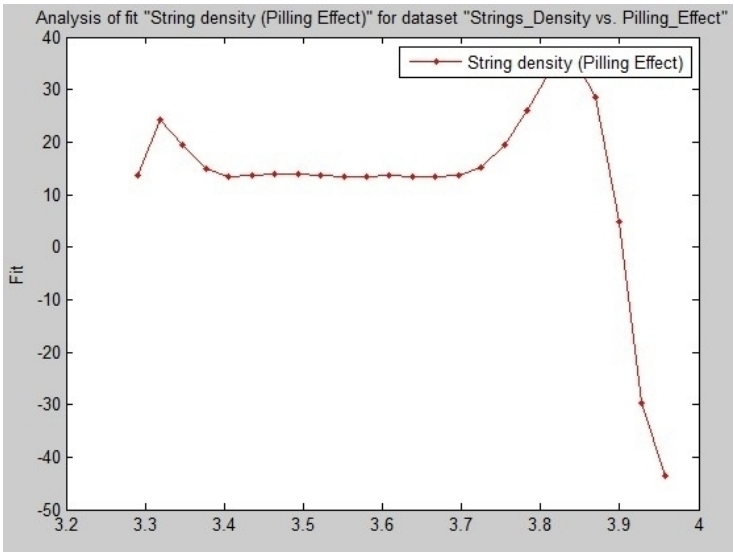

Figure 2. Fitting value analyzing for string density

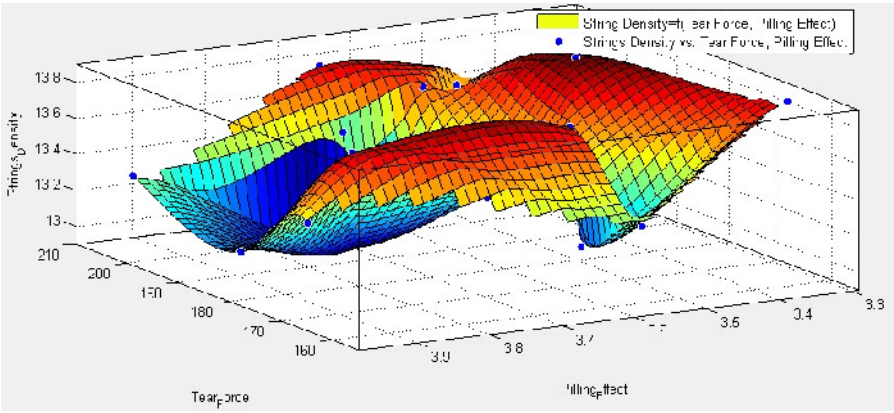

Figure 3. String density -3D analyze in function of tear force and pilling effect

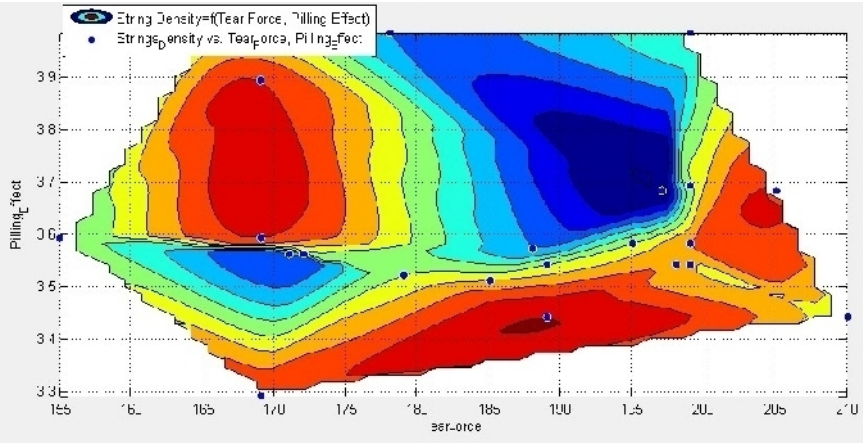

Figure 4. Contour analyzing –string density vs. tear force, pilling effect

By analyzing the residuals values can conclude that string density in function of tear force and pilling effect don't have residual values and respect the 3D fitting.

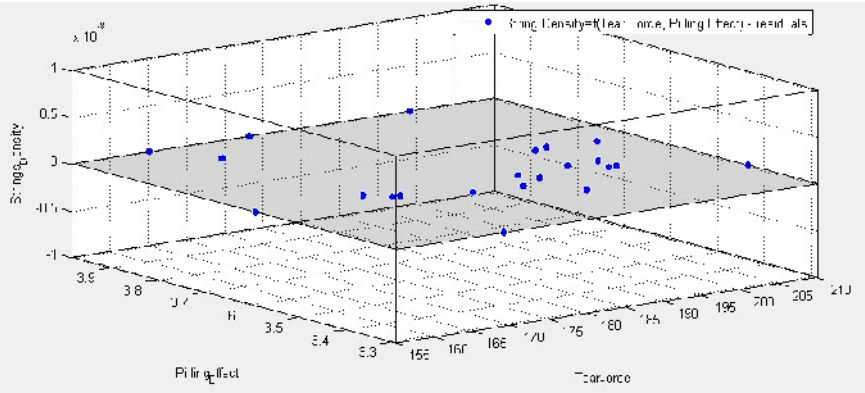

Figure 5. Residual values for model analyzed

CONCLUSIONS

From analyzed data can conclude that plasma nanotechnology treatment for knit samples made from cotton and having medical usage destination area is ecofriendly method for surface functionalization and conduct to:

- Reducing pilling effect
- Surface activation
- Traction resistance improvement
- Electrostatic tension reduction in knit surface
- Improvement finishing treatment and increasing the life cycle of treated surface

- Improvement of hydrophilic capacity
- Contact angle reduction
- Economy on chemicals used for finishing treatment
- Reduction of steps required for treat the materials used for medical area.

Acknowledgements

This paper presents experimental results obtained in *Innovative Production Chain For Textile By Plasma Nanotechnology*, Multitexfunction CrossTexNet project, 7-062/ 2012.

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MEDICAL TEXTILE MULTIFUNCTIONALIZATION BY USING PLASMA TREATMENT

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This paper expose aspects regarding design, development and plasma nanotechnology treatments required for obtain a textile product for medical usage. The multifunctional products presented in this paper are based on natural or synthetic fibers treated in plasma. This work presents fundamental research regarding plasma treatment actions to the physic-mechanical, physic-chemical and structural parameters of the textile materials with different fibrous composition made by natural and synthetic fibers. This work exposes the benefit of the plasma treatment for textile materials characteristics improvement.

Keywords: textile, plasma, medical.

INTRODUCTION

The textile industry directions at EU level are to develop and produce raw materials, converting them into finished products for satisfy customer expectations with respect for health and environmental safety (Bhosale *et al.*, 2013). For a sustainable production systems in environment safety, economies of industrial communities, and consumer interests it is required to develop new products, alternative raw materials, and processing technologies more "environmentally friendly" obtained by using new natural processes and increasing environmental concerns (Gorenšek *et al.*, 2013). Synthetic fibers in combination with natural one can conduct to obtaining of new yarn with improved parameters. The nanotechnology plasma treatment can be used for reducing the energy consumption in textile finishing process, chemicals, and time waste in textile processing (Gulrajani et Gupta, 2011). Plasma technology is an alternative that will have a significant impact on the quality of life if will be used in industrial textile processes even if at European level is an increased interest for natural fibers, for their many outstanding properties including aesthetics, comfort, and biodegradability (Buyle, 2009).

EXPERIMENTAL PART

The textile surface designated for medical articles and with composition 65% polyester and 35% cotton was activated by using oxygen plasma. The goal of plasma nanotechnology usage was to obtain surface preparation for colloidal silver and chitosan submission by using foulard method.

The textile surface, treated in plasma for 10, 20, 30 and 90 minutes, was tested in laboratory for evaluating the tear force parameter improvement or depreciation. The results obtained are presented in Table 1.

Table 1. Maximal tear force [N] – in warp direction for samples treated in plasma (Woven fabric 65% polyester 35% cotton, 180 g/m², width 160 cm, density: 43 yarns/cm warp direction and 22 yarns/cm in weft direction

Sample No.	Standard sample	Sample treated in plasma 10 minutes	Sample treated in plasma 20 minutes	Sample treated in plasma 30 minutes	Sample treated in plasma 90 minutes
1	1044	1230	1240	1235	1150
2	1167	1160	1235	1230	1152
3	1195	1240	1231	1229	1151
4	1209	1065	1206	1228	1149
5	1200	1200	1207	1226	1148
6	1120	1203	1208	1224	1146
7	1037	1210	1209	1204	1147
8	1229	1207	1210	1208	1068
9	1204	1208	1204	1201	1059
10	1207	1209	1205	1203	1086
11	1230	1235	1206	1208	1056
12	1200	1200	1207	1203	1089
13	1203	1204	1208	1201	1099
14	1151	1206	1209	1206	1203
15	1210	1209	1208	1119	1117
16	1210	1205	1207	1118	1116
17	1131	1200	1205	1117	1115
18	1012	1203	1208	1113	1112
19	1012	1210	1209	1114	1113
20	1154	1156	1201	1113	1123
Average value	1156.25	1198	1211.15	1185	1119.95
ST DEV	73.34121768	36.97509831	10.72270096	47.79892093	37.0312457
CV	6.3430%	3.0864%	0.8853%	4.0337%	3.3065%

RESULTS AND DISCUSSIONS

The textile woven with 65% polyester and 35% cotton present a high uniformity due to the uniformity of polyester and cotton fibers presence in the yarn structure (figure 1 and figure 2). In this way structural uniformity conducts to different values for tear force in warp direction (figure 1). It knows that in plasma treatment the maximal values for tear force for cotton are after 10 minutes oxygen plasma treatment and for polyester are after 20 minutes oxygen plasma treatment. In discrete cases occur high level values after 10 minutes plasma treatment.

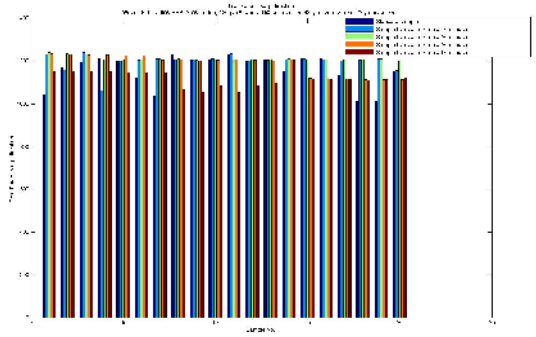

Figure 1. Tear force – warp direction for samples treated in plasma

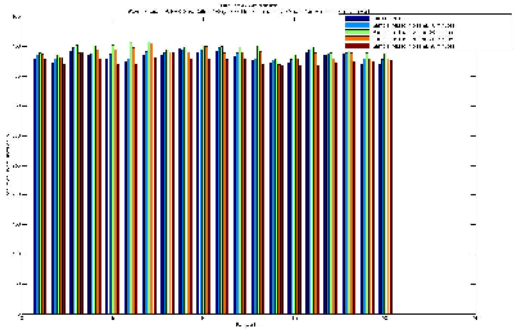

Figure 2. Tear force-weft direction for samples treated in plasma

For the weft direction we can observe that the maximal tear force values are after 20 minutes of oxygen plasma treatment.

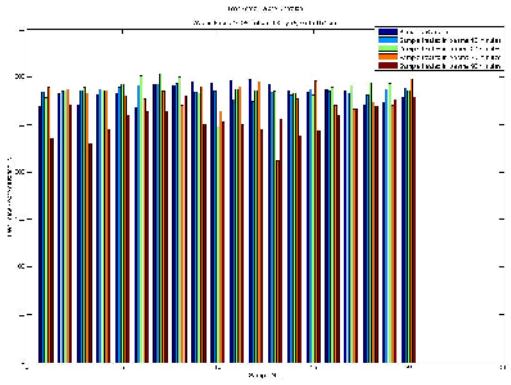

Figure 3. Tear force-warp direction for cotton samples treated in plasma

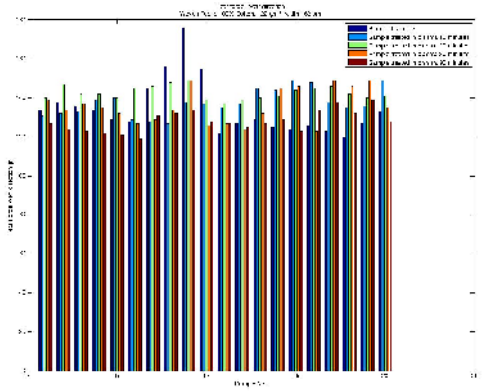

Figure 4. Tear force – weft direction for cotton samples treated in plasma

By using comparative analyze we can conclude that the woven fabric resistance is increased by polyester fibers presence.

The yarn structure is not one ideal and we can have different resistance due to the cotton or polyester predominance.

The polyester can increase the values obtained after 20 minutes plasma treatment and cotton can increase the values after 10 minutes oxygen plasma treatment.

From all cases analyzed it can see that the values obtained for untreated sample are higher than values obtained after 90 minutes plasma treatment. The explanation for this phenomenon can be that after 90 minutes plasma treatment the polyester and cotton yarns are destroyed due to the increased depolymerization (figure 4).

From experimental results it can conclude that higher values for tear force are obtained after 20 minutes plasma treatment and this is due to the polyester yarns presence on woven structure.

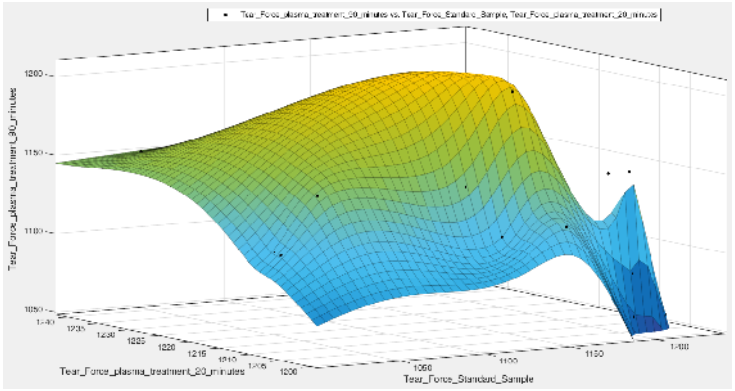

Figure 5. Tear force after 90 minutes plasma treatment in function of value obtained after 20 minute and values for standard sample

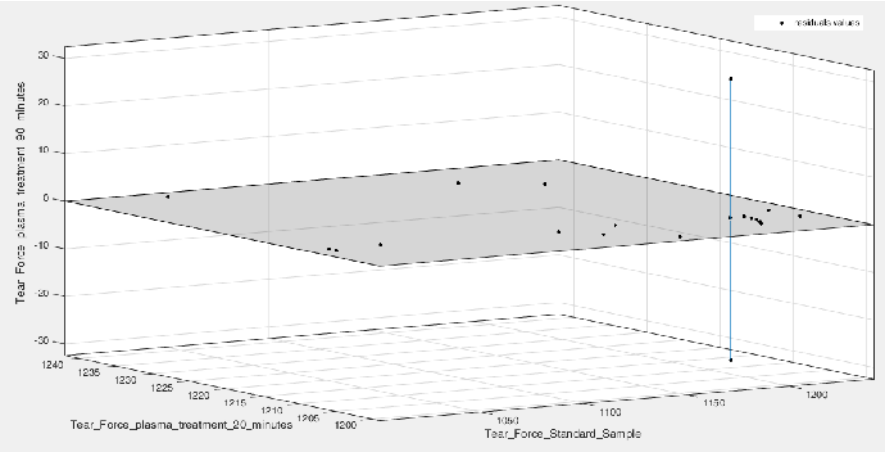

Figure 6. Tear force – residuals values

In the figure 6 it is observed that we have 2 residuals values. Here we have the case of analyzing data by polynomial model was observed that the model doesn't respect a polynomial law and the residuals values are increased (figure 7).

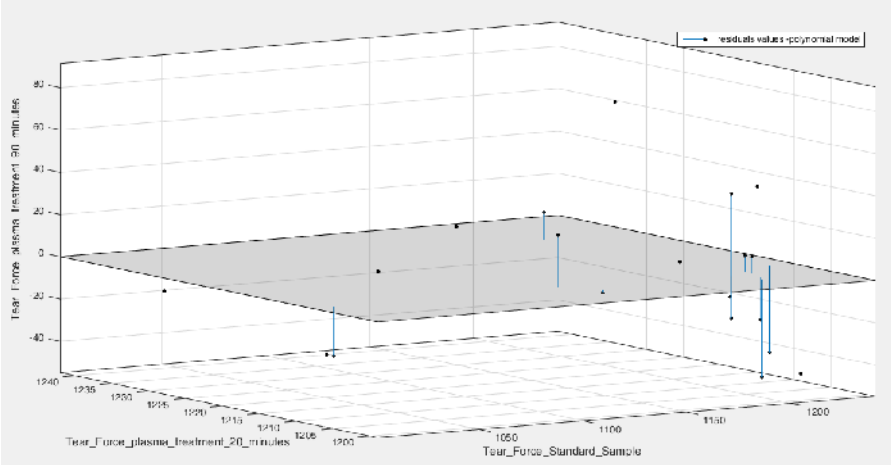

Figure 7. Tear force – residuals values for polynomial model

CONCLUSIONS

The advantages of using plasma treatment for woven fabric with polyester and cotton composition are that in this way is increased tear resistance.

The percent 65% polyester yarns presence on woven fabric conduct to obtaining higher values for tear force than are obtained when we are using woven fabric made from cotton yarns.

The higher values for tear force in case of use cotton woven fabric is after 10 minutes plasma treatment.

The tear force values for standard sample, sample treated in plasma 20 minutes and samples treated in plasma don't fit to polynomial modeling.

The woven fabric treated in plasma has the pilling effect minimized. This will contribute to chemicals substance (chitosan, colloidal silver) economy in the textile materials finishing and treatments for antimicrobial effect.

The high irregularities for values obtained are because the structure of yarns is not ideal one.

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This paper presents experimental results obtained in *Innovative Production Chain For Textile By Plasma Nanotechnology*, Multitexfunction CrossTexNet project, 7-062/ 2012.

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OPTIMIZATION OF ASSEMBLE UPPERS SYSTEMS USING CAD/CAM

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The paper presents the optimization of assembly uppers using CAD/CAM systems based on bibliographic study and analysis of existing manufacturing tehnologies in the enterprise shoe ISC „Cristina Mold-Rom Simpex" SRL, Chisinau city. The purpose of this paper is to improve existing technologies uppers assembly in the company implementing new ways of designing manufacturing technologies footwear with uppers of leather. The practical value is to: develop automated design of technological process of assembling uppers of leather; developing unified database assembly faces technological operations; expanding the base of information about the process of assembly. The need for rapid introduction of high design constantly pressuring all companies producing shoes. Be the first to bring a new style shelves is often the difference between success and failure in business. The case study was conducted at the footwear company EFC "Cristina Mold-Rom Simpex" LLC, with conventional tehnologies and modern tehnologies. They have been made 10 models of shoes, is found the following: between clasical and modern tehnologies is a difference of 1 to 5.0 min; number of workers performing traditional sewing machines, ranging from 1 to 2 workers on the automatic sewng machine made to engage a worker. Active introduction CAD/CAM systems in light is an innovative tehnique that allwows improving the manufacturing uppers enchancing the quality uppers; decreasing the number of workers involved.

Keywords: technology, uppers, CAD/CAM systems.

INTRODUCTION

Technological preparation of production of footwear includes a complex set of operations that must be performed in the shortest time with minimum cost and high quality. One of the main characteristics of the production of footwear is a frequent change models, and the large number of factors influencing technology assembly uppers. The error in assembling uppers are often associated with lack of experience of the designer. With the rapid development of science and technology, new fundamental technologies are developed and implemented immediately. Decision making in the manufacturing process can not be achieved by increasing the number of technologies, but in the process due to the use of computer aided design innovations (Volocariu, 1999).

OPTIMIZATION OF ASSEMBLE UPPERS SYSTEMS USING CAD/CAM

The different industries have been introduced and implemented computer-aided systems design processes. Design automation of technological processes take account of the nature and relationship factors that determine the final quality of the finished product, cost effective technologies, structural and parametric optimization of the technological process designed.

Automates processes is due primarily to the scientific development of technology also mathematical methods and technical means. Advances in technology development and automation of computer aided engineering can determine this direction as one of the most successful.

Introduction of automatic sewing machines flow raises organizational issues, technical and technological (Papaghiuc, 2003; Papaghiuc and Ionescu, 1999; Volocariu, 1999). When installing automatic sewing machines must ensure:

- larger surface location;
- easy access to carry out adjustments;
- connectable installations air or vacuum;
- climatic conditions.

The use of the possibilities of automatic machines is possible only through rational organization technologies. The ideal situation is when they make a single model, as time period. The existence of automatic sewing machines raises models and designers who need to consider the shape and size of the product being processed. To this end, we can apply the principles of constructive typing and unification models. The need for rapid introduction of high quality designs constantly pressuring all companies producing shoes. Be the first to bring a new style shelves is often the difference between success and failure in business. To be competitive on the domestic footwear company management EFC "Cristina Mold-Rom Simpex" LLC purchased **CES_2000** program. Using software facilitates the assembly CES_2000 upper assembly, reducing manufacturing errors. The advantages of this program are: high productivity, the possibility of drafting drawing step applied at the seam, compatibility with any version of Windows, minimal energy consumption. To use the is required automatic sewing machine **CEM 350** (fig. 1) (http://www.embroid.ru).

Figure 1. Automatic sewing machine CEM 350

Sewing operations on this machine consists of phases whose content is determined by the number and order of the calculation technology. Realization phase is successful during an operating cycle of the machine, but can overlap in time over some stages of the preceding or following.

COMPARISON OF TECHNOLOGIES TO ASSEMBLE UPPERS

For a good collection of comparative analysis of the patterns is present during the sewing thereof, the number of workers involved, the cost of a pair, both made from the automatic machine and simple sewing machines (fig. 2-11 and tab. 1).

Figure 2. The analyzed 1 (MA 1)

Figure 3. The analyzed 2 (MA 2)

Figure 4. The analyzed 3 (MA 3)

Figure 5. The analyzed 4 (MA 4)

Figure 6. The analyzed 5 (MA 5)

Figure 7. The analyzed 6 (MA 6)

Figure 8. The analyzed 7 (MA 7)

Figure 9. The analyzed 8 (MA 8)

Figure 10. The analyzed 9 (MA 9)

Figure 11. The analyzed 10 (MA 10)

Table 1. Comparative analysis of the models studied

Symbol models	Number of parts	Sewing time, min/pair		Number of workers needed		The cost of a pair, lei	
		The automatic machine	Simple machine	The automatic machine	Simple machine	The automatic machine	Simple machine
MA 1	8	4	6,1	1	2	2	6
MA 2	20	11	16,8	1	3	2	15
MA 3	8	3,5	4,5	1	2	2	6
MA 4	14	6	7,2	1	2	2	7,6
MA 5	2	3	4	1	1	2	3,6
MA 6	2	2	3,6	1	1	2	3,6
MA 7	14	6	7,2	1	3	2	7,6
MA 8	16	6	8	1	3	2	9
MA 9	12	4,5	6,6	1	2	2	6,8
MA 10	14	5	6,6	1	3	2	7,6

Analyzing the results shows that for performing automatic stitching machine CEM 350 is required for sewing smaller than if the sewing machine simple. The offset is from 1 to 5,0 min / pair. Regarding the number of workers who perform sewing is a reduction to a minimum when using automatic machines, more than that depending on the organization of the manufacturing process and range made a working could serve 2-3 automatic. The cost of a pair of semi plain sewing the machine is much higher than the cost of semi automatic machine sewn. The price of the machine semi automatic sewing is set by chief engineer and is 2 lei / pair, considering the complexity of the pattern and volume of production conducted in a month.

CONCLUSIONS

1. Analysing the possibilities it offered CES_2000 overall drive system CEM 350, is found to be a system that does not require sophisticated computers and great effort from the user to very way work. The program allows drawing drawing any version of Windows.

2. The combination of new designs for the seams be obtained by a varied appearance, which leads to the development of the collection of shoes has a given season, a healthy competition improve the appearance and quality.

3. By the simultaneous phase and the operations landmarks in different positions to achieve a considerable increase in labor productivity.

4. Implementation of automatic sewing machine CEM 350 allowed increasing the precision and execution of stitches.

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INNOVATIVE TECHNOLOGIES OF CUSTOMIZED FOOTWEAR FOR ELDERLY AND PROMOTION OF ACTIVE AGING

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This paper highlights the importance of designing and producing customized footwear for elderly while taking into account the profound demographic changes and considering the reduced functioning, capacity and performance, because of ageing changes of the feet. Functioning, capacity and performance are described according to ICF, WHO 2001 (The International Classification of Functioning, Disability and Health). The authors are analysing the phenomenon from an active ageing perspective. Neuromusculoskeletal and movement – related functions may be impaired by ageing itself or by certain health conditions which could make old people more vulnerable. Standing and walking are fundamental for the well-being and preserving the physiologic ranges of these functions is an important goal. The main feature of this domain is the multidisciplinarity which requires the work of multiple professionals: physicians, rehabilitators, engineers, technical staff. The Active Ageing concept includes interventions and policies that aim to reduce premature physical ageing and to empower older people to live healthy, productive, participative and independent lives. Romania has devised the Strategy for Protection of the Elderly and Promotion of Active Ageing supported by the EU, as a part of The National Reform Program. Romania's commitment to Active Ageing concept is substantial for achieving Europe's 2020 strategical goals.

Keywords: active ageing, old foot, The International Classification of Functioning, Disability and Health

INTRODUCTION

Population aging is a process more and more evident in the contemporary society, both in the developed and developing countries. It appears on the agendas of the international meetings of all kinds.

Traditionally, aging is associated with decreased functional capacity and difficulties in coping with the changing of the environment, resulting in an increased vulnerability and making older people more susceptible to illness and disability .

From a more challenging perspective, the aging of population could offer unexpected opportunities for the society on the condition of encouraging and empowering the older people to live healthy, productive, participative and independent lives.

Documents on population aging issued by International organisations clearly state the guidelines for further actions : growing old in security and dignity, participation in social life as citizens with full rights, improvement of reciprocal intergenerational relations (United Nations,World Population Aging 1950-2050, Population Division).

Romania's Current Demographic Profile

The current demographic trends of the population of Romania, follows the trends recorded all over the world. Romania becomes, little by little an aging society,

according to the latest demographic statistics. Three main causes of the issue were identified:increased life expentancy, fertility rate decline, strong emigration flows.

Having these phenomenaas a background, the projection of the statistic data for the next years, by 2060 (according to Eurostat population projection) shows that the share of population aged 65 and over is expected to double (from 15% to 30 %) while the working age population, aged 20 to 64 is decreasing (with 30% by the year 2060), dysplaing one of he deepest decline in Europe. In the same time, the strong net emigration reduced The cohort currently aged 25- 30 by 20%.

The effects of The demographic phenomena mentioned above could be also estimated by projecting *the demographic dependency rate* over the same period. *The demographic dependency rate* is defined as the number of people aged 65 and over and those under 20 for every 100 people aged 20 to 64. The current value of The ratio is 55 but it is expected to rise dramatically and reach the value of 100 by the year 2055, showing a sharp reverse (INSSE Statistical DB Tempo. Romania).

All these phenomena result in negative effects on the economic growth and impact the health care systems and the sustainability of the pension schemes.

The increase ot life expectancy is basically a positive issue and it means more years lived in The conditions of an active and productive life. But the reverse must not to be neglected because more those more years could be associated with illness, disability vulnerability and dependency. Special attention needs to be paid for promoting harmonious, phisyologic aging both by the health care and social assistance systems.

Harmonious, phisyologic aging is defined by the capacity to maintain a low risk and vulnerability, high level of phisical and mental performances and a high motivation for living (Donca, 2008).

The "Active Aging" Concept

The World Health Organisation introduced the Active Aging concept and it refers to a number of action plans aiming to prevent the negative economic and social effects of population aging.

In response to the WHO initiative, The European Union has uptakes the concept as a major objective for its own politics.

The WHO defined *Active Aging* as: "the process of optimizing the opportunities for health, participation and security in order to enhance the quality of life as people age both within the labour force through delaying retirement and within society through the participation in a range of social, economic, civic or cultural activities" (European Commission, 2012). The policies and strategies of the EU offer the framework for the practical approach of the concept. The goals of European Commission policies to encourage older people to remain active as long as possible, to delay retirement and to promote the social participation by civic engagement and volunteering.

In order to support the national agendas in the field of active aging The European Commission developed helpful statistical tools and a policy framework.

The special Euro barometer Survey#378 was aimed to understand people's opinions and attitudes regarding old people. It also searches people's readiness to adapt the new demographic conditions and to accept reforms in the field of active aging.

Developed by the European Commission and the United Nations Economic Commission for Europe (UNECE), the *Active Aging Index (AAI*) is a composite tool which attempts to evaluate the extent to which older people can reach their full potential in four domains": a) employment, b) social participation, c) long independent, healthy

and secure life, d) capacity an enabling environment for active aging. The index offers the opportunity to compare data from different countries, for each category.

CUSTOMIZED FOOTWEAR - FUNCTIONING VS. DISABILITY

Neuromusculoskeletal and movement–related function present impairments inevitably induced both by the aging process and to diseases to which older people are more vulnerable.

Standing and walking are fundamental functions and preserving their performance is an imperative demand for active aging. The main feature of this domain is the multidisciplinarity which requires the work of multiple professionals: physicians, rehabilitators, engineers, technical staff.

Age-related changes of the feet affect the bones, the joints and the soft tissues (skin and muscles) as well. Impaired nervous coordination of ambulation and balance may cause difficulties to accomplish the activities of daily living, increase the frequency of falls, disability and dependence and a low quality of life.

Impaired biomechanics of the feet in geriatric population is caused by the changing of size and shape, degenerative alterations, arch flattening, orthopaedic problems such as tendonitis, osteoarthritis. Skin disorders (itchy rash, calluses, keratosis), changes in plantar tactile sensitivity and alteration in muscles tone makes walking and standing very difficult.

Impaired ambulation due to age-related changes of the feet might be prevented by designing and producing customized footwear for elderly, adapted to their demands and necessities of an active life.

From the perspective of The International Classification of Functioning, Disability and Health (ICF-WHO, 2001) aging is seen as a health problem which needs special approach and compensatory measures in order to prevent disability. The ICF states a framework and definitions in order to understand age-related disability.

The structural an functional loss induced by the aging process are *impairments.* When severe enough, impairments generate *acivity limitation* and *participation restriction* in social life. The *disability* reveals the negative aspects of the interaction of the elderly with the environment.

The policy makers in health care and in social assistance should set as a main goal the maintaining or even increasing the *capacit*y of the elderly (describes the individual's ability to execute a task or an action) and their *performance* as well (what the older people can do in their current environment).

Speaking of performing in real life environment, work at older ages is an important issue. The increased life expectancy is expected to affect the individual behaviour concerning the decision to remain employed longer. Professional longevity, "the greying of the workforce" could have positive effects both for the individuals and for the developing societal norms of more active lives at older ages. Professional longevity is related with more opportunities for employment for older people, with life-long learning and the ensuring of an healthy and age-friendly work environment.

Coping with work promotes a healthy and independent life and becomes more and more important as the work force is aging.

Walking and staying are essential for work and they are impaired by the aging process itself and by the health conditions associated with older ages. Developing researches an innovation in the field is a great challenge.

Changes of the traditional model of employment, becoming more flexible in accepting older people have a direct impact on the sustainability of pension schemes both public and private. Basically, the viability of an aging society relies on the adaptability of the labour market and the sustainability of pension schemes (Scardino, 2009).

Social participation in the form of civic engagement and volunteering are profitable forms for activating the elderly. The measure of the benefits are a good physical health, the lowering of the risk for depression and the increased longevity.

A new Strategy for the Protection of the Elderly and Active Aging ,was developed in the frame of Romania's National Reform Program, supported by European Structural and Investment Funds (ESIF. Romania's commitment to Active Aging Concept is a essential for achieving the goals of broader *Europe 2020 Strategy*. An important part of, this engagement aims to shape the attitudes and opinions of the Romanian society towards elderly and aging in general, to make it more permissive to new societal models, to challenging demographic, economic and social changing.

The guidelines of the new policies which define the Strategy are: to prolong life and achieve healthy living; to increase employment rates among the older population; to encourage the social participation of the elderly; to reduce dependence and the providing of long-term care.

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SETTING THE ANATOMICAL AND MORPHS-FUNCTIONAL PARTICULARITIES OF THE DIABETIC FOOT PATIENTS USEFUL WHEN DESIGNING SPECIFIC FOOTWEAR

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The study allowed us to establish a set of criteria for rational classification of foot typologies in patients with diabetic based on parameters obtained from the planting footprint analysis. Pathological deviations were determined by comparison with normal foot plantografic parameter values. Thus coefficient previous area in patients with diabetic foot pathology-associated longitudinal flat foot (K1 = 1.24 ± 0.51, RF, K1 = 1.09 ± 0.14 - LF) presents the deviation as both adducted and the abduction of the area and flat foot pathology associated with hallux valgus transverse (K1 = 0.94 ± 0.35, RF, K1 = 0.85 ± 0.83 - LF)- slight deviation in abduction. Transverse arch flattening coefficient and angle of deviation of the big toe associated longitudinal flat foot - K2 = 0.39 ± 0.02, α2 = 8.9 ± 4.5 (RF) K2 = 0.38 ± 0, 01, α2 = 9.2 ± 4.3 (LF) is degree and flat foot hallux valgus associated with cross grades II and III. Longitudinal arch flattening coefficient and the angle of deflection of a flat foot heel longitudinal axis associated - K3 = 1.23 ± 0.62, α3 = -7.4 ± 1.35 (RF), K3 = 1.18 ± 0, 91, α3 = -6.8 ± 1.47 (LF) indicates the deviation degree and valgus. The design of diabetic footwear must be based on a in-depth understanding of the interaction between the footwear design features and the biomechanical characteristics and anatomic-morphs-functional parameters of the foot.

Keywords: foot, diabetes, pathology, anatomic-morphs-functional parameters

INTRODUCTION

Morphological changes of feet encountered within the framework of the different pathologies represent a challenge for the specialists of footwear industry. Thus in terms of their frequency they occupy first place in the structure of orthopedic disease. Diseases of the foot may occur in the case of diseases which are not related to the musculoskeletal system such as, for example, diabetes mellitus.

Diabetic foot is a common complication of diabetes. Diabetic foot care is very important because any injury, even a minor one can lead to serious complications. Due to peripheral nerve lesions (diabetic neuropathy) and blood vessel injuries, small injuries occurring at this level can be easily overlooked and can get over infected. The patient does not feel pain caused by various injuries, in some cases even the discomfort caused by inappropriate footwear. These injuries can cause the foot deformation.

The callus is commonly encountered in people with diabetes, without the peripheral pulse, at the Pedi or posterior tibia, in people with peripheral nerve damage, peripheral neuropathy in people with foot deformities, such as "hammer toes" Charcot arthropathy or "Charcot foot ". All these things can increase the risk of developing diabetic foot ulcers. The blisters are a special form of callus appearing on the fine hairless surfaces of the skin, due to pressure forces acting in an ellipsoidal direction. The most common location for blisters being the dorsum of the foot and the fingers. Unlike callus the blisters have a specific structure with a central core very hard and a soft peripheral area. Often these calluses are painful when putting pressure on their level. They can be removed surgically, but in the most cases they reappear and is a recurring problem for diabetic foot.

THE DESCRIPTION OF THE SUBJECTS INCLUDED IN THE STUDY AND RESEARCH METHODS

The work was developed based on complex examinations of 158 diabetic patients (316 feet) of which 92 were women and 66 men, aged between 21-68 years and weighing 47-110 kg. Examinations and studies have been conducted mainly in the Republican Center Experimental Prosthesis, Orthopedics and Rehabilitation (CREPOR), Republican Clinical Hospital and the Hospital "St. Spiridon" in Chisinau, Republic of Moldova during 2006-2013.

In the study group 38.4 % of the patients (61 persons) were examined by addressing in the clinic of the CREPOR center, and 97 people (61.6%) – in ambulatory conditions. In the study group the patients with diabetes of the type I (insulin-dependent) constituted 68 patients (42.8%), type II (insulin-nondependent) - 82 patients (51.6 %) and diabetes latent (or hidden) - 8 patients (5.6 %).

The primary patients (diabetes was detected for the first time to his addressing) constituted 14.5% (23 patients). The duration of diabetes was to 10 years 67 patients (42.1%), while 68 patients (43.4 %) - more than 10 years. When examining the patients were detected neuro-trophic complications in the distal part of the foot and lower limbs of different forms of severity at 41 patients (25.8%). In 9 patients (5.7%) were determined lesions, ulcers or wounds with relapse in the forefoot and in 32 patients (20.1%) - cracks and fissures in the heel area. The histories of the diseases, these complications were followed from 68 (42.8%) of the patients to who were detected and trophic and fungal damages of the toes' nails and mutilations (hammer toes, claws, etc.). In order to establish the typology of the foot were taken the fingerprints images tree front and side views of the lower limbs. Retrieving images and fingerprint legs planting and its processing was performed using computerized photometric system "Plantovizor".

The digital photometer APC "Plantovizor 2006" is composed of: a plantoscop mirror, double lighting, thick glass graduated two digital cameras, two cameras telescopic stands (Figure 1).

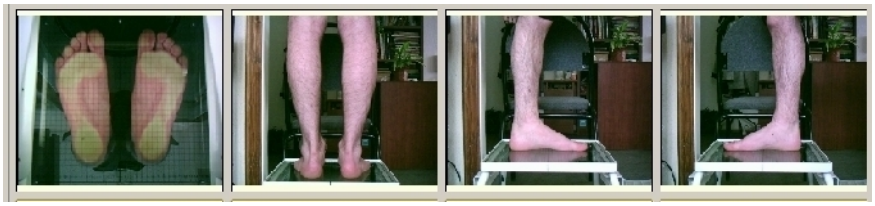

Figure 1. Images taken by shooting for the study

The studied subject climbs on the plantoscop in an orthostatic position. Afterwards are take four pictures from different views: the bottom (plantar); the back (posterior); of the middle side (including the knee and ankle) for the right and the left foot. After shooting, the obtained pictures are downloaded to your computer and processed using specialized software "Casting Sozvezdie" according to the scheme shown in Figure 2.

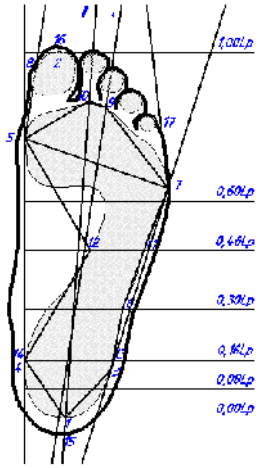

Figure 2. The processing scheme of the Footprint

The analysis of the footprint enables the establishment of the transversal and longitudinal parameters and of the specific angles:

1) The coefficient (shape) of the anterior leg - K1 determines the position of the front part of the foot against the back part and the foot shape which can be: straight K1 = 1.08; flared towards the heel (pes abductus) K1 > 1.08, flaring to the top (pes abductus) K1 < 0.92.

2) The coefficient of the transverse flattening - K2 usually it is between 0.30-0.35. In the case of the transverse flattening there are three degrees of deformation:

3) The coefficient of the longitudinal flattening - K3, is determined as the ratio of the footprint's width, and the width of the contour, measured perpendicular to the tangent line. For the normal foot this coefficient value between 0.51 to 1.00.

Angles:

1) The angle of the Chopart joint (angle α1) - characterizes the lateral deviation of the foot in the mediotarsian area. The value of this angle is in the range of 170-180° for the normal foot and open to the outside. Reducing the amount of the angle down to 130-140 ° indicates the valgus position in the medial zone of the leg. If there is the varus position angle α 1 is open on the inside.

2) The angle of deviation of the big toe (hallux) - α2 is obtained at the intersection of the tangent lines of the thumbprint. Characterizes the position of the thumb, the normal value of which is up to 10°. Exceeding the normal value indicates the presence of the hallux valgus abnormality.

3) The deflection angle of the heel α3 – characterizes the heel's position to the vertical line. Normative value of this angle is between -6° to 1°. The deviation of the heel from the standard values outside leads to the foot anomaly-valgus and the deviation to the inside-the anomaly varus.

The "Plantovizor" enables printing of the results of the investigation on the paper with the form of some records containing information about anthrop-functional and parameter values of the investigated leg (Figure 3).

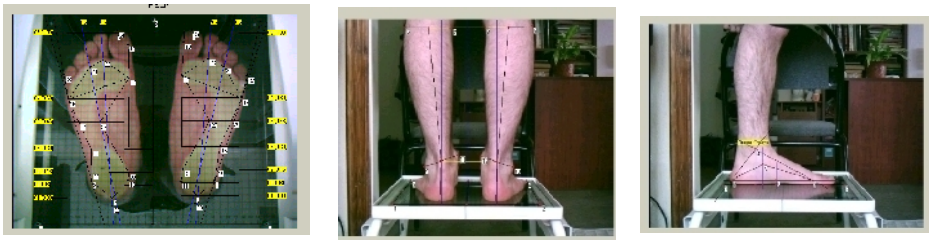

Figure 3. Images resulted from processing the "Plantovizor" system

EXPERIMENTAL RESULTS AND INTERPRETATIONS

Estimation of the Amount and Severity of Foot Pathologies in Patients with Diabetes

Depending on the type of pathology in patients with diabetic foot have been divided into 12 distinct groups, to which was added the group with diabetes without anatomical changes at the level of the leg (Table 1).

Table 1. The proportion of the foot pathologies in patients with diabetes

Group	Pathologies and deseasof the foot	The number of legs	The percentage of the total number of legs %
1	Flat foot flattening of the longitudinal arch	89	28.2
2	Flat foot flattening of the transversal arch	97	30.7
3	Flat foot flattening of the transversal arch associated with the flattening of the longitudinal arch	86	27.2
4	Hollow foot (pes cavus)	27	8.5
5	Hollow foot it associated with the flattening of the transversal arch	39	12.3
6	Hallux valgus	51	16.1
7	Hallux valgus associated with the flattening of the transversal arch	54	17.4
8	Hindfoot valgus (pes valgus)	48	15.2
9	Hindfoot varus (pes varus)	12	3.8
10	Hammer fingers or claws	69	21.8
11	Blisters, calus and hyperkeratosis	204	64.6
12	Amputation of the fingers	48	15.2
13	Lack of anatomical changes in the foot	36	11.4

Anatomic-morphed-functional parameters of the foot in patients with diabetes

The results of investigations carried plantographic lower limb and foot diabetic patients divided into two groups, enabled the setting global parameters which were calculated anatomic-morph-functional statistical indicators (Table 2). Statistical significance of values for the left leg, respectively as compared to the mean left foot /

right foot was tested using Student t test (T -test). In the case of an n number of subjects with a high degree of freedom, df = 2 x (n-1), an acceptable probability of P = 65 % and the value of α = 0.05, Student's t test is applied (T-test). As a working hypothesis it is considered that the variation in group 1 (left leg) is not significantly different statistically from the change to group 2. The obtained value of the test statistic, the value of t and p, where p = 0.05 compared, the higher is acceptable thus, the initial hypothesis, whereby the size of the group 1 (left foot) is not significant statistically different those of group 2 (right foot).

Table 2. The global values of anatomic-morphs-functional parameters in patients' foot who have diabetes

The anatomorphofunctional parametres	The foot of the patients with diabetes				Normal foot
	With the associated longitudinal flat foot patology		With the transversal flat foot associated with hallux valgus		
	Right foot (RF) M±σ	Left foot (LF) M±σ	Right foot (RF) M±σ	Left foot (LF) M±σ	
The coefficient of the anterior zone of the foot (K1)	1,24±0,51**	1,09±0,14*	0,94±0,35***	0,85±0,83*	0,92-1,08
The coefficien of fllatening of the transversal vault (K2)	0,39±0,02*	0,38±0,01*	0,45±0,18**	0,43±0,07*	0,25-0,35
The coefficien of fllatening of the longitudinal vault (K3)	1,23±0,62**	1,18±0,91*	1,01±0,47*	0,89±0,60**	0,51-1,00
The angle of the Chopart jointα1,º	175,7±1,2**	174,7±1,6*	145,7±1,3**	132,7±1,9***	170-180º
The deviation angle of the big toe α2,º	8,9±4,5*	9,2±4,3**	18,4±3,8*	19,2±4,1***	till 10º
The deviation angle of the sole's axis towards the vertical α3,º	-7,4±1,35***	-6,8±1,47*	-2,7±1,08*	-4,6±1,16**	from -6 till +1º
The deviation angle of the leg's axis towards the vertical,º	1,2±0,5*	0,9±0,47**	1,3±0,45*	1,4±0,32***	0-5º

Note: * - p<0.05, ** - p<0.01, *** - p<0.001

Pathological deviations were determined by comparison with normal foot plantografic parameter values. Thus coefficient previous area in patients with diabetic foot pathology - associated longitudinal flat foot (K1 = 1.24 ± 0.51, RF, K1 = 1.09 ± 0.14 - LF) presents the deviation as both adducted and the abduction of the area and flat foot pathology associated with hallux valgus transverse (K1 = 0.94 ± 0.35, RF, K1 = 0.85 ± 0.83 - LF)- slight deviation in abduction. Transverse arch flattening coefficient and angle of deviation of the big toe associated longitudinal flat foot - K2 = 0.39 ± 0.02, α2 = 8.9 ± 4.5 (RF) K2 = 0.38 ± 0,01, α2 = 9.2 ± 4.3 (LF) is degree and flat foot hallux valgus associated with cross - K2 = 0.45 ± 0.18, α2 = 18.4 ± 3.8 (RF), K2 = 0.43 ± 0.07, α2 = 19.2 ± 4.1 (LF) grades II and III. Longitudinal arch flattening coefficient and the angle of deflection of a flat foot heel longitudinal axis associated - K3 = 1.23 ± 0.62, α3 = -7.4 ± 1.35 (RF), K3 = 1.18 ± 0,91, α3 = -6.8 ± 1.47 (LF) indicates the deviation

degree and valgus and flat foot hallux valgus associated transverse K3 = 1.01 ± 0.47, α3 = -2.7 ± 1.08 (RF), K3 = 0.89 ± 0.60, α3 = -4.6 ± 1.16 (LF) degree without axis deviation heel.

CONCLUSION

The anatomical proportions of the foot in patients with diabetes have specific features and represent an association of pathologies and disorders. The study allowed us to establish a set of criteria for rational classification of foot typologies in patients with diabetic based on parameters obtained from the planting footprint analysis. Examination of the fingerprints planting is a quick and simple method which led to obtaining configuration parameters for the quantification of the foot and creation of a data base. The usefulness of this classification can be found both in the subsequent analyzes of anthropometric parameters and in modeling and designing specific footwear. The design of diabetic footwear must be based on a in-depth understanding of the interaction between the footwear design features and the biomechanical characteristics and anatomic-morphs-functional parameters of the foot. The obtained information by morph-structural setting foot type designer footwear is used in the design of the customized products for each subject, depending on the complex manifestations of structural change identified and the risk in the foot areas.

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INNOVATIVE PROCESSING OF LIGNITE COMBUSTION ASHES TOWARDS CERAMICS SYNTHESIS

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In the present study, novel two-step sintering of lignite combustion highly-calcareous ashes is investigated. So far, solid-state sintering of fly ash through conventional thermal treatment has already been considered for the development of ceramic materials. Although conventional sintering is generally a preferred manufacturing technique for industrial ceramics, a new two-step method is proposed in the last few years for sintering dense and fine ceramic microstructures. Innovative processing of ashes – produced in massive quantities from lignite-fed power generation units – in the elaboration of value-added ceramic materials represents a challenge with important environmental, technological and economic aspects, due to the intrinsic characteristics of these industrial by-products. For that purpose, disc-shaped compacts from fly ash, bottom ash and ash mixtures were prepared by cold pressing, and then consolidated using two-step sintering procedures: the specimens were first heated at a higher temperature to achieve an intermediate starting density, then cooled down and held at a lower temperature to approach higher densities. The sintered specimens were characterized by means of XRD and SEM-EDX analyses as well as shrinkage, apparent density, water absorption capability and Vickers microhardness measurements. According to the results, effectively solidified ceramic materials are obtained with interesting specific microstructural features and properties.

Keywords: Two-step sintering, lignite combustion ashes, ceramics.

INTRODUCTION

Management of fly ash and bottom ash, produced in massive quantities from lignite combustion for power generation, is of great environmental concern, as only a limited amounts of ashes are currently used, while the rest is landfilled, a situation that will possibly cause severe long-term environmental effects (Zacco *et al.*, 2014, Karapanagioti *et al.*, 2012, Sun *et al.*, 2011, Polic *et al.*, 2005). Nevertheless, the chemical, mineralogical and morphological properties of these by-products render their valorization as secondary raw materials into value-added products a challenge with important technological, environmental and economic aspects (Karayannis *et al.*, 2012, Blissett and Rowson, 2012, Badanoiu and Voicu, 2011, Ahmaruzzaman, 2010, Komnitsas, 2009).

In particular, the utilization of lignite combustion ashes for ceramics production represents a significant research area. So far, solid-state sintering of fly ash through conventional thermal treatment has already been considered for the development of ceramic materials. Although conventional sintering is a generally established manufacturing technique for industrial ceramics, a new two-step method (TSS) was proposed in the last few years for sintering powdery materials to produce dense and fine ceramic microstructures without detrimental final-stage grain growth, thus leading to improved mechanical properties (Chen and Wang, 2000). This sintering method uses two steps in the heating schedule: the sample is first heated to a higher temperature to achieve an intermediate but sufficiently high starting density, then cooled down and held at a lower temperature to approach full densities or even for pore size control. The

feasibility of densification without grain growth relies on the suppression of grain-boundary migration while keeping grain-boundary diffusion active. The two-step sintering procedure appears an important milestone for modern technical ceramics, and its feasibility has been verified in various ceramic systems (Domopoulou *et al.*, 2014, Kim *et al.*, 2014, Xiong *et al.*, 2013, Isobe *et al.*, 2012, Zhang *et al.*, 2012, Lourenco *et al.*, 2011, Li *et al.*, 2010, Maca *et al.*, 2010, Li *et al.*, 2008, Mazaheri *et al.*, 2008, Wang *et al.*, 2008). The efficiency of this method is considered more pronounced in ceramics with crystalline phases of higher symmetry, whereas its applicability is questioned only when the activation energy for consolidation is higher than that for grain growth. This new process provides sufficient motivation to investigate its potential for the treatment and valorization of industrial by-products in ceramics development, as an efficient alternative to currently employed traditional heating procedures.

In the present research, innovative two-step sintering of lignite high-Ca fly ash and bottom ash is attempted. This rich-in-Ca ash composition can possibly be expected to yield an interesting mineralogy in the sintered materials, while the Ca-bearing phases may also act as a flux enabling melting to begin at lower temperatures, thus using less energy. Furthermore, the low thermal conductivity of the ashes, as they are mainly consisted of hollow sphere-shaped particles (cenospheres), should also influence the sintering result. In previous studies, ashes of similar composition have been tested by the authors for microwave as well as conventional furnace sintering (Karayannis *et al.*, 2013a, Karayannis *et al.*, 2013b, Moutsatsou *et al.*, 2008). By heating the lignite ashes under investigation employing the two-step method, interesting solidification processes, microstructure and properties can be attained. The sintering results are evaluated as a function of the two-step sintering program employed and the ash specimen composition.

EXPERIMENTAL

Materials

The fly ash (FA) utilized as a secondary raw material in the present research, a fine powder, was obtained by the electrostatic precipitation of dust-like particles from the flue gases of a lignite combustion power plant situated in Northern Greece (Region of Western Macedonia where the main lignite deposits of the country are located). The bottom ash (BA) that was used, a granular material much coarser than FA and also formed during lignite firing, was removed from the bottom of dry boilers of the same power plant. The chemical analysis results for these ashes are given in Table 1. It can be seen that, particularly FA, is characterized by high % CaO content, similarly to other fly ashes from Northern Greece power units belonging to Class-C ashes (ASTM C 618). BA is less abundant in Ca, but contains higher residual (unburned) carbon (≈5%).

Table 1. Chemical analysis of Greek fly ash (FA) and bottom ash (BA)

	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	SO_3	Na_2O	K_2O
FA	30.16	14.93	5.10	34.99	2.69	6.28	1.01	0.40
BA	48.63	21.62	7.29	6.83	2.75	2.78	0.89	2.97

Two-Step Sintering of Ash Specimens

Disc-shaped green compacts (13 mm diameter) from FA, BA and 50-50 wt% FA-BA mixtures were prepared by uniaxially cold pressing in a stainless steel die using a

hydraulic press (Specac, 15011), and then consolidated using two-step sintering procedures in a laboratory chamber programmable furnace (Thermoconcept, KL06/13).

A temperature slightly lower than the melting point of the ashes was selected for the 1st first sintering step (T1=1150°C). As soon as T1 was attained, the samples were rapidly cooled down in the furnace, and held at a lower temperature, this of the 2nd sintering step (T2=950°C). Two different sintering programs where tested: TSS1, where the samples remained at T2 for 2h, and TSS1, where the samples remained at T2 for 4h. In order to evaluate the intermediate sintering result at the end of the 1st sintering step, a series of specimens were sintered only up to 1150°C and then taken out of the furnace. Finally, the specimens were gradually cooled to ambient temperature in the furnace.

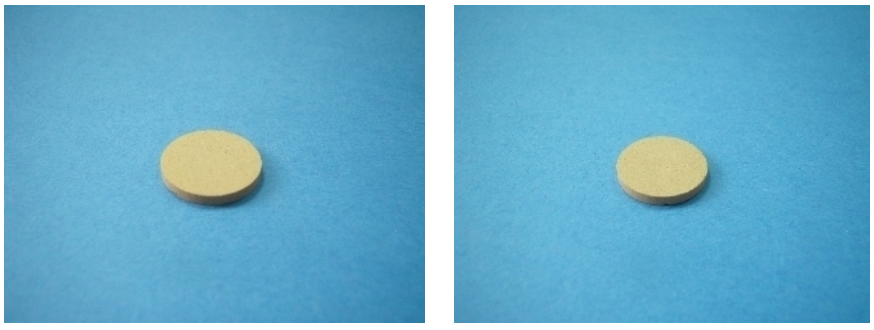
Characterization of Two-Step Sintered Specimens

Phase characterization of green and two-step sintered specimens was realized by X-Ray Diffraction (XRD) (Siemens, Diffractometer D-5000). The microstructures produced were studied using Scanning Electron Microscopy (SEM - Jeol, JSM-6400).

Shrinkage of the samples was calculated as the volume change (%) upon sintering. Apparent density was measured according to the Archimedes principle by means of a specific apparatus (Shimadzu, SMK401-AUW220V). In order to determine water absorption capacity, sintered specimens were first oven dried to constant weight, cooled to room temperature, and weighed (W1). Then, the specimens were immersed in distilled water for 24h, and subsequently weighed again (W2), after the excess water had been removed from their surfaces by wiping with a damp cloth. The water absorption (%) was calculated as the increase in mass as a percentage of oven dried mass. Vickers microhardness was measured with a load of 200g and a dwell time of 15s (Shimadzu, HMV-2T). In order to enable reliable comparisons, mean microhardness values were calculated over five valid indentations per specimen.

RESULTS AND DISCUSSION

Photographs of ash mixtures (FA/BA:1/1) sintered through the two-step method (TSS1 (a) and TSS2 (b)) are provided in Figure 1. It can be seen that successfully consolidated integral and similar between them earth-yellowish specimens are obtained.



(a) (b)

Figure 1. Photographs of specimens (diameter: 13 mm) made of ash mixtures (FA/BA:1/1) and sintered via the two-step method: (a) TSS1 and (b) TSS2

The main mineral phases present in the green specimens prepared of ash mixture (FA/BA:1/1), as well as in those sintered only up to 1150°C and in the two-step sintered sintered ones (TSS1 and TSS2), as determined by means of XRD, are shown in Figure 2.

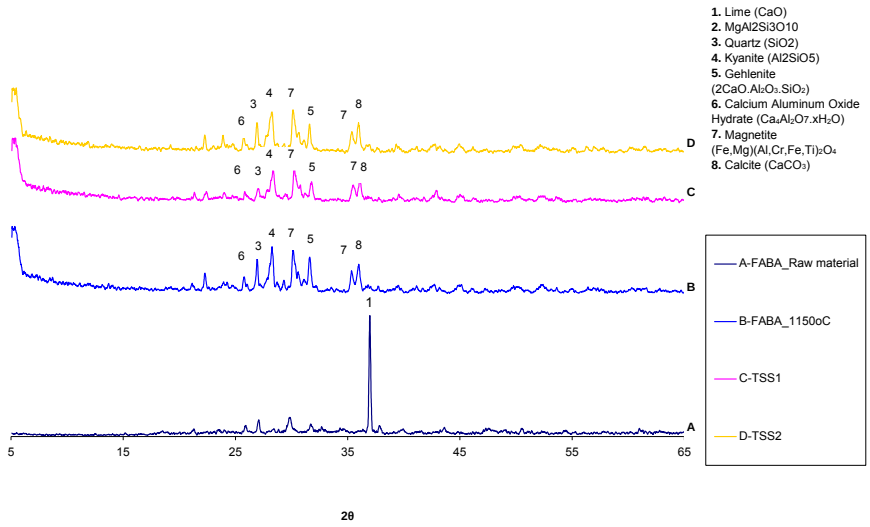
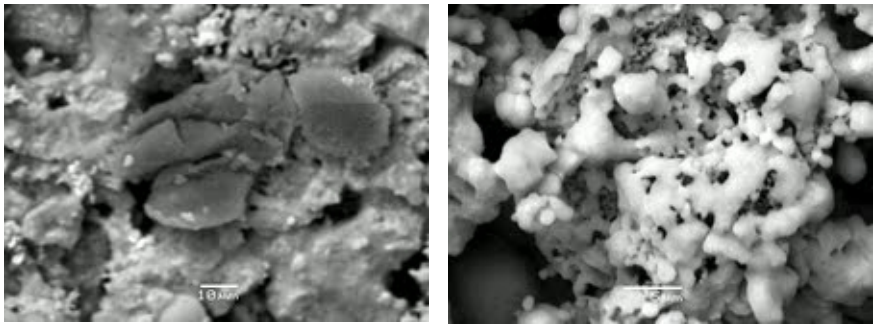


Figure 2. Typical XRD spectra of ash mixtures (FA/BA:1/1):
(A) green, (B) sintered up to 1150°C, & sintered by 2-step method (C:TSS1 & D:TSS2)

Obviously, the intensity of the peak associated with lime (CaO) predominates in the diffractogram of the green specimen (Figure 2, A). This intense presence of lime in the ashes is mainly due to the high percentage of limestone ($CaCO_3$) in the feedcoal (lignite) of the power unit. The ash mixtures sintered only up to 1150°C (Figure 2, B) as well as those sintered via the two-step procedure (Figure 2, C and D) exhibit quite similar mineralogical compositions between them. Therefore, it can be concluded that the mineralogical phases detected in the two-step sintered specimens were already generated in the first sintering step. Actually, an interesting ceramic microstructure and more complex than that of the raw materials is revealed, whose major crystalline phases are kyanite, magnetite, quartz, gehlenite and calcite.

The result of the consolidation process can be evaluated upon microstructural observation of two-step sintered specimens via SEM analyses (Figure 3). From Figure 3, when using the TSS1 heating program (2h-holding-time in the second step), a reasonably sintered and rather rough matrix is shown where quartz crystals are located. On the other hand, a clearly finer microstucture is obtained when the TSS2 program (4h-holding-time in the second step) is employed. Hence, the consolidation process and the specific microstructural features not only depend on the temperatures chosen for the two sintering steps but also on the holding time in the second step. In order to achieve a higher densification degree, even higher holding times should be considered for the second sintering step.



(a) (b)

Figure 3. SEM micrographs of ash mixtures (FA/BA:1/1) sintered using two-step programs: (a) TSS1 and (b) TSS2

Shrinkage of the two-step sintered fly ash specimens is lower than 5%. Density lies in the range of 2.3-2.4 g.cm^{-3}, whereas mean Vickers microhardness attains 120 HV. Water absorptivity varies up to 20 %, strongly depending on the existence of open and interconnected pores that can be verified from the SEM analyses. Moreover, rough pore wall surfaces are frequently indicated in the micrographs, revealing a high specific surface area facilitating water adsorption on the pore wall (following the penetration of water in the open pores and its diffusion along the interconnected pores). Precise preparation of porous ceramics having various pore size and porosity two-step sintering program from Al_2O_3 powder compact are also reported from other researchers (Isobe et al. 2012). From a technological point view, such microstructures may be of interest for porous ceramic applications.

CONCLUSIONS

Lightweight ceramic microstructures with pronounced crystallinity are obtained from lignite high-Ca fly ash and bottom ash employing two-step sintering processes.

The highly-calcareous nature of fly ash and the residual carbon of bottom ash influence the sintering results but do not hinder the synthesis of ceramics via this method.

TSS approach depends on the heating program applied. It should be noticed that sintering time is kept at lower temperatures, thus leading to possible energy consumption reductions. Further investigation of the heating conditions would enable a tailoring of the ceramic microstructures to meet the needs for specific applications.

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FINISHING TECHNOLOGIES FOR WOOLEN SHEEPSKINS

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In this paper, a finishing composition and technology for nappalan woolen sheep skins are presented. The finishing composition contains a new feel agent – Wax-AGE 7 – based on beeswax, lanolin and triethanolamine monostearate and stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide. The performance of finishing film was investigated by physical-mechanical analysis, optical microscopy and ATR-FITR. Good results for feel and physical-mechanical properties were obtained.

Keywords: woolen sheepskin, finishing, nappalan, feel agent

INTRODUCTION

Disperse systems used for finishing nappalan leather and fur contain various auxiliaries: pigments, binders, dyes, natural and synthetic waxes, preservatives, plasticizers, thickening agents, fillers, penetrating agents, solvents (Lange, 1982; Heidemann, 1994).

Nappalan furs are processed from lower quality finished suede leather, with superficial flaws which can be corrected by coating with polymer films.

Nappalan leather finishes differ from classic ones in terms of the requirements imposed. Thus, film thickness must be minimal, so as not to alter softness and extensibility specific to suede furs on which they are deposited. Film-forming polymers must be selected so as to be compatible with those embedded in the impregnation layer, deposited on the velour surface before applying the film, and the adhesion of film to the substrate must have a high value. As nappalan finish is applied to clothing items to be worn in the cold season, the film-forming mixture must maintain its highly elastic properties up to temperatures of about 12°C (Maier, 2008; Maier, 2010).

In nappalan fur finishing operations there are restrictions regarding the use of heavy metals in pigment pastes, ethoxylated alkylphenols, formaldehyde and other toxic crosslinking agents (OSPAR, 2004).

The paper presents finishing technologies for nappalan finished sheepskin furs with matte or glossy effect. In the final dressing a new feel agent was used, Wax-AGE 7, obtained by emulsifying a mixture of beeswax, lanolin and ethanolamine monostearate and stabilized using lauryl alcohol ethoxylated with 7 moles of ethylene oxide (Niculescu, 2013).

EXPERIMENTAL

Materials

Woolen sheep skins chrome tanned, retanned, fatliquored and dyed (black and brown), 0.9-1.2 mm thick from Taro tannery, Romania.

Finishing auxiliaries from Triderma, Germany (Triderma, 2010).

Feel agent – Wax-AGE 7 – obtained by a process described in Niculescu (2013). The product was used as handle modifier containing beeswax, lanolin and triethanolamine monostearate and stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide with the following characteristics: dry substance – 18.96, pH (10% solution) – 7.3, Ford cup viscosity Φ4 – 27 s, kinematic viscosity, cSt – 10.48, density – 0.975 g/cm^3.

Methods

Optical microscopy images were captured using a Leica stereomicroscope S8AP0 model with optic fiber cold light source, L2, with three levels of intensity, and Magnification 40X.

Physical-mechanical analyses were performed for the finished leathers: tensile strength and elongation using the Shopper dynamometer pendulum (Louis Shopper, Germany), according to SR EN ISO 3376:2003; colour resistance to friction using the Veslic device (Giuliani, Italy), according to SR EN ISO 5402:2003.

Attenuated Total Reflectance Fourier transform infrared spectroscopy (ATR-FTIR) measurements were run with a Jasco instrument (model 4200), in the following conditions: wavenumber range – 600-4000 cm^{-1}; data pitch – 0.964233 cm^{-1}; data points – 3610: aperture setting – 7.1 mm; scanning speed – 2 mm/s; number of scans – 30; resolution – 4 cm^{-1}; filter – 30 kHz; angle of incident radiation – 45°.

Nappalan Woolen Sheepskin Finishing Technologies

Surface finishing technologies have been developed for black (P1) and brown (P2) nappalan sheepskins (Niculescu, 2007 and 2014). Finishing composition of nappalan type finishing of woolen sheepskins included acrylic and polyurethanic binders in the base coat and a special wax in final coat, namely 20-50g/L Wax-AGE 7 wax emulsion was added to improve the final feel of finishing films. Finishing composition was applied on dermal side of the furs.

The finishing technology and composition are presented in Table 1 for black nappalan and Table 2 for brown nappalan.

Table 1. Technology for finishing black nappalan woolen sheepskins (P1)

Operation	Composition of dispersion/Method of application
Applying dispersion I (basecoat)	200 g/L Roda-base 4088 200 g/L Roda-base 4095 600 g/L water Application by spraying (2 passes)
Intermediate plating	Hydraulic press using mirror or steam plate, parameters: 50-60°C, 50-80 bar
Applying dispersion I	By spraying (2-3 passes)

Applying final dressing (fixing)	700 g/L Roda-pure 5011 50 g/L Wax -AGE 7 250 g/L water Application by spraying (2 passes of final dressing)
Final plating	Hydraulic press using mirror plate, parameters: 60-70°C, 50-80 bar

Table 2. Technology for finishing brown nappalan woolen sheepskins (P2)

Operation	Composition of dispersion/Method of application
Applying dispersion I (basecoat)	80 g/L Casicolor Brown R 40 g/L Roda wax MONO 100 g/L Roda-cryl 510 100 g/L Roda-pur 302 100 g/L Roda-bind TLC 327 560 g/L water Application by spraying (2 passes)
Intermediate pressing	Hydraulic press using mirror or steam plate, parameters: 50-60°C, 50-80 bar
Applying dispersion I	By spraying (2-3 passes)
Applying final dressing (fixing)	700 g/L Roda-lac 93 20 g/L Wax -AGE 7 280 g/L water Application by spraying (2 passes of final dressing)
Final plating	Hydraulic press using mirror plate, parameters: 70-90°C, 50-100 bar

RESULTS AND DISCUSSION

Optical microscopy images show that the finishing films are uniformly on the dermal support for nappalan finished woolen sheepskins. Magnification was 40X for surface of finished fur.

P1 – black nappalan

P2 – brown nappalan

Figure 1. Images for nappalan finished woolen sheep skins (40X)

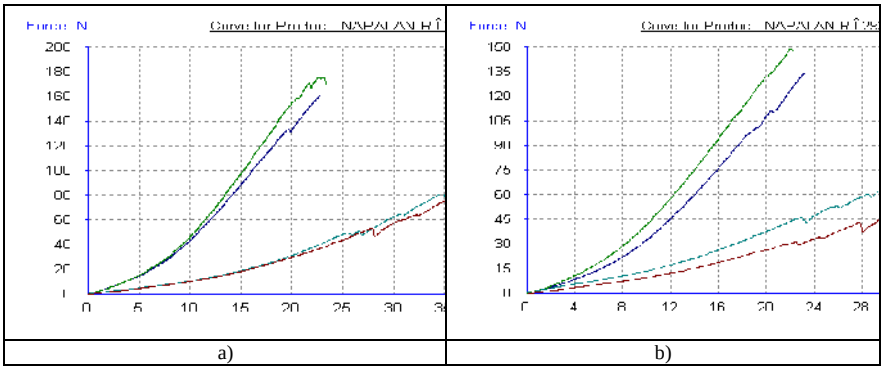
Physical-Mechanical Characteristics of Nappalan Finished Woolen Sheepskins

As Table 3 shows, physical-mechanical characteristics of nappalan finished woolen sheepskins P1 and P2, fall into the limits provided by the standards in force.

Table 3. Mechanical performances of fur samples P1-P2

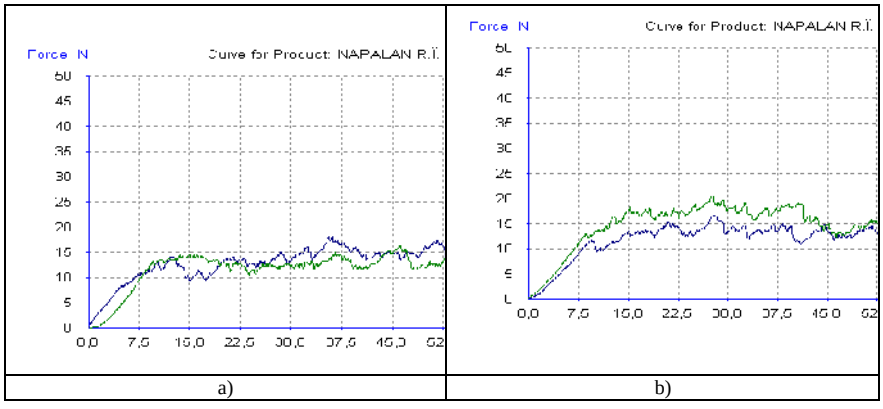
Leather sample	Tensile strength (N/mm^2)	Tear Resistance (N/mm^2)	Elongation (%)	Resistance to dry friction (mark)	Resistance to wet friction (mark)
P1	Parallel -168 perpendicular-94	17	Parallel - 46 Perpendicular-80	5/5	4-5/4
P2	Parallel-141 Perpendicular-80	18	Parallel-45 Perpendicular-65	4-5/4	4/3
SR EN ISO 3376:2003	Direction Parallel-min.100 perpendicular min.70	min. 15	Direction Parallel-min.40 perpendicular min.45	min. 4-5/3	min. 4/3

Physical-mechanical characteristics of nappalan finished woolen sheepskins are also presented in Figure 2 and Figure 3.



a) b)

Figure 2. Tensile strength for nappalan finished woolen sheepskins P1 and P2



a) b)

Figure 3. Tear Resistance for nappalan finished woolen sheepskins P1 and P2

FT-IR Characterization of Obtained fur Assortments

Figure 4 presents the spectral characteristics of the untreated fur samples M1, compared with those of the treated ones P1, used polyurethane final dressing containing AGE 7 wax emulsion. The main spectral bands of the unfinished fur samples M1 are found in the following regions: 2922 and 2853 cm^{-1} ($-CH_3$, $-CH_2-$), 1632 cm^{-1} (-OC-N), 1540 cm^{-1} (NH), 1450 cm^{-1} (C–H), 1229 cm^{-1} (NH-CO), 1085, and 1037 cm^{-1} (C-O). The spectra of the top coated fur samples P1 have the following characteristics, compared to those of the untreated ones: the intense peak at about 1632 cm^{-1}, characteristic for C=O and –OC-N groups, from the spectrum of the untreated fur, is absent in the spectra of the treated samples; these last ones present a peak at about 1724 cm^{-1}, corresponding to C=O stretching in saturated ester of polyurethane top coat; the two peaks at about 1237 and 1187 cm^{-1} assigned to the couplings of C-O and C-C stretches and to the stretching vibration of C-O-C of acrylates appear in the spectra of the finished fur; the broad band in the region 3200-3500 cm^{-1}, assigned to hydroxyl and amide groups vibrations, is diminished in the spectra of the top coated leather samples compared with the untreated ones.

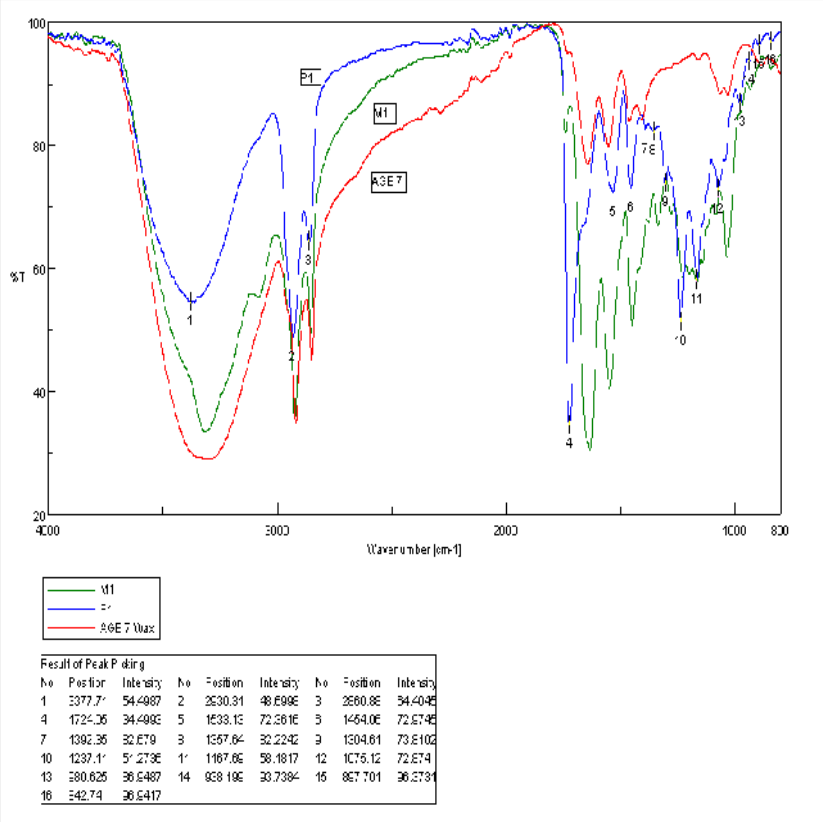


Figure 4. Superposed FT-IR spectra of unfinished dermal surface (M1), with finishing (P1) and of Wax AGE 7 emulsion used in the final dressing

The above differences between the IR spectra of finished and unfinished dermal surface may be considered as a proof for the chemical bonding of the top coat on base coat components. The polyurethane top coat containing the Wax AGE 7 emulsion is also bound chemically on base coat. Thus, the band from about 1645 cm^{-1} characteristic for C=O and –OC-N groups from the spectrum of the emulsion film is absent from the spectrum of the finished fur samples.

FTIR spectra of fur sample treated with polyurethane coating agent, to which the Wax AGE 7 emulsion was added, show that the latter is chemically bound to the leather surface.

CONCLUSIONS

In this paper a finishing composition and technology for nappalan woolen sheepskins are presented. Finishing composition contain a new feeling feel agent – Wax-AGE 7 – based on beeswax, lanolin and triethanolamine monostearate and stabilized with lauryl alcohol ethoxylated with 7 moles of ethylene oxide.

The investigations on performance of finishing film showed good results of feel and physical-mechanical properties.

Acknowledgements

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CASE STUDY ON FAILURE MECHANISM FOR REINFORCED CONCRETE FRAME STRUCTURE WITH DIFFERENT INFILL MATERIAL

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Reinforced concrete frame structures are a wide spread structural system all around the world. Considered to be flexible structures, they are strongly recommended in areas with height seismicity. Several problems may occur due to different stiffness between the infill material and the reinforced concrete frame structure. Two major failures may appear – to crack the infill material or to damage the columns from the structural system. The second one is more unfavorable, and the only solution is to demolish the entire structure. The paper aim is to present a solution for this problem. For this purpose several numerical simulation are done using traditional material for the infill wall and an innovative solution. The results show that a flexible infill material lead to a better behavior for the system.

Keywords: infill material, stiffness, polyurethane.

INTRODUCTION

Reinforced concrete (RC) frame buildings are a widely used structural system due to its fast execution and good behaviour at horizontal loading. Even so, problems may appear due to non-structural elements that are not always taken into consideration in the design process, but have a great influence on the overall behaviour of the structure. These elements bring supplementary stiffness to the structure and can cause unfavourable failure mechanisms. Some of them are cause by the stiffness differences between the infill walls and the RC frame structure – producing either the failure of the infill wall or damaging the columns at the extremities (Olteanu, 2011). Separation between masonry walls and frames is often not provided and, as a consequence, walls and frames interact during strong ground motion. This leads to structural response deviating radically from what is expected in the design, Figure 1 (Elwood *et al.*, 2000).

Experimental research on the response of RC frames with masonry infill walls subject to static and dynamic lateral cyclic loads have shown that infill walls lead to significant increases in strength and stiffness in relation to bare RC frames (Mehmet, 2011). Intense research had been conducted starting with Polyakov (1960), Stafford-Smith and Carter (1969), Klingner and Bertero (1978), Mehrabi *et al.* (1996), Stavridis and Shing (2009) to more recent research conducted by Dolsek and Fajfar (2008), Sagttar and Liel, 2010, and Pujol and Fick (2010).

Figure 1. Damaged RC frame with hollow clay tile infill masonry, Izmit 1999 (Elwood *et al.*, 2000)

It is recognized that infill materials significantly influence the seismic performance of the resulting infill frame structures. The present study focuses on the effect of different types of infill materials (commonly used and a new one) on the seismic performance of an infill RC frames compared using SAP2000 and Axis (Olteanu *et al.*, 2011).

The paper presents several comparisons between classical infill material and a new one. The innovative masonry block is based on polyurethane.

POLYURETHANE

Polyurethane is a resilient, flexible and durable manufactured material that can replace rubber, metal or wood in thousands of applications. Can be manufactured in any color, can take any shape, size or geometrical complexity. Since its invention during the 1940s, polyurethane has been used in a wide range of items.

In Romania, the polyurethane was introduced in 1978 and it is manufactured by Oltchim SA.

Polyurethane is used in construction since 1950 in the shape of insulation panels for roofs, walls, ceilings and floors. Metal-faced polyurethane sandwich panels are widely used for large industrial buildings, refrigerated and other warehouses, office blocks, exhibition halls, fair pavilions, schools and sports halls. Prefabricated sandwich wall and lightweight roofing consist of metal facings bonded tightly together by a core of rigid polyurethane foam.

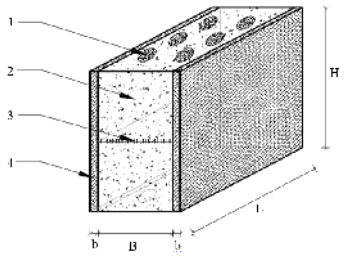


Figure 2. FlexyBrick: 1. Gaps; 2. Polyurethane; 3. Reinforcement mesh; 4. Cement plates

Polyurethane foam sandwich panels are recommended for facilities where a constant temperature or strict hygiene maintenance is required.

Polyol and isocyanate are the main components of polyurethane, which have to be mixed mechanically at a temperature of 25°C. The mixture expands and because of the limited dimensions of the mold, physical properties of the polyurethane bricks are obtained.

FlexyBricks, as the masonry blocks are called, can be produced in a variety of shapes and sizes. In Figure 2 a prototype is shown for a polyurethane masonry a block that has fibre cement boards on both faces, in order to increase the mechanical strength.

By creating an appropriate mold, FlexyBrick can be produced with circular cross section, similar with the branch of a tree. In this way, polyurethane concrete block can replace logs used for constructions in the country-house.

SOFTWARE ANALYSES

In order to compare the behaviour of bare RC frame with that having infill of different materials, static and modal analysis were performed, using computer software AxisVM and SAP2000. Both of them are based on the finite element method (Pastia *et al.*, 2013).

A 3 stories 2D RC frame structure was considered, each level of 3 m high and opening of 6 m. The dimensions of the columns are 50x50 cmxcm, and for the beam are 30x50 cmxcm. The structure was loaded only with self weight. Four cases were considered: the bare RC frame and 3 cases with different infill material –clay tile, aerated light weight concrete (A.A.C) and FlexyBrick. The considered material characteristics are presented in Table 1.

Table 1. Materials characteristics

Material	Modulus of elasticity, E (N/mm^2)	Poisson coefficient, υ	Unit weight (kg/m^3)
Concrete, C20/25	29000	0.2	2500
Clay tile	1210	0.2	2700
A.A.C.	2500	0.1	1100
FlexyBrick	100	0.2	180

Results for Static Analysis

In the static analysis the total internal efforts were evaluated. These values determine the structural system elements dimensions for the cross section and for the reinforcement. The values for the axial force and shear force at the base of the frame are shown in Figure 3 and Figure 4.

These results are directly proportional with the weight of the considered structures, which are shown in Figure 5. The values for the weight were extracted from SAP2000. The main conclusion from this analysis is that the proposed brick, FlexyBrick, brings the smallest load to the structural system.

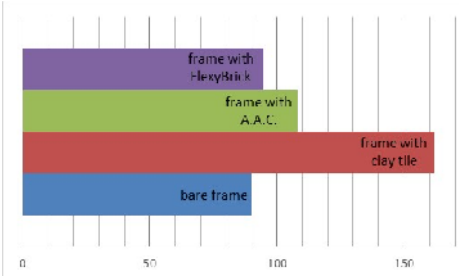


Figure 3. Axial force reactions results

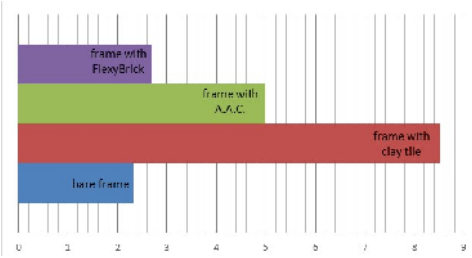


Figure 4. Shear force reactions results

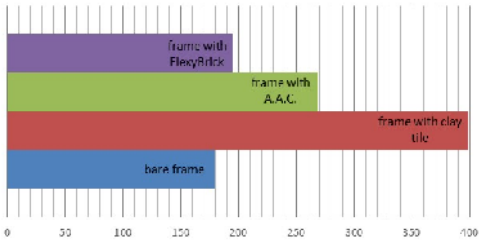


Figure 5. Structure weight, G (kN)

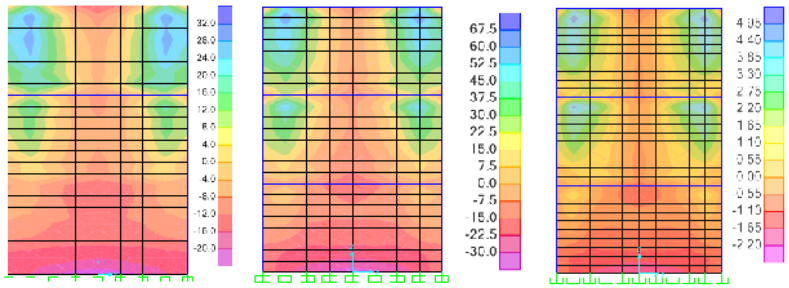
If the maximum stresses that appears in the masonry are compared, it can be noticed in Figure 6 that even though the distribution is similar in all 3 cases, the maximum values for the clay infill is 13 times higher than the values obtained for FlexyBrick and the case in which we consider A.A.C. infill, the values are only 6 times higher.

Results for the Modal Analysis

The results of this analysis are: characteristics of the models considered – periods of vibration, frequencies, eigenvalues, percentage of participation of the masses, adding modal participation rates and structural modal participation factors.

The first comparison realized was for horizontal displacement at the top of the structure, Figure 7. The maximum value is for the bare frame, 0.36 mm, and the minimum one is for the case in which the infill material is clay. In this case the displacement reaches 0.23 mm. It can be observed that the displacements for the first

mode of vibration in case of a frame with FlexyBrick infill and a bare frame, are similar, differing only with 5%.



(a) (b) (c)

Figure 6. Maximum stresses in the infill material for: (a). A.A.C., (b) clay tile and (c) FlexyBrick

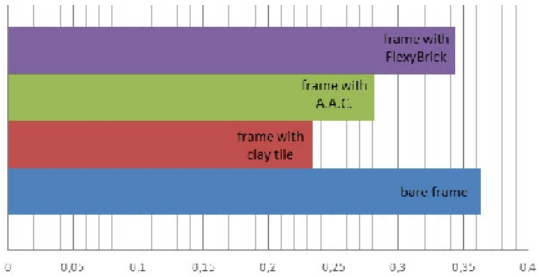


Figure 7. Horizontal displacement at the top of the structure, (mm)

The modal analysis was performed in SAP200 and Axis software. It appears that differences in values between the two computer programs vary between 0.24% and 5%, differences that may be considered negligible, Figure 8. The model with FlexyBrick infill has the closed fundamental period with the bare frame case.

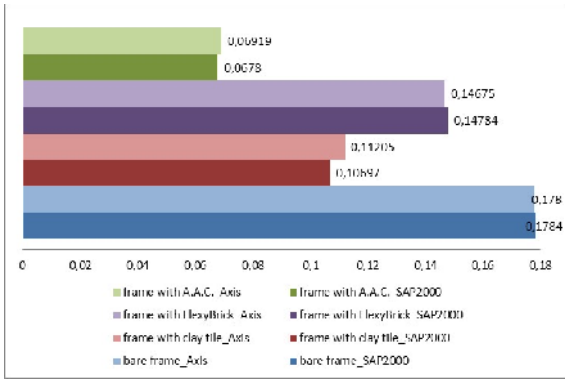


Figure 8. Period for first mode of vibration in SAP200 and Axis (s)

Comparing the stiffnesses for the four considered cases, Figure 9, it is observed that the A.A.C. has the higher value, and the FlexyBrick has the lowest one. This is in accordance with the initial hypothesis that the proposed infill material will bring for the structural system sufficient stiffness without changing the failure mechanism. Beside this the FlexyBrick infill is recommended for the envelope because of its thermal insulating properties.

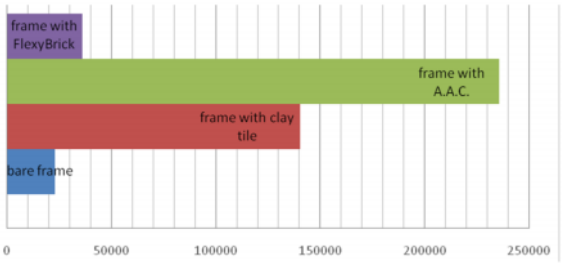


Figure 9. Assembly stiffness comparison, k (kN/m)

CONCLUSION

The main conclusion is that the behavior of reinforced concrete frame structures can be improved by changing the material characteristics of the infill. The proposed polyurethane masonry block have a flexible behavior, with good properties for thermal insulation and mechanical ones. The main advantage is the low self weight, respectively the low load that is transmited to the structural system.

Further analyses will be made in order to determine physical properties, costs and detailed behavior with nonlinear analysis for the FlexyBrick product.

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FOOTWEAR PLANTAR MECHANICAL COMFORT: PHYSICAL MEASURES AND MODERN APPROACHES TO THEIR APPROXIMATION

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Comfort is a fairly complex subjective phenomenon consisting of physiological, psychological and physical aspects. Foot mechanical comfort is defined and two major groups are distinguished: dorsal and plantar. Plantar mechanical comfort is concerned with the interaction of the foot with the footwear sole and the ground. The most important mechanical phenomena and quantities related to plantar mechanical comfort and their relation to foot anatomy and physiology, footwear design and use activities are discussed. Though measurement and prediction tools for comfort aspects exist, there is limited evidence regarding integration of different approaches towards the complete measurement, calculation or approximation of plantar mechanical comfort aspects. The most appropriate measures for complete plantar mechanical comfort evaluation are proposed. Taking into account the modern trend towards applying computer simulation and optimization techniques, an account of attempts to evaluate mechanical plantar comfort for upright human standing and walking with the aid of Finite Element Analysis is given.

Keywords: footwear design, mechanical comfort, plantar comfort evaluation.

THE CASE FOR FOOT COMFORT

Comfort is “lack of pain”. Richards defined comfort as subjective wellbeing in reaction to a situation, whereas Slater as a pleasant state of physiological, psychological and physical harmony with the environment (Vink *et al.*, 2005; Fan, 2009a). Its perception is a cognitive process influenced by previous experiences with the product, the physiological and psychological state of the user and the environment. It includes physical, physiological and psychological aspects becoming apparent after the end of the use of goods. Comfort is now recognized as a subjective phenomenon, a reaction to the environment or a situation and that it has physical, physiological and psychological aspects, prior, during and after use. A product is never comfortable. It may provide the user with no discomfort experience under certain conditions (Vink *et al.*, 2005). Therefore, when referring to comfortable footwear, product effects on foot comfort shall be considered rather than product features.

Interest in foot comfort is strong. Impact mechanics have attracted the attention of sport shoes brands (Thompson *et al.*, 1999). In podiatry, solutions for patient pressure relief are well documented (Tyrrell and Carter, 2009). In addition, occupational health and safety research has attributed impaired cognitive and physical employee performance to low clothing permeability, whereas in military textiles investigations on the psycho-physiological comfort aspects are now established (Cardello, 2008).

PHYSICAL, PHYSIOLOGICAL AND PSYCHOLOGICAL ASPECTS

Luximon and Zhang (2006), Fan (2009a) and Kilinc-Balci (2011a) provide accounts of foot anatomy and physiology that are useful in understanding comfort. Skin supports cutaneous sensations: tactile (touch, pressure, vibration) and pain. Sensation is the awareness of changes (stimuli) in temperature, pressure, mechanical energy or chemical energy activating sensory neurons. When stimulus amplitude exceeds the ‘sensing’ threshold of a receptor, stimulus energy is converted to electrical impulses conducted through nervous pathways to the central nervous system and the cerebral cortex.

Establishing the thresholds is important in discomfort studies. For example, prickle (acute, localized, painful sensation) threshold is 75mN per $10cm^2$ and, recognizing this fact, fibre protrusion models referring to the rod buckling theory have been proposed to explain discomfort arising from fabric roughness (Fan J., 2009a).

Sensory mechanoreceptors are either free dendrite cells, associated with tickle and touch or encapsulated Merkel discs, Meissner, Ruffini and Pacinian corpuscles associated with pressure, vibration, and some touch sensations. Pain nociceptors belong to the first group. The spatial distribution of receptors in the skin is not even (Kilinc-Balci, 2011b). Typically, it ranges from 2.5mm on finger tips to 50mm at the back and the calf. This has complications with regard spatial stimuli discrimination for different body parts. Another characteristic of receptors is adaptation occurring when the duration of the stimulus is long, causing sensory magnitude decrease. The perception of a sensation may alter and even disappear even though the magnitude of the stimulus remains constant. Differences in adaptation exist. Free nerve endings adapt quickly but Merkel discs, Ruffini corpuscles and nociceptors slowly.

Touch is localized and mostly related to free nerve endings. Pressure sensation originates from deformation of larger tissue areas. Vibration sensation is realized through repetitive signals from Meissner and Pacinian corpuscles, detecting low and high-frequency vibrations respectively. Pain is caused by excessive stimulation of sensory receptors and large deformations.

Sensation is about receiving and partly integrating low level stimuli information. Context augmented stimuli receive attention depending on sensory or cognitive thresholds. Attention involves selective processing of some sensorial and environmental aspects to the expense of others. Attended input is recognized and organized into meaningful information involving higher brain processes. Organized information is interpreted and inferences are made thus leading to perceiving a phenomenon. Thus, perception is an active transformational mental process involving the selection, organization, structuring and interpretation of information to make inferences, create experiences and give meaning to sensorial information. It is driven by both sensory data and cognitive processes influenced by beliefs and attitudes about the product and its use in a specific context. These are not restricted to function. They are often shaped by financial, self flattering, cultural, religious, social and peer influences (Fan, 2009b).

Comfort, as subjective perception of physiological effects of physical quantities, is addressed by a combination of psychophysics, objective physical measurements and psychological scaling (Fan, 2009a). The focus, in this paper, is on the identification and presentation of physical-mechanical effects in foot comfort studies.

DEFINING AND DETERMINING MECHANICAL PLANTAR COMFORT

Foot comfort is related to sensations and homeostatic response. Mechanical comfort is about interaction of the foot with footwear and the ground, mainly related to the upright stance and gait mechanics that are well documented (Kirtley, 2006). It concerns foot deformation, vector mechanics and walking kinetics and kinematics (Braune and Fischer, 1987; Keller *et al.*, 1996; Luximon and Zhang, 2006; Whittle, 2007). Instrumentation exists for measurements and verification of analytical and numerical techniques (Cobb and Claremont, 1995).

The plantar foot side contacts the ground, either directly or through the footwear sole. Most loads are applied to the foot and the human body through this side (Goonetilleke, 1999). Sole geometry and material properties affect foot plantar

mechanics. Geometry affects insole shape conformance (fitting) to the foot and the resulting areas of contact (Williams and Nester, 2010), as well as contact between the outsole and the floor. It also affects stress-strain distribution internally within the solid bodies of the sole parts. Reverse heeling, wedged soles and rocker bottoms complicate force transfer characteristics (Tyrrel and Carter, 2009). Plantar mechanical comfort is concerned with interactions between footwear sole geometry and materials with the plantar side of the foot and the ground, for different environmental conditions and activities. On the other hand, the dorsal foot side does not bear any significant loads, though it is subject to upper assembly restrictions. Restrictive pressures or touch forces are much smaller compared to plantar loads. Dorsal mechanical comfort is limited to fitting and stability (Fong *et al.*, 2007).

Loads, due to the reaction of the ground to the action of body weight while standing or on impact with the floor, are of utmost importance in plantar mechanics (Whittle, 2007). The normal vector of the plantar forces is transmitted to the leg and body through the long rod-like shin bone. Shear loads are minor compared to normal ones (Whittle, 2007). Shear transverse and longitudinal forces are not important to body loading, however they cause skin deformation and superficial damages (Frederick and Wojcieszak, 2005). Touch concerns localized loads. Contrary to pressures spreading over greater areas, these concentrated loads may cause pain and local tissue damage.

Besides loading, cushioning is also fundamental. In biomechanics, it is the ability of material to reduce forces that may cause injury (shock absorption). In ergonomics, the bias is on material hardness or compression characteristics related to fatigue and discomfort. Goonetilleke (1999) reviewed cushioning and proposed metrics of hardness, compression and deceleration. Instead of deceleration, rebound resilience or percentage energy lost can be used, extracted from stress-strain hysteresis graphs. If recovery time for the sole material exceeds gait cycle time, then residual compression affects sole mechanics (Alcántara *et al.*, 2001).

Footbed conformance to foot shape has also attracted attention. Insole shape affects plantar pressure distribution. Experiments on patients with plantar fasciitis support claims that footbed shapes affect comfort perception (Witana *et al.*, 2009). The midfoot area is critical since it can change shape independently of the forefoot and heel. Comparison between flat and contoured shapes has been carried out to establish effects on the comfort experience, however, such investigations are complex due to effects of hardness and percentage energy lost on the perception of touch and pressure sensations (Mills *et al.*, 2011).

Bending and torsional deformation of the sole are of no less significance to other aspects. The influence of longitudinal bending characteristics on push-off forces during walking has been demonstrated (Cikajlo *et al.*, 2007). Also the relationships between longitudinal rotation and rotation along the toe break line with comfort have been established along with relevant measurement arrangements (Hillstrom, 2005).

Another aspect of plantar footwear comfort is insole roughness affecting sensation of touch and pressure mechanoreceptors. Relevant research is still limited and the phenomenon not well understood (Nurse *et al.*, 2005). The opposite of roughness is softness; however, certain researchers use this term to describe materials that are not very stiff rather than surface properties (Kilinc-Balci, 2011b)

Static and dynamic balance issues, as affected by footwear sole materials and geometry, also affect the comfort experience (Emery, 2003; Menant *et al.*, 2008). Static balance is approached in terms of centre of mass and base of support statics. Dynamic balance is related to staying upright while moving and impacting the floor. Wikstrom *et*

al. (2005) have proposed the Dynamic Postural Stability Index, based on plantar force vectors, as a candidate dynamic balance metric.

Identification of stimuli, related mechanical phenomena and their effects on physiology is the first step on defining mechanical plantar comfort. Comfort is related to tactile psychophysics, therefore amplitude, spatial distribution and adaptation effects analysis is necessary. Objective analysis should be followed by subjective analysis (e.g. psychological scaling) and corresponding objective phenomena to subjective perceptions (Kilinc-Balci, 2011b; Fan, 2009a). Most research on comfort stimuli and mechanical aspects focus on specific aspects (e.g. pressures, prickle, shock absorption) and it appears that in the wider apparel field a truly integrated approach is missing even in distinct comfort sectors (e.g. tactile). This is a view supported also by Kilinck-Balci (2011b). The footwear industry, however, has to demonstrate an interesting multi-aspect approach proposed by the European CEC-MADE-SHOE project and called Virtual Shoe Test Bed (Azariadis *et al.*, 2007). This was based on the concepts of normal plantar pressure, cushioning, shock absorption, bending, torsion, friction, stability, footwear weight and thermal aspects of footwear. Simplified differential and analytical models were developed for simulation purposes. The VSTB approach is fairly integrated; however, a complete view of mechanical plantar comfort would require that the following phenomena and quantities be considered: plantar loading (normal and shear), compression, hardness, rebound resilience, compression recovery time, longitudinal bending and torsion, torsion along the toe brake axis, shape conformance (on deformation), static balance, dynamic postural stability index and weight. Given current scientific knowledge, insole roughness cannot be considered as a comfort metric. These aspects can be measured by analytic, numerical and laboratory tools and most can be normalized to body weight (Shorten, 2002).

Arrangements for measuring and analytic tools for predicting individual comfort aspects have been developed (Kilinc-Balci, 2011b; Fan, 2009a). Modern trends are moving towards numerical and simulation techniques. Finite Element Analysis (FEA) is one of these techniques and it is suited to analyzing complex structures, combining different materials, loading and boundary conditions. Accuracy depends on the accuracy of geometric models, initial and boundary conditions and meshing density. FEA has been used for calculating stress-strain relationships on tissues due to the interaction of the foot with the sole and the floor. Early works by Nakamura *et al.* (1981), Lemmon *et al.* (1997), Jacob and Patil (1999), and Chen *et al.* (2001) resulted in simple though adequate models. FEA was used for material sensitivity studies by Lewis (2003) and Cheung *et al.* (2005) and for investigating the effects of insole geometry on plantar pressure distribution (Chen *et al.*, 2003). Modern models (Verdejo and Mills, 2004; Cheung and Zhang, 2005) and simulation cases (Cheung *et al.*, 2005; Yu *et al.*, 2007; Hsu *et al.*, 2008; Antunes *et al.*, 2008; Gu *et al.*, 2010) are realistic, accurate, verified (through testing) and validated.

Modern biomodels for FEA are developed from CT and MRI medical imaging scans acquired from the feet of healthy research subjects in neutral position. Areas of bones, soft tissue and anatomical features are extracted from density based segmentation, with the aid of medical imaging software and 3D models of these elements are developed. So far, researchers have made certain assumptions (e.g. cartilage is treated as extension of osseus tissue and tarsal bones are considered as one large solid) to facilitate calculations (Cheung and Zhang, 2006; Chen *et al.*, 2001). Then, the 3D model of the foot, containing both hard and soft tissues, is assembled. FEA of foot structures is often

combined with surfaces other than simple flooring. Sole multi-layered multi-material assemblies, have been designed, meshed and simulated by several researchers.

A similar approach is followed in the OptShoes (2012) project, in which it is attempted to develop an integrated tool for plantar comfort simulation for different multi-layer and multi-material sole arrangements.

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SEISMIC RESPONSE OF BUILDING STRUCTURES WITH PASSIVE FLUID DAMPERS

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This paper presents the numerical results of several passive viscous fluid dampers implemented to a real three-storey building to improve the seismic structural performance. For strong earthquakes, a large amount of input energy will be dissipated by inelastic deformation which means structural damages take the form of localized plastic hinges. Energy dissipation demand of the main building elements can be reduced by transferring this energy dissipation demand to the viscous fluid dampers. These devices operate on the principle of the flow of special compressible fluids through orifices and are characterized by a high cycle-fatigue life. Some examples of experimental studies to understand the principles of the operation of the fluid devices for seismic energy dissipation are briefly described. A common mathematical model for describing the linear or nonlinear behavior of viscous fluid dampers in terms of force-velocity curves is presented. Numerical simulations have been performed in order to assess the performance of a structure protected with such devices. The additional of passive viscous fluid dampers demonstrates a reduction of the input energy and of the deformation in structure, this way improving the structural seismic protection.

Keywords: viscous fluid damper, seismic protection, energy dissipation

INTRODUCTION

The traditional approach of decreasing vibrations due to earthquake and wind loads is applied in building structures with enough resistance and capacity of deformation in a ductile way. This approach, based on insuring a combination resistance-ductility of principal elements of a structure, understands the strong seismic action as a load at which the structure must resist and remain functional, accepting a certain level of structural and non-structural degradations.

In seismic design, the input energy of an earthquake is typically dissipated through hysteretic behaviour of main structural elements, which allows the structure to undergo inelastic deformations without compromising the stability of the structure (Banu *et al.*, 2012). Furthermore, inelastic behaviour translates into some level of damage on these elements. This damage leads to high cost for repair works, in the cases when repairs are possible. Sometimes, the damage is so large that repairs are not viable, even though the structure has not collapsed, and the building must be demolished.

The passive dampers dissipate energy on principles as phase transformation in metals, deformation of viscoelastic solids, flow of special compressible fluids through orifices and sliding friction (Budescu *et al.*, 2010; Olteanu *et al.*, 2011; Pastia and Luca, 2012; Stefancu *et al.*, 2011). In this paper, passive viscous fluid dampers are designed such that the most dissipation energy demand is concentrated on these devices. The concept is described in this study in a mathematical relationship for linear or non-linear behaviour of viscous fluid dampers in terms of force-velocity curves. Numerical simulations have been performed in order to assess the performance of the three-degree of freedom lumped mass structure protected with such devices.

Viscous damping devices and tuned mass dampers were used in London Millennium Bridge in order to reduce vertical and horizontal vibrations due to pedestrian induced forces. This reduction corresponds to increasing the basic damping ratio of the structure

at 20% for the assumed loads. 8 TMDs were used to provide secondarily additional damping in horizontal direction and a total of 26 pairs of TMDs were installed to supplement primarily the damping in vertical direction. Horizontal and vertical damping is provided by 37 viscous dampers, of 7 different types (Taylor, 2002). Only 4 supplemental viscous damping devices were used to decrease resonant deflections in vertical direction. All viscous dampers increase damping primarily for the lateral and lateral-torsional structural modes. One of viscous damping devices is illustrated in Figure 1, during installation (Dallard *et al.*, 2001).

Figure 1. Installation of viscous dampers beneath the deck of the London Millennium Bridge

MATHEMATICAL MODELING OF VISCOUS FLUID DAMPER BEHAVIOUR

These dampers operate on the principle of the flow of special compressible fluids through orifices and are characterized by a high cycle-fatigue life. Construction of a device is shown in Figure 2 (Symans and Constantinou,1995).

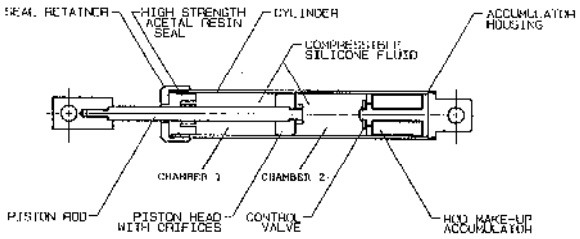


Figure 2. Description of passive viscous fluid damper

It consists of a stainless steel piston rod with a bronze orifice head and a piston rod make-up accumulator. The device is filled with a thin silicone oil (kinematic viscosity = 100 cSt, specific weight = 9.78 KN/m^3). The force generated by the fluid damper is due to a pressure differential across the piston head. When the damper is subjected to a compressive force, the fluid volume is reduced by the product of travel and piston rod area. This change in fluid volume is accompanied by the development of a restoring force. This is prevented by use of an accumulator and a control valve. An alternative construction of this device is a balanced piston rod.

A balanced piston rod is one in which the rod enters the damper, is connected to a piston head, and then continues out through the opposite end of the device. The orifice

flow around the piston head is compensated by a passive bi-metallic thermostat that allows operation of this device over a wide temperature range (-40°C to 70°C) (Symans and Constantinou, 1995).

The linear force-displacement response of a fluid viscous device has commonly been characterized by mechanical models consisting of combinations of linear springs and dashpots. The cyclic response of fluid viscous devices is generally dependent on the deformation frequency and can be mathematically formulated using a classical Maxwell model in which a dashpot and a spring are joined in series. The tested device demonstrated that, below a cut-off frequency less than about 4Hz, the storage stiffness was negligible while the damping coefficient was nearly constant. This cut-off frequency depends on the accumulator design (Symans and Constantinou, 1995). Hence, the device provides supplemental damping to the natural modes of vibration of the structure having an important contribution to the structural response. These natural modes must have frequencies less than the cut-off frequency. Also, the higher modes of vibration do not contribute significantly to the structural response because the damper provides both supplemental damping and stiffness.

The non-linear force-velocity relationship of the passive fluid damper below the cut-off frequency is expressed as (Symans *et al.*, 2008)

$$f_{df}(t) = C\left|\dot{x}(t)\right|^{\alpha} \operatorname{sgn}\left[\dot{x}(t)\right] \tag{1}$$

where $\dot{x}(t)$ is the relative velocity of the piston head with respect to the damper housing, C – the damping coefficient and α – the exponent which is determined by the piston head orifice design and is located in a range of approximately 0.5 to 2.0. For seismic applications the exponent α is a value from 0.5 to 1. A design with α=1 appears to be the most desired for earthquake engineering applications because the damper behavior becomes as an ideal linear viscous dashpot (Symans *et al.*, 2008).

SEISMIC RESPONSE OF 3DOF FRAME STRUCTURE WITH PASSIVE FLUID DAMPERS

A series of numerical analyses was performed to a three-story frame structure with one degree of freedom per floor at which are attached damping devices. The dampers were placed at the first story (consisting in 2 fluid devices), at the second story (consisting in 4 fluid devices) and at the third story (consisting in 6 fluid devices).

Description of the Frame Structure

The frame structure constructed inside the ELSA (European Laboratory for Structural Assessment) has three stories. It consists of a steel frame with floors constituted by sheet metal and concrete properly connected. The inter-storey hight is 2 meters because the scale was considered 2/3 of a real structure. The structure has been tested with dynamic and pseudodynamic techniques (Marazzi, 2003). Without entering into details, the mass, stiffness and damping matrices used in the analytical model are as follows:

$$M = \begin{bmatrix} 5000 & 0 & 0 \\ 0 & 5000 & 0 \\ 0 & 0 & 5000 \end{bmatrix} \text{(Kg)}, \quad K = \begin{bmatrix} 45774000 & -25936000 & 647000 \\ -25936000 & 36260000 & -17555000 \\ 647000 & -17555000 & 12600000 \end{bmatrix} \text{(N/m)},$$

$$C = \begin{bmatrix} 5854 & -3547 & 1347 \\ -3547 & 5073 & -1571 \\ 1347 & -1571 & 2273 \end{bmatrix} \text{(Ns/m)}.$$

With the above matrices, the identified natural frequencies and the damping ratios are reported in the following table:

Table 1. Frequencies and damping ratios

Frequencies	f_1 3.018 (Hz)	f_2 10.29 (Hz)	f_3 19.09 (Hz)
Damping ratios	ξ_1 0.8 %	ξ_2 0.32 %	ξ_3 0.8 %

These low damping ratio values are normal for a steel structure.

Numerical Results

The structural system has been modeled with MATLAB and several simulations have been performed using as input El Centro and Bucuresti'77 earthquake accelerations and a synthetic ground acceleration time history with a response spectrum compatible with Eurocode 8 (EC8) for stiff soils. Characteristics of the fluid device are: damping coefficient C=20000 Ns/m and exponent α=1 for the linear behavior.

A comparison of peak responses (relative displacement and absolute acceleration at each floors) are shown in Table 2 supposing that the structure works in linear elastic domain and it is equipped with fluid dampers connected through diagonal bracings to the structure. The damper force $f_{df}(t)$ acting on the structure is obtained by considering an angle θ=30^o of the damping element with respect to the horizontal axis. For a rigid brace the damper force can be written as

$$f_{df}(t) = C\left|\dot{x}(t)\right|^{\alpha} \operatorname{sgn}\left[\dot{x}(t)\right]\cos^2\theta \tag{2}$$

Table 2. Peak relative displacements and peak absolute accelerations

Excitation	No. Dampers	d1 (cm)	d2 (cm)	d3 (cm)	a1 (m/s^2)	a2 (m/s^2)	a3 (m/s^2)
El Centro	0	0.9473	2.4811	3.4862	5.3126	9.8528	13.910
El Centro	2	0.8510	2.2892	3.2419	3.4284	7.8442	11.234
El Centro	4	0.6531	1.7724	2.5316	2.9957	5.9727	7.7222
El Centro	6	0.5470	1.4851	2.1233	2.2269	4.6444	6.1147
Buc'77	0	0.3523	0.9140	1.2748	1.4565	3.4807	4.5943
Buc'77	2	0.3269	0.7749	1.0339	1.2054	2.9085	3.8114
Buc'77	4	0.3041	0.7520	1.0235	0.8112	1.9162	2.5047
Buc'77	6	0.2968	0.7354	1.0025	0.6453	1.5654	2.1093
EC8	0	1.4372	3.5653	4.8242	8.3443	14.792	18.006
EC8	2	1.0395	2.6071	3.5607	3.9973	9.5597	12.526
EC8	4	0.7563	1.9075	2.6216	3.2745	6.7703	8.9416
EC8	6	0.5831	1.4684	2.0127	2.7919	5.5471	7.6382

Figure 3 shows a comparison among the time histories of displacement response of the structure's third floor, with and without the 6 fluid dampers.

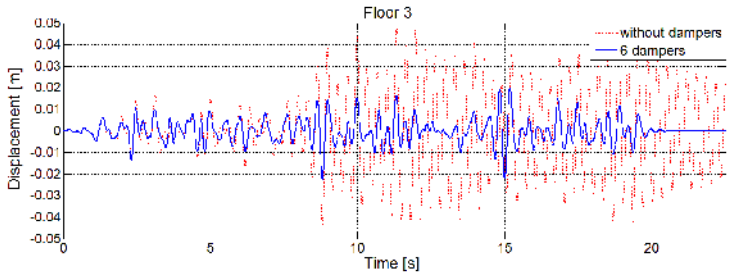


Figure 3. Time histories of displacement response of structural models to EC8 earthquake motion without and with 6 fluid dampers

In all analyzed cases, the effect of fluid dampers is to reduce the response of the 3DOF structural model from 10% (case of 2 dampers) to 65% (case of 6 dampers). The placement of the viscous fluid devices at first story did not have any adverse behavior. In general, the effect of fluid dampers is improved by placing them at those stories where the largest interstory velocities are expected.

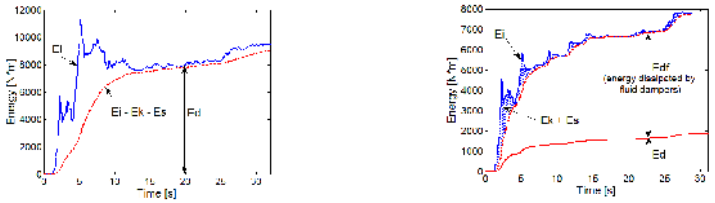
From view point of energy balance, the work done by external forces acting on a system is equal to the sum of the mechanical energy temporarily stored in the structure (kinetic and recoverable strain energies) and the energy transformed to another form, through either viscous damping energy or irrecoverable hysteretic energy. The relative energy balance equation takes the following time-dependent conservation of energy form (Symans, et al., 2008):

$$E_i = E_k + E_s + E_d + E_h + E_{df} \tag{3}$$

where, at time t, E_i is input energy in structure by the earthquake motion, E_k - kinetic energy stored in the mass, E_s - recoverable strain energy stored by the structure, E_d - viscous damping energy dissipated by the principal elements of the structure, E_h - hysteretic energy dissipated by the principal elements of the structure, E_{df} – viscous energy dissipated by the supplemental fluid dampers.

The sum of kinetic and recoverable strain energies indicates the level of deformation in the structure while the hysteretic energy dissipated by the principal elements of the structure shows the level of the structural inelastic action. However, due to the use of additional fluid viscous dampers, the structure is expected to deform into the linear elastic range.

Figure 4 shows time histories of the energy dissipated at third story by viscous damping and kinetic plus strain energies for the linear structural models without and with 6 fluid dampers under El Centro motion. The demand of energy absorption capacity on the main structural members is reduced. When the maximum response of the system is achieved, the peak of input energy is decreased by 50% approximately.



(a) (b)

Figure 4. Time histories of energy dissipation: (a) linear system without fluid dampers, (b) linear system with fluid dampers

CONCLUSION

The response of a 3DOF structural system with conventional and added 2-4-6 passive viscous fluid dampers was analyzed and interpreted. Linear time-history analyses were presented to assess the effect of adding viscous damping devices. Simulation results showed that the addition of 2-4-6 devices is beneficial for reducing the seismic response of the 3DOF frame structure.

The approach using energy dissipation mechanisms consists in transferring as much energy as possible from the primary structural members to the devices attached to structure. It is clearly observed that the increasing of damping ratios of the dominant modes of vibration by supplemental fluid dampers reduces the input energy, the kinetic plus strain energy and the energy dissipated by the principal elements of the structure, this way improving seismic protection of structures.

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DESIGN CRITERIA OF TUNED MASS DAMPER SYSTEMS TO CONTROL VIBRATIONS OF BUILDING STRUCTURES

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This paper investigates the effectiveness of a passive Tuned Mass Damper (TMD) attached to a three story building in reducing the response of the structure to harmonic and seismic excitations. Some examples of existing building structures that contain tuned mass dampers are briefly described. Generally, inertial mass is attached near the top, through springs and viscous damping mechanisms. The frequency of the TMD is normally tuned to a particular frequency of the structure so that the two peaks of the frequency response curve of the damped system have the same dynamic amplification, when expressed in terms of displacements. Design charts and equations to determine the optimum values of mass, damping, and stiffness for a passive TMD are illustrated. Numerical simulations have been performed to assess the optimum TMD efficiency in reduction of the seismic and harmonic response of the structure. In addition, this paper shows that a TMD is more effective to mitigate the vibrations induced by harmonic loads than earthquakes.

Keywords: tuned mass damper, vibration, seismic excitation

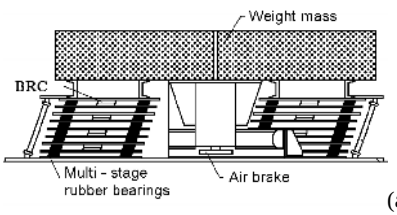
INTRODUCTION

A tuned mass damper (TMD) might be an efficient passive vibration suppression device consisting of a mass, springs and damping mechanisms (e.g. fluid damper) that is connected to a building in order to reduce the dynamic response of the building structure subjected to wind or earthquake loads. A fluid damper is normally incorporated into the TMD to allow the TMD's motion to quickly decay when the vibration input stops. Reduction of vibrations is accomplished by transferring some of the structural vibration energy to the TMD and dissipating the energy by the inertia force of the TMD acting on the structure. Usually, the frequency of TMD is tuned to one of the dominant frequencies of the structure.

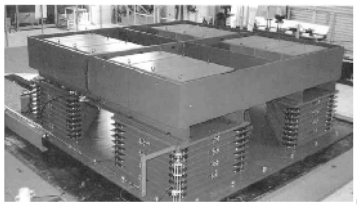
The first structure in which a TMD was installed appears to be Certerpoint Tower in Sydney, Australia (Housner *et al.*, 1997). Also, the first major buildings using a TMD, in the USA were the John Hancock Tower in Boston, completed in 1975, the Citicorp Center in New York, completed in 1977 (Housner *et al.*, 1997). The Citicorp building is 279m high and has a fundamental period of around 6.5s with the viscous damping ratio of 1% along each axis. The TMD is installed on the 59th floor in the crown of the structure and has a mass about 2% of the effective modal mass that corresponds to the first mode. The TMD is designed to work in biaxially direction with a variable operating period of 6.25±20%, adjustable linear damping from 8% to 14%, and a peak relative displacement of ±1.4m. The damper is expected to reduce the building response about 50% to wind loads. An equivalent of around 4% damping ratio for the fundamental modes of the structure is the estimated TMD's performance. The concrete mass block (400 tons) is about 2.6m high with a plan cross section of 9.1m by 9.1m and is supported on a series of twelve hydraulic pressure-balanced bearings. During operation, the bearings are supplied oil from a separate hydraulic pump, which is capable of raising the mass block about 2cm to its operating position. The damper system is activated automatically whenever the horizontal acceleration exceeds 0.003g for two consecutive cycles and will automatically shut itself down when the building acceleration does not exceed 0.00075g in either axis over a 30 minute interval (Conner, 2003).

In Japan, the first TMD was installed in the Chiba Port Tower, completed in 1988, followed by other installations. Chiba Port Tower is a steel structure with 125m high and 1950 tons weight. The first and second mode periods for the X direction are 2.25s and 0.51s and for Y direction are 2.7s and 0.57s. The damping ratio for fundamental modes is estimated at 0.50%. For the TMD, the dynamic characteristics of are: the period in X direction is 2.24s; the period in Y direction is 2.72s; the damping ratio is 15%. The maximum relative displacement of the TMD is ±1m in each direction. Reductions in peak displacements and peak bending moments of the structure to wind loads are expected around 30% ÷40% (Conner, 2003).

Newer versions of TMDs employ multi-level elastomeric rubber bearings, which function as shear springs, but which provide viscoelastic damping capability. The device do not requires sophisticated controls, is multidirectional, and is easily assembled and modified (Conner, 2003), as in Figure 1(a). Figure 1(b) shows an actual installation in Huis Ten Boch Tower, Japan (Nagasaki Prefecture).



(a)



(b)

Figure 1. Tuned mass damper: (a) a simple scheme; (b) installed in Huis Ten Boch Tower, Japan (Conner, 2003)

Also, the TMDs and viscous dampers were used in London Millennium Bridge in order to reduce vertical and horizontal vibrations due to pedestrian induced forces. This reduction corresponds to increasing the basic damping ratio of the structure at 20% for assumed loading. 8 TMDs were used to provide secondarily additional damping in horizontal direction and a total of 26 pairs of TMDs were installed to supplement primarily the damping in vertical direction. Horizontal and vertical damping is provided by 37 viscous dampers, of 7 different types (Taylor, 2002). The vertical TMDs are located on top of the transverse arms beneath the deck. They are situated along the length so that they are approximately at the antinodes of the vertical modes that they are damping (Dallard *et al.*, 2001). Two of the dampers are contained in Figure 2.

Figure 2. Tuned mass dampers beneath the deck of the London Millennium Bridge

DESIGN ELEMENTS OF A TMD

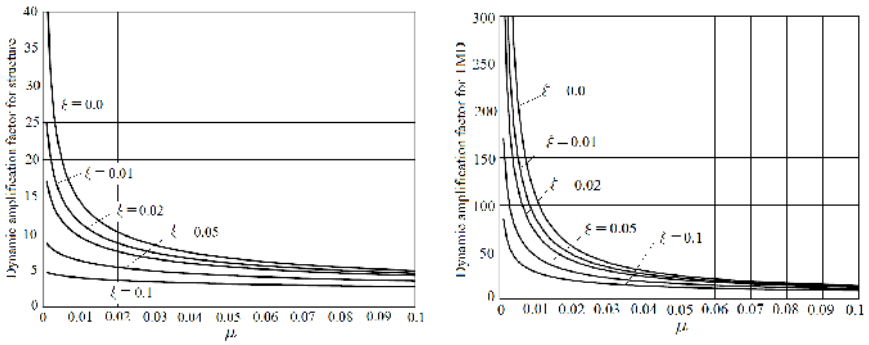
The first theoretical investigation of TMD design was carried out by Ormondroyd and Den Hartog in 1928 and detailed discussions of optimal tuning, damping parameters and design curves derived from the dynamic equations of motion are available (Conner, 2003; Hartog, 1947; Heinemeyer *et al.*, 2009; Pastia and Luca, 2013). A TMD can be very effective if it is precisely tuned on the resonance frequency, which we want to reduce it. The mass ratio, μ, between the TMD's mass and one of the dominant structural modal mass should be chosen typically between 1/100 and 1/10. Figure 3 represents graphs of the effectiveness of a TMD at various mass ratios typically found cost-effective for structures.

A design procedure of a TMD follows the next steps:

- Establish the desired responses of the structure and the TMD for design loads. Choice TMD's mass, m_d, and determination mass ratio, μ, see Figures 3(a) and 3(b).
- Determine from Figure 3(c) the optimum tuning frequency ratio, r_{opt}, expressed as ratio between optimal TMD's frequency, $f_{opt,d}$, and dominant structural frequency.
- Calculation of the TMD's spring constant k_d.
- Determine from Figure 3(d) the optimal damping ratio of the TMD, $\xi_{opt,d}$.
- Calculation of the TMD's damping constant c_d.
- Determine from Figure 3(e) the performance of a TMD which is usually expressed as an equivalent viscous damping ratio, ξ_e.

Simpler, if one does not want to use the design curves, the classical formula for optimal tuning parameters of a TMD as function of mass ratio, μ, is given in literature as:

$$f_{opt,d} = \frac{f}{1+\mu} \text{ and } \xi_{opt,d} = \sqrt{\frac{3\mu}{8(1+\mu)^3}} \tag{1}$$



(a) (b)

Figure 3. Effectiveness of a TMD (after Conner, 2003)

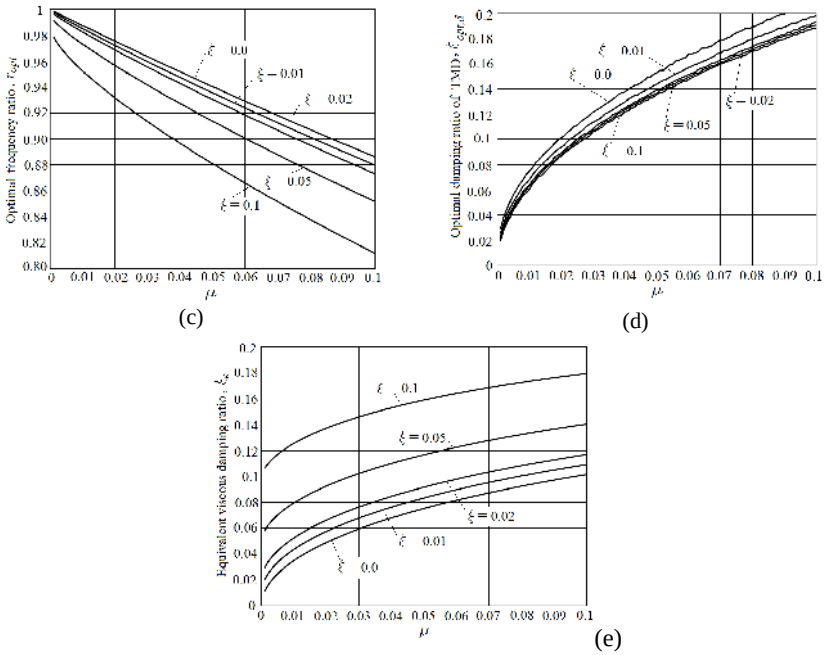


Figure 4. Effectiveness of a TMD (after Conner, 2003)

CASE STUDIES

The models for the numerical analyses are three story shear buildings with a tuned mass damper installed at the third floor. The governing equations of lumped mass structure as those of the models can be written as:

$$\begin{cases} M\ddot{X} + C\dot{X} + KX = \begin{bmatrix} 0 \\ 0 \\ c_d\dot{x}_d + k_d x_d \end{bmatrix} + \begin{bmatrix} -m_1\ddot{x}_g + P_1 \\ -m_2\ddot{x}_g + P_2 \\ -m_3\ddot{x}_g + P_3 \end{bmatrix} \\ m_d\ddot{x}_d + c_d\dot{x}_d + k_d x_d = -m_d(\ddot{x}_g + \ddot{x}_3) \end{cases} \quad (2)$$

where: $\ddot{x}_g$ represents the horizontal components of a recorded ground acceleration, $\{P\}$ is a vector containing the horizontal harmonic forces and mass, damping and stiffness matrices (M, C, K) are as follow (Olteanu *et al.*, 2011):

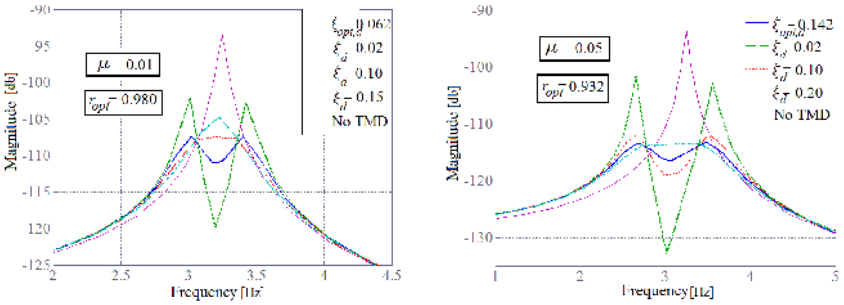
- Case (**A**) where f_1=3.247Hz, T_1=0.308s, damping ratio ξ = 0.01,

$$M = \begin{bmatrix} 7 & 0 & 0 \\ 0 & 7 & 0 \\ 0 & 0 & 7 \end{bmatrix}[t],\ K = \begin{bmatrix} 3.1 & -1.55 & 0 \\ -1.55 & 2.6 & -1.05 \\ 0 & -1.05 & 1.05 \end{bmatrix} \cdot 10^4 \left[\frac{kN}{m}\right],\ C = \begin{bmatrix} 30.8 & -15.4 & 0 \\ -15.4 & 25.3 & -9.9 \\ 0 & -9.9 & 9.9 \end{bmatrix} \left[\frac{kNs}{m}\right].$$

- Case (**B**) where f_1=0.230Hz, T_1=4.355s, damping ratio ξ = 0.01,

$$M = \begin{bmatrix} 70 & 0 & 0 \\ 0 & 70 & 0 \\ 0 & 0 & 70 \end{bmatrix}[t],\ K = \begin{bmatrix} 15.5 & -7.75 & 0 \\ -1.55 & 13.0 & -5.25 \\ 0 & -5.25 & 5.25 \end{bmatrix} \cdot 10^2 \left[\frac{kN}{m}\right],\ C = \begin{bmatrix} 22.4 & -11.2 & 0 \\ -11.2 & 18.4 & -7.2 \\ 0 & -7.2 & 7.2 \end{bmatrix} \left[\frac{kNs}{m}\right].$$

The magnitude curves of the transfer function between the excitation input and the system output (displacement of third floor, case **A**) for some values of TMD damping ratio and mass ratio equal to 0.01 and 0.05 are showed in Figure 4. It is observed that outside of the frequency range, about $\pm 0.15 f_1$, centered on the first natural period of the structural model, the response is not significantly influenced by the TMD.



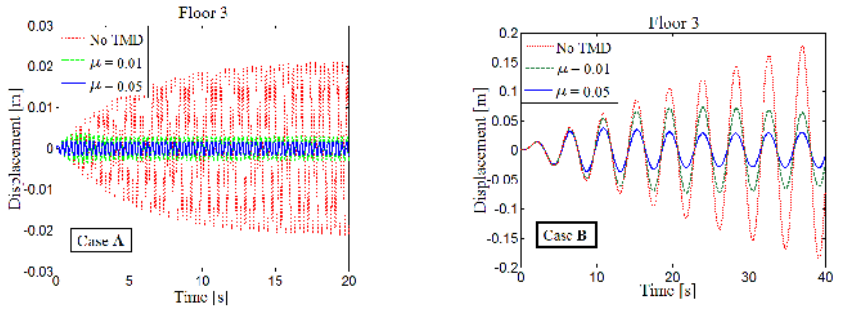
(a) (b)

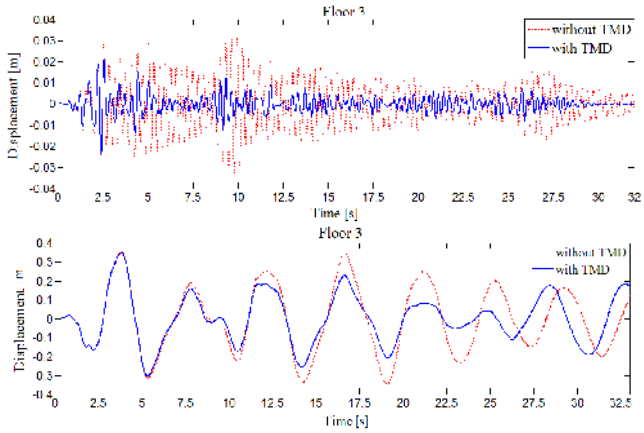
Figure 5. Response curves for magnitude of structural model (case A)

The optimal parameters for the TMD were considered and several simulations have been performed using as input El Centro acceleration and harmonic excitations. The harmonic forces are used at the frequency f =3.247Hz for case (**A**) and f =0.230Hz for case (**B**), with the force amplitude equal to 1000N. Table 1 and Figures 4 and 5 show the comparisons among the responses of the structural models in the uncontrolled case and controlled one with TMD.

Table 1. Peak displacements and peak accelerations of the third floor

Excitation	Case (**A**)	d3 (cm)	a3 (m/s^2)	Case (**B**)	d3 (cm)	a3 (m/s^2)
seism	Without TMD	3.21	14.38	Without TMD	35. 24	3.518
seism	TMD (μ = 0.01)	2.70	12.84	TMD (μ = 0.01)	34. 77	3.516
seism	TMD (μ = 0.05)	2.07	11.72	TMD (μ = 0.05)	33. 21	3.504





Case (**A**), $\mu = 0.05$, $T_1 = 0.308$s

Case (**B**), $\mu = 0.05$ $T_1 = 4.355$s

Figure 6. Displacement responses of structural models under El Centro earthquake

CONCLUSION

In this paper the effectiveness of the TMD using the proposed tuned parameters has been investigated through numerical analyses. Significant reduction in the responses of the structures under harmonic loads is observed. The results of the responses of the structural model with high fundamental period show that the performance of TMD is ineffective for seismic excitation versus harmonic excitation. The disadvantages of the TMD are the very narrow band of suppression frequency and the sensitivity problem due to detuning. The advantage of TMD systems is that they are relatively simple, inexpensive and reliable in suppressing the undesired vibrations of structural systems under assumed loads.

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PATHOLOGICAL CONDITIONS REQUIRING THE USE OF CUSTOMIZED LASTS

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A customized last is a last used for a subject whose pathological condition does not allow the use of a mass-manufactured last. This paper evaluates foot pathomechanics that can influence the design of customized lasts for therapeutic footwear. It investigates ways of evaluating a pathological condition that causes morphological changes that require the use of customized lasts. The result of this study is a method of defining pathological conditions that lead to consideration of morphological parameters which are not required for mass-manufactured lasts. Designing the customized lasts necessary for medical shoes only based on the mass-manufactured last design system leads in most cases to failure. In conclusion, the customized last designer must have knowledge regarding the evaluation of those pathological conditions that require customized lasts. In the absence of such knowledge the customized last can only be the basis of a final functional and comfortable therapeutic product after a lengthy process based on gaining experience through trial and error.

Keywords: therapeutic footwear, pathological conditions, customized last

INTRODUCTION

There are numerous studies on the use of sophisticated data processing techniques in last design. For this purpose, work has been focused on different directions of development such as:

- anthropometrics: by using anthropometric foot measurements to define the optimal last shape that meets the comfort criteria (Luximon and Luximon, 2009),
- techniques of deforming the virtual last structure (Leng and Du, 2005),
- fuzzy systems for comfort analysis based on last shape (Peng *et al.*, 2009),
- CAD-CAM systems for designing shoe lasts for people with diabetes (Bernabéu *et al.*, 2013),
- customized footwear - "mass customization". In this sense, "customized" is viewed as the possibility of the customer to choose footwear and last properties from a given number of options (Leng and Du, 2006).

It is noteworthy that the vast majority of this work is oriented towards mass-produced lasts and not lasts for footwear used in the treatment of foot pathomechanics. The issues most often discussed are the anthropometric ones (size and morphology). René Rigal (1991) defines the last as "a piece of wood, plastic or metal, representing the volume of the foot and is used in the manufacture of footwear." Although there is a rich literature oriented towards CAD-CAM last design, we can see that it is not oriented towards customized last design for orthopedic footwear, given that the experience of the last technician plays an essential role in this case (Bernabéu *et al.*, 2013). Work on therapeutic/orthopedic footwear insists on anthropometric and morphological aspects, namely the required sizes and shapes as basic information for last design. While older works insist on the traditional methods of making lasts for orthopedic footwear, recent works focus on biomechanical aspects specific to pathologies for which footwear is designed. In this respect, some authors suggest, in terms of design elements, the technical parameters of lasts to be modified depending on the biomechanical objective that the shoes must meet (Bernabéu *et al.*, 2013). Thus, in

the case of diabetic footwear, dimensional parameters of the last such as toe girth, toe width, instep girth, heel girth, etc., are correlated with meeting biomechanical objectives such as minimizing friction in the forefoot area, minimizing stress in Achilles tendon and pressure under the forefoot, minimizing pressure on the back of forefoot and toes, minimizing pressure under metatarsophalangeal joint heads 1-5 and hallux.

What is very important to note is that the above mentioned authors believe that the medical specialist is the one who "measures biomechanical characteristics" required to design the last, this measurement being performed in the orthopaedic shop (Bernabéu *et al.*, 2013). The footwear also is delivered and tested in the orthopaedic shop. This is in full accordance with Directive 93/42/EEC concerning medical devices, transposed into national legislation by Law no. 176/2000, in which the "custom-made device" is referred to as "any device specifically made in accordance with a duly qualified medical practitioner's written prescription which gives, under his responsibility, specific design characteristics and is intended for the sole use of a particular patient. The abovementioned prescription may also be made out by any other person authorized by virtue of his professional qualifications to do so".

This paper addresses the way in which the evaluation of foot pathomechanics can influence customized last design for orthopedic footwear. This evaluation of pathological conditions must be correlated with the design features of customized lasts.

METHOD

The education system focusing on the design of mass-manufactured footwear offers no solutions to investigate ways of evaluating a pathological condition requiring the use of customized lasts. These solutions must be sought both in the medical field and in the field of biomechanics of the musculoskeletal system. Depending on the morphology resulting from pathology evolution, it is required to use footwear designed based on customized lasts or based on normal lasts in terms of structure but whose volume allows insertion of different types of medical devices (orthotics and prosthetics) for the lower limb. In the second case the footwear is referred to as "depth shoe", being essentially a normal shoe but with an increased inner volume. It must also be mentioned that in the case of certain pathologies, more than a single type of footwear may be indicated, as there is always a relation between "depth shoes" and customized ones according to the size of the morphological changes that occur. In the Romanian footwear industry a number of geometrical parameters (lengths, widths, angles) are used, defined based on an orthogonal reference system of the plantar footprint in bilateral orthostatic position. In terms of destination, the "deformities" category is generally mentioned; in some cases pathologies such as "flat feet" (pes planus), "hollow foot" (pes cavus), neurological disorders, diabetes and bunions are mentioned. The correlations between these parameters and the pathological condition and how these correlations can influence last design are present to a very small extent and generally with no scientific basis. Unlike this situation, in the field of podiatry a definition system for pathologies was created which, based on the definition of "normal" and "pathological" states in relation to a set of criteria for normality, makes a prediction of how a certain pathology will influence gait biomechanics. Even if this system of thinking was fought over time and has led to the emergence of new operational models for the foot (Petcu and Colda, 2012), evaluating pathological conditions in relation to this system can influence the design of customized lasts.

RESULTS AND DISCUSSIONS

In the operating model of the foot around the neutral position of the subtalar axis M. Root suggests that the normal and pathological shape of the bone structure determines the type, degree and direction of movement in different foot joints (Petcu and Colda, 2012). Moreover, the bone structure plays a decisive role in how the muscular system provides foot mobility and stability. Based on personal clinical experience and published research when developing this model, Root introduces, as the central concept of his classification system for normal and pathological foot structure, the concept of subtalar joint neutral position, a position in which the foot is neither in pronation nor supination. In this system structural deformations of the foot can be identified and measured and their influence on foot function can be estimated during walking. Another key element of Root's paradigm is to establish criteria for normality, the differentiating element from other systems being the neutral position of the subtalar axis. To assess dysfunctions caused by a pathology, the frontal plane was mainly chosen due to the reduced range of motion in this plane, required to "absorb" the negative effects of imbalances. Normality criteria set forth by Root and his colleagues represents the ideal relationship between bone segments of the lower limb that must be met for gait to be performed with maximum efficiency. Also these relationships are the basis for evaluating the existing deformation degree in a certain pathology.

According to this system, the criteria for normality (Figure 1) are as follows:

- the distal third of the lower limb is vertical,
- the subtalar axis is placed in neutral position: neither in pronation nor supination,
- the bisector of the posterior surface of the calcaneus is vertical or in eversion of about 3-4 degrees from the vertical,
- the mediotarsal joint is in full pronation position,
- metatarsophalangeal joints are positioned in a transverse plane perpendicular to the calcaneal bisector and contain the distal end of the medial calcaneal tubercle (Figure 1a).
- the foot is rotated outwardly (abduction) with a mean angle of 7-10 degrees,
- there are no abnormal rotational or torsional influences in the lower limb.

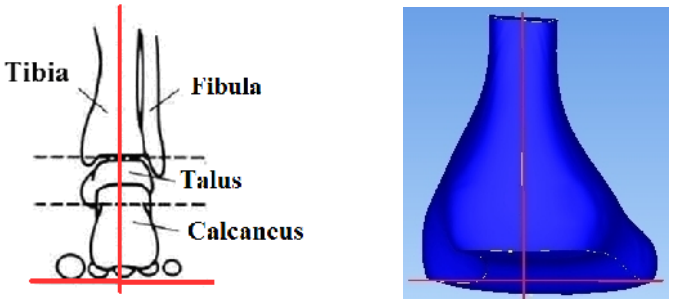
Deviations from normality criteria of the biomechanical system can generate abnormal movements that induce additional tension in the lower limb structure. These additional tensions may cause pathologies over time. It can be seen that the perpendicularity relationship between the bisector of the rearfoot and the forefoot line is also found in normal lasts (Figure 1b).

Thus, relative to the fundamental criterion of Root's model – the neutral position of the subtalar axis –the following types of foot pathology are identified:

1. Forefoot in inversion ("forefoot invertus")

1a. forefoot varus - is a specific fixed deformation, in which the forefoot plane is in varus position relative to the calcaneal bisector when the subtalar joint is placed in neutral position and the midtarsal joint is fully pronated relative to both axes.

1b. forefoot in supination - is an acquired deformation (contraction) of the forefoot around the longitudinal axis of the midtarsal joint.



a) b)

Figure 1. a) Schematic representation of normality criteria; b) transposition of normality criteria to the last

2. Forefoot in eversion ("forefoot evertus")

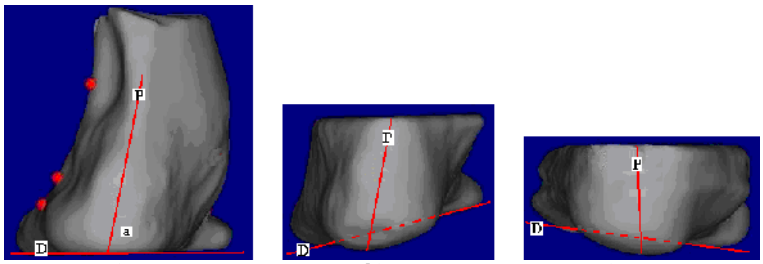
2a. forefoot valgus - is a fixed congenital bone deformation, in which the forefoot is placed in eversion relative to the calcaneal bisector given that the subtalar joint is in neutral position and the mediotarsal joint is fully pronated.

2b. first ray in plantar flexion represents a deformation in eversion of the forefoot that can be congenital or acquired.

3. Rearfoot varus - is a structural deformation in which the calcaneus is positioned in inversion relative to the support surface when the subtalar joint is in neutral position.
4. Rearfoot valgus - is a structural deformation in which the calcaneus is in eversion relative to the support surface when the subtalar joint is in neutral position.

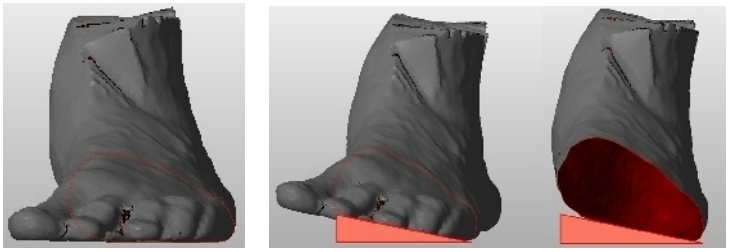
Pathologies associated with deformations described above could be hallux abductus and hallux abducto-varus; mediotarsal joint subluxation; deep callosity under the 2nd metatarsal head; friction callosities under the 2nd, 3rd and 4th metatarsal heads; tibialis posterior disorders; genu valgum; hammer toes, hallux limitus, hallux valgus, interdigital neuroma, plantar fasciitis, tarsal tunnel syndrome, tailor's bunion, tendinitis, callosities under the metatarsophalangeal joint heads, etc.

As an example, in figure 2 a, b, c, a forefoot varus associated with rearfoot valgus is shown, whose plantar surface has been captured by three different methods: 3D scanning, moulding with suspended foot, and foam molding (Petcu *et al.*, 2010). One can see the differences between the appearance of the plantar surface of the same foot area depending on the method of capturing it. These differences will be undertaken by the medical device inserted in the shoe, with a direct effect on the internal volume of the shoe. An objective of conservative treatment in this case may be to limit excessive pronation movement of the subtalar joint during the gait cycle by supporting the forefoot varus deformation. It is important to note in this case that the measurement of the toe girth and its transfer it in the last girth, without taking into account the objective of the treatment, namely of supporting the first metatarsal head, may result in a shoe that creates a high pressure on the back of the first metatarsal phalangeal joint and an abnormal functioning of the foot.



a) b) c)

Figure 2. Comparison between three methods of capturing the 3D surface of a rigid flat foot (left foot). The line of MF joints and rearfoot direction. P - calcaneal axis, D - forefoot direction, a - scanned foot, b - mould of suspended balanced foot, c - mould of partially loaded foot



a) b)

Figure 3. Foot position in the case of a forefoot varus in relation to biomechanical assessment leads to reconsidering the toe girth: a) position during scanning, b) position according to treatment objectives

At the same time, reducing last girth in the metatarsophalangeal joint area (basic operation in designing mass-manufactured lasts), without taking into account the shape of the medical device to be placed in the shoe, will cause high pressure in this area, conditioning the functionality of footwear and decreasing the chances of a successful conservative treatment. A last built with a toe girth calculated on the criteria found in mass production will force the foot to adopt a non-functional position while walking. Also one can observe that in this case, a shoe built on a last with vertical rear side (Figure 1b) can create problems related to adjustment of the foot in the shoes and also promote abnormal biomechanics during walking.

CONCLUSIONS

The last represents a fundamental element of the design process for orthopedic footwear with major implications in successful conservative treatment using this type of medical devices.

Several conclusions can be drawn from this work:

- Last definitions take into account anthropometric, morphological and design aspects, reflecting an orientation towards mass-produced lasts for the normal foot and not for a customized last specific to pathological conditions;

- The conventional last design system is based on an orthogonal reference system without a connection with biomechanical and ergonomic aspects of using footwear in the conservative treatment. The last designer must adapt his thinking to a system that would allow him to understand the pathological condition and the prescribed conservative treatment;

- Last changes can be influenced by the type of existing pathology. Anthropometric measurements performed without taking into account the specific pathology and treatment objectives generally lead to failure or to achieving positive results after a large number of trials without a thorough understanding of the causes that have led to success. The last designer must have a good understanding of pathology definitions in terms of functionality, and of the implications they have on gait biomechanics.

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ASPECTS REGARDING NATURAL DYEING OF ENZYMATICALLY PRE-TREATED CELLULOSIC BLENDED YARNS

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The objective of this study was to investigate the effects of preliminary enzyme treatments and natural dyeing on the characteristics of yarns made from fibrous blends of cotton and enzymatically cottonized hemp. To attain the cottonized hemp fibres, enzyme and ultrasound treatments were simultaneously applied. The cottonization of technical hemp fibres was performed to remove lignin and pectin from the middle lamella in order to obtain small bundles of elementary fibres, with similar features as those of cotton. To investigate the impact of enzyme processes on the characteristics of hemp fibres, physical-mechanical and physical-chemical investigations, including RAMAN spectroscopy and AFM were used. The cottonized hemp and cotton fibres were used to make yarns that were subsequently pre-treated with pectinase, thereafter a natural dyeing with *Allium Cepa* extract was applied. The enzyme pre-treatment was followed by a treatment with tannic acid and pre-mordanting with potassium or iron alum. To determine the efficiency of the enzyme pre-treatment process on natural dyeing, colour measurements and dyeing fastness to washing, light, acid and alkaline perspiration were carried out.

Keywords: enzymatic pre-treatments, natural dyeing, *Allium Cepa*

INTRODUCTION

Among natural fibres, pluricellular cellulose fibres (flax, hemp, ramia, jute) are distinguished from the monocellular ones (cotton) by complexity of the chemical structure, the more accentuated crystal architecture and the presence of the middle lamella with a role of cementing the constituent elementary cells (Asandei and Grigoriu, 1983). In the case of bast fibrous plants, fibres shall develop in bundles that can be subsequently released from the cellular tissue by means of refining and individualization treatments: retting, scutching, carding, cottonization, thus obtaining fibres that can be spun individually or in blends with other natural or synthetic fibres (Thygesen *et al.*, 2011).

In the area of innobilation processes, the development and application of various enzyme systems play an important role, as they are a feasible and easy alternative to the conventional processes in the textile industry and represent new ways to solve the environment-related problems (Kozlowski *et al.*, 2006). Enzyme pre-treatments of textile materials could improve the dyeing process performances through dye uptake increase.

In this sense, this study presents an investigation of the impact of enzyme pre-treatment processes over dyeing behavior and physical-mechanical and physical–chemical characteristics of blended yarns of 70% cotton-30% cottonized hemp. Laccase enzyme treatment was conducted to obtain cottonized hemp fibres and ultrasonication was used in order to accelerate the enzymatic process. In order to ensure a reduced residual content of impurities and an adequate hydrophilicity for the subsequent dyeing

in good conditions with natural dyes, the cotton yarns with cottonized hemp content were subjected to enzyme pre-treatment with pectinase.

EXPERIMENTAL PART

Materials

Technical hemp fibres and cotton fibres were used as raw materials for achieving blended yarns. Cottonization enzyme treatment of hemp fibres was performed with laccase Denimcol LAC-LRE (Bezema AG), under pilot conditions, on installations belonging to SC FIRI VIGONIA SA. For the enzyme pre-treatment of cotton/cottonized hemp blended yarns, BioPrep 3000L (Novozymes) pectinase was used. Natural dye extracted from *Allium Cepa* in aqueous solution was used for the dyeing process. Mordanting was carried out with potassium alum and iron alum supplied by Sigma Aldrich and, respectively, with tannic acid supplied by Consors Romania.

Cottonization of Hemp Fibres

The cottonisation of technical hemp fibres was conducted in two stages, the first one consisting in the treatment at 40°C, for 50 minutes, with a chelating agent-EDTA (5g/L), in an alkaline bath with NaOH (pH=11). The second stage consisted in the fibre treatment in a ultrasonic bath at 70°C, for 30 minutes, with 1% laccase in the presence of a mediator (HOBT), at pH=4.5. After the rinsing, squeezing and drying operations, the cottonized hemp fibres were processed on blow room in view of their mechanical cleaning and pre-individualization, being followed by mixing bed formation with cotton fibres and the other operations prior to spinning. Spinning was conducted on an OE unconventional spinning machine, the obtained yarn having a composition of 70% cotton/30% hemp. These yarns were subjected to subsequent enzyme pre-treatment, pre-mordanting and natural dyeing.

Preliminary Treatments

Treatments were conducted on Ugolini laboratory equipment. Classical pre-treatment (P_1) was performed according to the classical alkaline procedure at boiling temperature. Enzymatic pre-treatment with pectinase (P_2) was conducted with 0.2 g/L BioPrep 3000 L at 55°C, for 30 minutes, the pH of the solution being set at 8 with sodium carbonate. Pre-mordanting with metal salts was carried out at concentration of 5% (owf) at 80°C (Hm 1:30), for 45 minutes. The yarns thus treated were washed with hot and cold water, squeezed and dried. Prior to mordanting, a treatment with 4% concentration (owf) tannic acid at 60°C was carried out for 4 h, the samples being afterwards rinsed, squeezed and freely dried.

Natural Dyeing Process

The *Allium Cepa* infusion was prepared by boiling for 45 minutes 20 g of dried onion peels in 1000 mL of distilled water. The resulted mix was stored for 24 hours and afterwards filtered. The dyeing was carried out at 95°C, for 60 minutes, followed by rinsing at 60°C, soaping, hot and cold rinsings, squeezing and drying.

Methods

To highlight the influence of enzyme treatment on the integrity of the hemp fibre as well as to determine the content of residual non-cellulose impurities on the fibre after cottonisation, the following characteristics were determined: average degree of polymerization (SR ISO 5351-1:1999), waxes content (SR 7690:1993), hydropectin content, pectic substances, hemicellulose and lignin (Rusanovschi and Dragnea, 1981). The cottonized hemp fibers were analyzed using a combined AFM-Raman equipment: NTEGRA Probe NanoLaboratory AFM (NT-MDT, Russia)-inVia Raman Microscope (Renishaw, Wotton-under-Edge, Gloucestershire, United Kingdom). The AFM investigations were carried out in a semi-contact mode using NSG30/Au probes from NT-MDT, Russia, and the Raman spectra were recorded at λ = 785 nm. The efficiency of the preliminary treatments performed with pectinase over natural dyeing, in terms of colour difference attributes and colour fastness, was determined through colour measurements (ISO 105 J03: 2001) and colour fastness to washing (SR EN ISO 105-C 10: 2010), acid and alkaline perspiration (SR EN ISO 105-E 04:2013) and light (SR EN ISO 105-B02:03).

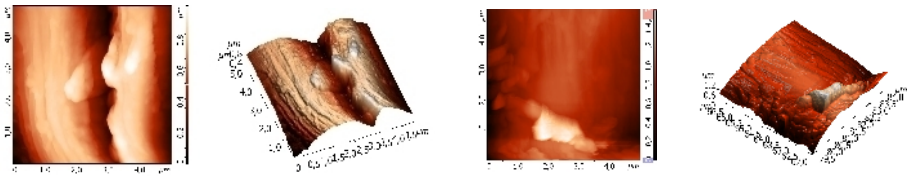
RESULTS AND DISCUSSIONS

The analysis of the characteristics obtained for the hemp fibres cottonized with EDTA/laccase (Table 1) reveals a decrease of impurities content in the fibre, compared with the control raw fibres (untreated), the lignin content decreasing by over 60% and the pectin content by over 40%. The enzymatic cottonization treatment applied has determined a decrease of the average degree of polymerization compared to the control sample without affecting the macromolecular chain integrity of cellulose from hemp fibre.

Table 1. Physical–chemical characteristics of the enzymatically cottonized hemp fibres

Determined characteristics	Untreated	EDTA/laccase
Lignin content (%)	7.6	2.63
Waxes and fats content, (%)	2.4	1
Hydropectin content, (%)	2.03	1.5
Hemicellulose content, (%)	17.72	18.25
Pectin content, (%)	4.15	2.48
GMP	2631	2323

The morphologic structure of the hemp fibers has been revealed by performing AFM investigations and this is presented in Figure 1. The surface of untreated fibers is rough and not well delineated compared with smoother surface of treated hemp fibers. For the treated hemp fibers the cellulose fibrils are visible due to removal of waxes, fats, pectins, hemicelluloses, and probably exhibiting the primary cell wall (Le Troedec *et al.*, 2011).



a. b. c. d.

Figure 1. Topography: a. - 2D of raw hemp fibres, b.- 3D of the raw hemp fibres' surface, c.- 2D of laccase treated hemp fibres, d.- 3D of the surface of laccase treated hemp fibres

Raman spectra exhibited band positions similar with those reported in literature (Figure 2) (Kavkler and Demsar, 2011; Osterberg *et al.*, 2006). For both untreated and treated hemp fibers the bands atributed to cellulose were observed (1095, 1120, 2900 cm^{-1}). The Raman data confirmed that the enzyme treatment of the hemp fibers did not alter crystallinity type I of the cellulose and successfully removed lignin and pectins.

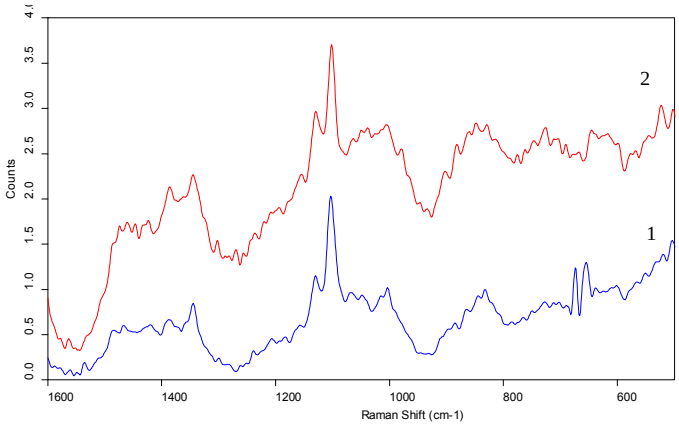


Figure 2. Raman spectra recorded for untreated hemp fibers (1), and laccase treated hemp fibers (2)

Table 2. Physical-mechanical characteristics of blended yarns before and after dyeing

Variant code	Physical–mechanical characteristics			
	Yarn count (Nm)	Tensile strength (N)	Breaking elongation (%)	Breaking length (km)
Raw yarn - untreated	17.2/1	4.49	10.65	7.70
P_1-Potassium alum + Dyeing	16.4/1	3.93	11.31	6.45
P_2-Potassium alum + Dyeing	15.8/1	3.99	10.80	6.30

The enzyme pre-treatment of blended yarns made of cotton and cottonized hemp, followed by mordanting and natural dyeing, leads to a decrease by 11.13 % of the breaking strength and respectively by 18.18 % of the breaking elongation, in comparison with the untreated sample yarns (Table 2). The classical variant of alkaline

pre-treatment, followed by mordanting and natural dyeing has a similar behaviour, leading to a decrease by 12.47 % of the breaking strength and, respectively, by 16.23 % of the breaking elongation. These decreases of strength characteristics are normal in the case of cellulosic yarns subjected to wet-thermal finishing treatments. It has also been noticed that the treatments applied do not influence in a negative manner the breaking elongation, the values being similar to those obtained for the raw yarn.

Table 3. Colour difference of blended yarns dyed with *Allium Cepa*

Variant code	X	Y	Z	Colour difference				
				DL*	DC*	DH*	DE*	Mark
P_1 + Potassium alum	17.11	15.21	4.76	REFERENCE				
P_2 + Potassium alum	17.85	16.07	5.13	1.15	-0.16	0.95	1.50	4

In the case of a similar dyeing process, preliminary treatments carried out in a differentiated manner (classically or enzymatically), have influenced the initial whiteness degree (or yellowness degree) of the sample and implicitly led to a total colour difference after dyeing of 1.5 between the enzymatically and classically treated samples (Table 3). The positive value for DL* (lightness difference) reflects a lighter colour for yarns subjected to enzyme preliminary treatment compared to the control sample subjected to classical treatment. The negative value obtained for DC* (difference in chroma) indicates a more unsaturated colour of the sample subjected to preliminary enzymatic treatment, compared to the classically treated control sample.

Table 4. Colour fastness of blended yarns dyed with *Allium Cepa*

Code	Colour fastness												
	Washing				Acid perspiration				Alkaline perspiration				Light 50ºC, RH 45% 84 h
	Colour change	Colour staining			Colour change	Colour staining			Colour change	Colour staining			
		CO	PA	WO		CO	PA	WO		CO	PA	WO	
Tannic acid + Potassium alum + Dyeing													
P_1	3-4	3	4	4	4-5	4-5	5	5	4-5	5	4-5	5	3-4
P_2	3-4	3	4	4	4-5	5	5	5	4-5	5	5	5	3
Potassium alum + Dyeing													
P_1	3-4	3	4	4	4-5	4-5	5	5	4-5	4-5	5	5	3-4
P_2	3	3	4	4	4-5	4-5	5	5	4-5	4-5	5	5	2-3
Tannic acid + Iron alum + Dyeing													
P_1	4	4	4-5	4-5	4-5	4-5	5	5	4-5	4-5	5	5	5
P_2	4	4	4-5	4-5	4-5	5	5	5	4-5	5	5	5	5
Iron alum + Dyeing													
P_1	4	4	4-5	4-5	4-5	4-5	5	5	4-5	4-5	5	5	5-6
P_2	3-4	3-4	4-5	4-5	4-5	5	5	5	4-5	4-5	5	5	5-6

Observations: Evaluation on the grey scale: *5- very good; 4/4-5-good; 3/3-4- moderate; 2/2-3-low; 1/1-2- very low;* ***Evaluation on the blue scale:*** *8- exceptional; 7-excellent; 6-very good; 5-good; 4-acceptable; 3- moderate; 2-low; 1- very low.*

Analyzing the data from Table 4 it is noticed that, irrespective of the pre-treatment method applied for cellulose fibres hydrophylization (classical or enzymatic variant), the colour fastnesses to washing and to light of the yarns variants pre-mordanted with/without tannic acid-potassium alum are in general moderate, while the colour fastnesses to acid or alkaline perspiration are good and very good. The pre-mordanting

treatments with iron alum improve the colour fastness to light, the marks obtained being higher with 1½-2½ tones compared to the samples pre-mordanted with potassium alum. Furthermore, in the case of pre-mordanting with iron alum better values for washing fastness are obtained, the ratings obtained both for colour change and for colour staining being higher by ½-1 tones compared to the variant subjected to potassium alum pre-mordanting.

CONCLUSIONS

Following the cottonization of hemp fibres with EDTA/laccase, in ultrasonic bath, the successful removal of lignin and pectin from the fibres surface was observed, the enzyme treatment being an efficient alternative to the classical chemical cottonization treatment. Starting from the enzymatically cottonized hemp fibres, it was possible to make spun yarns in blend with cotton by unconventional OE spinning technology. The preliminary treatment of the blended yarn with an enzyme from the pectate lyase class has provided adequate hydrophilization of the cellulose fibres in fibrous blend, leading to even dyeing performed with natural dye extracted from *Allium Cepa*. The pre-mordanting with iron alum provides better values for fastness to washing and to light, compared to the pre-mordanting with potassium alum.

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RADIATION VULCANIZATION OF NATURAL RUBBER USING TMPT AS POLYFUNCTIONAL MONOMER

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In our study, the effect of trimethylopropane trimethacrylate (TMPT) as polyfunctional monomer on radiation vulcanization (electron beam, EA) of natural rubber (NR) was evaluated. Gel fraction, crosslink density and FTIR of the natural rubber/TMPT samples have been investigated as a function of absorbed dose. The dependence of gel fraction and crosslink density on irradiation dose was determined from in the dose range of 100 to 250 kGy. The results showed an increase in gel fraction and crosslink density due to the introduction of TMPT.

Keywords: natural rubber, electron beam, trimethylopropane trimethacrylate, gel fraction, crosslink density, FTIR

INTRODUCTION

Natural rubber is a high molecular weight polymer of isoprene in which essentially all the isoprene's have the cis 1-4 configuration. Since it is of biological origin, it is renewable, inexpensive and creates no health hazard problems. NR is an interesting material with commercial success due to its excellent physical properties, especially high mechanical strength, low heat build-up, excellent flexibility, and resistance to impact and tear, and above all its renewability (Daniel *et al.*, 2005). The most important stage in the rubber processing technology is vulcanization/crosslinking. During crosslinking, rubber molecules with chain configuration are linked by chemical bridges/ bonds, and the rubber mass turns from its plastic mass into an elastic one. This is normally done by sulphur and accelerator for general purpose rubbers. The vulcanisation of natural rubber (NR) by sulphur in presence of organic accelerator is a complicated process (Stelescu *et al.*, 2011, Gonzalez *et al.*, 2005). The mechanism of vulcanization and its acceleration depends on the structure of the rubber, type and concentration of accelerators and activators (zinc oxide and fatty acid) and on the thermodynamics of each particular reaction. Vulcanization with peroxides is done by radical mechanism when bonds are formed between C-C macromolecules. The chain of free radical reactions is initiated by thermal decomposition of the peroxide into primary radicals formed by scission stable species (acetone and diacetylbenzene) and the second radical that continue the propagation in the presence of rubber. Besides the conventional techniques, crosslinking of NR can also be achieved by means of *high energy radiation*. This technology has been studied for a long time. Radiation can produce crosslink densities like those obtained by sulfur curing, but the net effects, while similar, are not identical. The type of crosslink formed in this method (–C–C–) give rise to better mechanical properties at higher temperature. EB vulcanization has demonstrated extremely positive results compared to the conventional curing system such as: no polymer degradation due to high temperature as EB cross-linking occurs at room temperature, no oxidative degeneration in polymers as observed in classical cross-linking, direct cross-linking by C-C linkage by EB, extremely strong bonds, high degree

of cross-linking, extremely short curing cycles, zero blooming effects; extremely high tensile strength; extremely high resistance to compression set; extremely high resistance to oils, grease, lubricants; highly improved accelerated ageing properties, very high productivity, perfect for thin products, lower material waste (MGM Rubber Company-Research and Development, 2007). However, the radiation cross-linking of rubbers was not used in larger technical applications because of the high cost of irradiation to bring about vulcanization, but could become an industrial process when the radiation dose decreased with the use of some sensitizers. Reported papers suggest that appropriate polyfunctional monomers (co-agents) in polymer matrix (Vijayabaskar & Bhowmick, 2005; Yasin *et al.*, 2005) could be used to obtain desired rubber physical properties at lower irradiation doses (Hafezi *et al.*, 2006). Co-agents are multi-functional organic molecules which are highly reactive towards free radicals (Alvarez Grima, 2007). They are used as reactive additives to boost the vulcanization efficiency (Endstra, 1990).

In our study, the effect of trimethylopropane trimethacrylate (TMPT) as polyfunctional monomer in radiation vulcanization (electron beam, EB) of natural rubber (NR) was evaluated. Gel fraction, crosslink density and FTIR of the samples have been investigated as a function of absorbed dose. The dependence of gel fraction and crosslink density on irradiation dose was determined in a dose range of 100 to 250 kGy.

EXPERIMENTAL

Materials

All the raw materials: natural rubber Crep 1X (Mooney viscosity is 74 ML_{1+4} at 100°C, 0.32% volatile materials content, 0.38% nitrogen content, 0.22% percentage of ash, 0.021% impurities content), pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate Irganox 1010, and polyfunctional monomer trimethylopropane-trimethacrylate Luvomaxx TMPT DL 75 (22% percentage of ash, pH 9.2, density 1.36 g /cm^3, 75 ± 3 % active ingredient), were used directly without purification.

Sample Preparation

Blends were prepared on an electrically heated laboratory roller mill. For preparation of NR with TMPT, the blend constituents were added in the following sequence and amounts: 100 phr NR and 3 phr TMPT. Process variables: temperature 25-50 ±5°C, friction 1:1.1, and total blending time 5 min. Plates required for physico-chemical tests were obtained by pressing in a hydraulic press at 110 ±5°C and 150 MPa.

Experimental Installations and Sample Irradiation

EB irradiation experiments were carried out with an electron linear accelerator of 6.23 MeV and 75 mA (ALIN-10). The optimum values of the EB peak current I_{EB} and EB energy E_{EB} to produce maximum output power P_{EB} for a fixed pulse duration τ_{EB} and repetition frequency f_{EB} are as follows: E_{EB} = 6.23 MeV; I_{EB} = 75 mA; P_{EB} = 164 W (f_{EB} = 100 Hz, τ_{EB} = 3.5 μs). The EB effects are related to the absorbed dose (D) expressed in Gray or J kg^{-1} and absorbed dose rate (D*) expressed in Gy s^{-1} or J kg^{-1} s^{-1}. For EB treatments, the rubber sheets were cut in rectangular shapes of 0.1 x 0.03 m^2 and

covered with polyethylene foils to minimize oxidation. Sandwiches consisting of ten layers of material were irradiated in atmospheric conditions and at room temperature of 25°C.

Laboratory Tests

The sol-gel analysis was performed on crosslinked NR/TMPT samples to determine the mass fraction of insoluble NR (the network material resulting from network-forming crosslinking process) samples (gel fraction). The samples were swollen in toluene and extracted after 72 h in order to remove any scissioned fragments and unreacted materials. The networks were then dried in air for 6 days, then dried in an oven for 12 h at 80°C to completely remove the solvent and reweighed. The gel fraction was calculated as:

$$Gelfraction = \frac{m_s}{m_i} x\,100 \tag{1}$$

where m_s and m_i are the weight of the dried sample after extraction and the weight of the sample before extraction, respectively (Stelescu, 2010).

The crosslink density (ν) of the samples was determined on the basis of equilibrium solvent-swelling measurements (in toluene at 23-25°C) by application of the well-known modified Flory-Rehner equation for tetra functional networks. The samples (2 mm thick) were initially weighed (m_i) and immersed in toluene for 72 h. The swollen samples were removed and cautiously dried to remove excess solvent before being weighed (m_g) and, during this operation, the samples being covered to avoid toluene evaporation during weighing. Traces of solvent and other small molecules were then eliminated by drying in air for 6 days, then dried in an oven for 12 h at 80°C to completely remove the solvent and reweighed.. Finally, the samples were weighed for the last time (m_s), and volume fractions of polymer in the samples at equilibrium swelling ν_{2m} were determined from swelling ratio G as follows:

$$\nu_{2m} = \frac{1}{1+G} \tag{2}$$

$$G = \frac{m_g - m_s}{m_s} \times \frac{\rho_e}{\rho_s} \tag{3}$$

where: ρ_e and ρ_s are the densities of elastomer samples and solvent (0.866 g/cm^3 for toluene), respectively.

The samples crosslink densities, ν, were determined from measurements in a solvent, using the Flory–Rehner relationship:

$$\nu = -\frac{Ln(1-\nu_{2m}) + \nu_{2m} + \chi_{12}\nu_{2m}^2}{V_1\left(\nu_{2m}^{1/3} - \frac{\nu_{2m}}{2}\right)} \tag{4}$$

where V_1 is the molar volume of solvent (106.5 cm^3/mol for toluene), ν_{2m} is the volume fraction of polymer in the sample at equilibrium swelling, and χ_{12} is the Flory-Huggins polymer-solvent interaction term (the values of and χ_{12} are 0.393 for toluene (Lopez-Manchado *et al.*, 2003; Arroyo *et al.*, 2003; Chenal *et al.*, 2007).

Fourier Transform Infrared (FTIR) Spectroscopy

Changes in the chemical structure of NR/TMPT samples were determined using a FTIR spectrophotometer-JASCO FT/IR 4200, by ATR measurement method. Samples spectra are the average of 30 scans realized in absorption in the range of 4,000–600 cm^{-1}, with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Polyfunctional monomers are effective on modification of polymer material by crosslinking. Generally speaking, there are two factors which affect the functionality of polyfunctional monomers in polymer: one is the unsaturation of polyfunctional monomers and the other is the solubility of polyfunctional monomers in polymer (Tawney et al. 1964). The polyfunctional monomers can participate in a number of radical reaction mechanisms, including grafting and radical addition. So, these polyfunctional monomers can be grouped according to their influence on cure kinetics and ultimate physical – mechanical properties: type I polyfunctional monomers are highly reactive and increase both the rate and state of cure (acrylate, methacrylate, or maleimide functionality), and type II polyfunctional monomers are based on allyl reactive sites and increase the state of cure only.

In this paper the induced crosslinking was evaluated by the *gel fraction* (mass fraction of the network material resulting from a network-forming polymerization or crosslinking process; the gel fraction comprises a single molecule spanning the entire volume of the material sample) and the crosslink density (number of crosslinks per unit volume in a polymer network) and the results are presented in Figure 1 and 2, respectively.

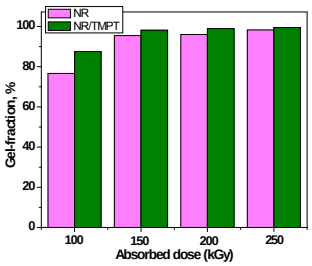

Figure 1. Gel fraction versus absorbed dose

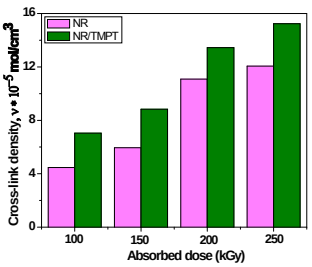

Figure 2. Cross-link density versus absorbed dose

The samples which were not subjected to crosslinking treatment (by irradiation with electron beam) were completely dissolved in toluene – in the same conditions as the irradiated samples. Sol-gel studies revealed higher sensitivity of NR and NR/TMPT to EB irradiation. Even at a low dose (100 kGy) gel fractions (Figure 1) was found to be 76.60% and 87.57% for NR and NR/TMPT respectively. A slow increase is observed for all samples when irradiation dose increases from 100 to 250 kGy. Similar results were obtained for crosslinking degree. Figure 2 shows that, for all samples the crosslinking degree increases with absorbed dose increasing. As expected the spectacular increase in the degree of crosslinking was obtained for NR/TMPT samples.

Thus, were obtained values of $7.04x10^{-5}$ mol/cm^3 for the smallest irradiation dose (100 kGy) and $15.24x10^{-5}$ mol/cm^3 for the biggest tested irradiation dose (250 kGy).

Figures 3 and 4 show the infrared spectra and characteristic infrared bands observed (in the region of 4,000–560 cm^{-1}) of NR/TMPT after irradiation. The main component of NR is cis-1,4-polyisoprene with a high degree of long chain branching generally associated with the presence of non-hydrocarbon groups distributed along the chains. The broad band in the region 3300-3270 cm^{-1} were identified to the proteins and both monopeptides and dipeptides present in natural rubber (Eng *et al.*, 1992; Manaila *et al.*, 2014). Absorption bands with a maxima at 3040-3030 cm^{-1} corresponding to CH stretching in the -CH=CH$_2$ group, were observed. Vulcanization of the samples results in consumption of the double bonds in NR molecules, so that the intensities of these absorption bands decrease. The characteristic bands of the saturated aliphatic sp^3 C–H bonds are observed in the region 2980 –2830 cm^{-1} which are assigned to ν_{as} (CH$_3$), ν_{as} (CH$_2$), and ν_s (CH$_2$), respectively (as three corresponding bends) (Ali *et al.*, 2008; Manaila *et al.*, 2014).

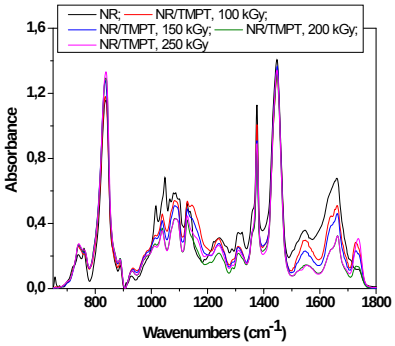

Figure 3. FTIR spectra of NR in range of 650-1800 cm^{-1}

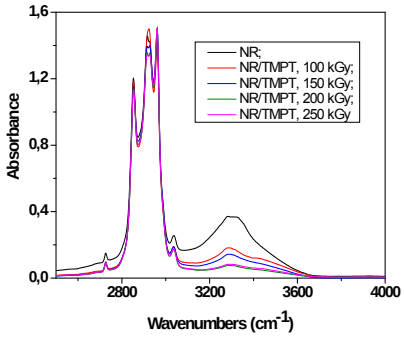

Figure 4. FTIR spectra of NR in range of 2500-4000 cm^{-1}

The presence of absorption bands in the spectral region located between 1670-1630 cm^{-1}, is due to valence vibration of homogeneous double bonds (C=C) in the NR structure. Their intensity decreases with the increasing of absorbed dose. The evidence for methylmethacrylate (MMA) group being present in TMPT, was observed at 1.730–1.720 cm^{-1} for C=O stretching and 1.130–1.120 cm^{-1} for the –C–O– moiety of the ester functional groups of MMA (Watcharakul *et al.*, 2011).

CONCLUSIONS

Radiation crosslinking has been promoted as a cleaner and more homogeneous cure process. This study demonstrated that chemical properties of NR can be improved as a function of irradiation absorbed dose. Sol-gel studies revealed higher sensitivity of NR and NR/TMPT to EB irradiation. Even at a low irradiation dose (100 kGy) the gel fraction was found to be 76.60% and 87.57% for NR and NR/TMPT respectively. A slowly increasing was observed for all samples when irradiation dose has increased from 250 kGy. Similar results were obtained for crosslinking degree. The obtained results demonstrated that gel fraction and crosslink density were improved as a function

of absorbed dose. When the EB dose increases, there is a increase of gel content (G %) and crosslink density (ν) of samples, due to the formation of a three-dimensional network structure.

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INVESTIGATION OF COLOR STABILITY OF NATURALLY DYED DENIM GARMENTS

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Natural dyes represent an essential part of the world's ecological and cultural heritage; their selection and usage to create colors are common to all civilizations. In the new era of synthetic dyes, research is focusing on identifying environmental friendly dyeing solutions and on the need for a sustainable "green economy". Natural dyes provide important alternative to petrochemical-based dyes and offers environmental and social benefits in comparison with synthetic dyes. Natural dyes are organic compounds and are, therefore, vulnerable in some degree to the action of destructive agents such as light, moisture, detergents, which may conduct in color fading. The study provides useful information regarding color fastness properties of naturally dyed denim garments, obtained within ERANET CROSSTEXNET project VEGDENIM, coordinated by MODAZEN Turkey. Using conditions for the tests specified in different ISO and EN standards, a direct comparison of L*, a*, b* values, for change in color and staining were undertaken. The results of the study indicated that using Punica Granatum and Walnut Shells deeper and more stable shades of colors are obtained in comparison with Indigofera Tinctoria dyed denim samples. All treated samples highlights a change in color in the sense of fading which has occurred to the highest extent when exposed to artificial light and washing.

Keywords: vegetable dyes, denim, color fastness

INTRODUCTION

People never stopped adding colour to their life, starting from the clothes they wear, the cosmetics they apply on their face and the way they dye their hair. Colour is a reflection of our mood, feelings and personality. Today, dyeing is a complex, specialized science. Nearly all dyestuffs are now produced from synthetic compounds. This means that costs have been greatly reduced and certain application and wear characteristics have been greatly enhanced. Synthetic dyes are being used in all commercial applications. Large amounts of water are used to flush conventional synthetic dyes from garments and then this waste water must be treated to remove the heavy metals and other toxic chemicals before they can be returned to water systems (Sengupta and Singh, 2003).

European regulations are more stringent in terms of dye environmental impacts. Many countries are rich in natural and renewable resources and they often have expertise on how to produce and process these resources in a sustainable way. Although the Earth possesses large plant resources, only little has been exploited so far. More detailed studies and scientific investigations are needed to assess the real potential and availability of natural dye-yielding resources. Almost all parts of the plants produce dyes. It is interesting to note that over 2000 molecules used for dyeing are synthesized by various parts of plants, of which only about 150 have been commercially exploited (Siva, 2007).

In developing countries with a textile tradition, natural dyeing is still practiced, but only as a handcraft. Recently, a number of commercial dyers have started looking at the possibilities to overcome environmental pollution caused by the synthetic dyes, by replacing them with natural dyes. Natural dyes produce soft shades as compared to synthetic dyes. In spite of the better performance at multiple washing, recently the potential use of natural dyes on textile materials has been attracting more and more scientist to study the natural alternative for dyeing due to the following reasons: wide spread of natural dyes sources and huge potential; available experimental evidence for allergic and toxic effects of synthetic dyes; available information on different natural colorants, including methods for their extraction and purification.

For successful commercial use of natural dyes, appropriate scientific techniques need to be established by scientific studies on dyeing methods, dyeing kinetics and compatibility of selective natural dyes, in order to obtain shades with acceptable colour fastness behavior and reproducible colour yield (Samanta, 2009).

In the last few decades, denim garments has gained popularity unimaginable for those who initially wore it for protection, rather than for fashion. Denim has become a wardrobe staple. Fit, comfort and price are the most important factors affecting the purchase of denim jeans. Due to longer life span of jeans, the denim industry continues to hold an advantageous position over other types of apparel (Nayak, 2010). In 2010, Greenpeace published a report denouncing the pollution caused by the denim industry (Greenpeace, 2010). Apart from conventional cotton production, which can be one of the most water-consuming industries, the report was also critical of jeans laundry, printing and dyeing processes, which involve high water usage and heavy toxic metals such as cadmium, lead, copper and mercury. A renewed international interest has arisen in natural dyes due to increased awareness of the environmental and health hazards associated with the synthesis, processing and use of synthetic dyes (Ali *et al.*, 2007). Most of the natural dyes have no substantivity for the fiber and are required to be used in conjunction with mordants. A mordant, usually a metallic salt, is regarded as a chemical, which will be fixed on the fiber and which will attach the dyestuff. A link is formed in this way between the fiber and the dye (Singh and Purohit, 2012). The uptake of the dye into the fibres depends on the nature of the dye and its chemical constituents (Zaharia and Suteu, 2012).

The heavy metals detached from the traditional mordants, however, may contaminate the water and the environment, thereby jeopardising the original intention of using environmentally friendly dye for better protection of the environment (Chan *et al.*, 2002).

Coloring components are derived from roots, barks, leaves, fruits and flowers of plant. All plants show a certain reaction to the increasing of toxic elements concentration in soil, depending upon their sensitivity and exposure intensity. Some species of plants disappear, while others are stimulated by these elements. Different plant parts contain different heavy metals quantities, the highest ones being contained in roots and leaves, and the smallest in flower buds and fruit (Smical *et al.*, 2008).

EXPERIMENTAL

In recent years there has been a revival of the use of dyes and color of natural origin for coloring food, pharmaceutical, cosmetic and textile products. Colours obtained with vegetable dyes are warm and have particular nuances. Nevertheless they have two problems that are the same of the industry: color fastness and reproducibility. Colour fastness means the resistance of the colour when exposed to different procedures textiles may suffer during manufacture and use.

Considering the latest trends, MODAZEN Company started to gain interest in using natural dyes within their industrial denim garment production. For this reason MODAZEN INC initiated VEGDENIM project, financed through ERANET CROSSTEXNET Programme.

Vegetable materials of indigo (Indigofera tinctoria leaves powder), Punica granatum (pomegranate bark powder) and walnut shells (Juglans Species) were used to dye denim fabrics at optimized dyeing conditions and the resulted colour fastness of the dyed samples was evaluated through the following tests:

- color fastness to washing, according to SR EN ISO 105 C06: 2010
- color fastness to acid perspiration, according to SR EN ISO 105 E04: 2013
- color fastness to alkaline perspiration, according to SR EN ISO 105 E04: 2013
- color fastness to water, according to SR EN ISO 105 E01: 2013
- color fastness to artificial light, according to SR EN ISO 105 B02: 2003

Materials Used

- Denim naturally dyed samples, dyed with extracts of pomegranate, madder, walnut shells and indigo – supplied by MODAZEN INC (dyeing process is protected by a patent owned by the project coordinator);
- Adjacent multi-fiber, purchased from James Heal, England;
- ECE Detergent with phosphate, without optical brighteners, purchased from James Heal, England.

Testing Equipments Used during Evaluation

- Scourotester – for washing fastness;
- Memmert oven – for water and perspiration fastness;
- Hunterlab – used for measuring color change.

RESULTS

A number of 9 denim samples dyed with vegetable natural dyes prepared by MODAZEN INC. were tested by INCDTP in order to evaluate their colour fastness properties. Preliminary chemical and physical–mechanical tests were performed in order to characterize the denim garments.

The change in color has been made by visual assessment, using the 9 grades grey scale from James Heal, and confirmed by instrumental analysis performed by using Hunterlab equipment. Grades according to ISO 105 A02 have been atributed to each tested sample. An interpretation of the attributed grades: 1 = Poor durability of the colour; 2 = Moderate durability of the colour; 3 = Good durability of the colour; 4 = Very good durability of the colour; 5 = Excellent durability of the colour. Intermediate grades were also attributed.

Determination of Colour Fastness Properties

Table 1. Colour fastness test results

Colour fastness Test	Walnut shells		
	Sample code B 1	Sample code B 2	Sample code B 8
Washing	1-2	1-2	1-2
Acid perspiration	4-5	4	4-5
Alkaline perspiration	4-5	4	4-5
Water	4-5	4	5
Light	1	1	1
Colour fastness Test	Natural Indigo		
	Sample code B 5	Sample code B 6	Sample code B 7
Washing	2	3-4	3
Acid perspiration	3	4-5	4-5
Alkaline perspiration	2-3	4-5	4-5
Water	2-3	4-5	4-5
Light	1	1	1
Colour fastness Test	Punica granatum		
	Sample code B 3	Sample code B 4	Sample code B 9
Washing	1-2	1	1
Acid perspiration	3	2-3	3
Alkaline perspiration	4	4	4
Water	3-4	4-5	4-5
Light	1	1	1

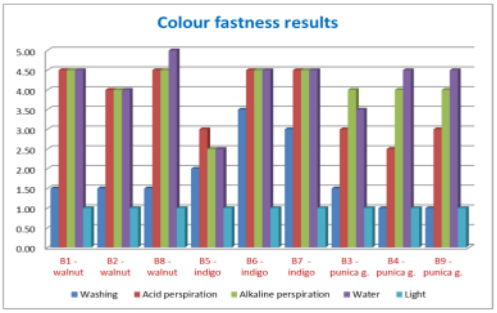

Figure 1. Graphic representation of colour fastness results

As it can be seen, the greatest modification of the colour has occured in the case of the following tests: colour fastness to light and colour fastness to washing. Acceptable results have been obtained for color fastness to water and perspiration in the case of using walnut shells and indigo dye.

Table 2. L*a*b* values obtained for naturally dyed denim samples

Colour fastness Test	B1 - Walnut shells		
Values	L*	a*	b*
Reference value:	55.07	1.11	7.34
Washing	62.90	-0.77	3.31
Acid perspiration	56.38	0.80	7.55
Alkaline perspiration	56.27	0.90	7.04
Water	55.52	0.94	6.58
Light	78.34	-0.43	4.57
Colour fastness Test	B 2 - Walnut shells		
Values	L*	a*	b*
Reference value:	54.84	0.87	6.89
Washing	63.63	-0.61	3.66
Acid perspiration	56.83	1.19	8.07
Alkaline perspiration	56.14	0.99	7.11
Water	55.98	1.00	6.68
Light	78.55	-0.32	4.54
Colour fastness Test	B 8 - Walnut shells		
Values	L*	a*	b*
Reference value:	58.52	3.22	17.29
Washing	65.18	2.22	16.74
Acid perspiration	59.15	3.66	17.88
Alkaline perspiration	58.08	3.87	18.58
Water	58.70	3.49	17.16
Light	70.52	1.03	13.64
Colour fastness Test	B 5 – Natural indigo		
Values	L*	a*	b*
Reference value:	69.99	-2.96	-11.16
Washing	76.94	-2.46	-7.02
Acid perspiration	74.25	-2.84	-8.44
Alkaline perspiration	74.62	-2.60	-8.12
Water	75.02	-2.85	-7.12
Light	88.71	-2.11	7.19
Colour fastness Test	B 6 – Natural indigo		
Values	L*	a*	b*
Reference value:	70.00	-3.02	-11.38
Washing	72.67	-2.56	-11.39

Acid perspiration	70.88	-2.70	-11.64
Alkaline perspiration	71.14	-2.72	-11.51
Water	69.56	-2.82	-11.95
Light	88.12	-2.21	6.07
Colour fastness Test		B 7 – Natural indigo	
Values	L*	a*	b*
Reference value:	71.38	-3.13	-11.04
Washing	73.59	-2.33	-11.03
Acid perspiration	71.03	-2.58	-11.44
Alkaline perspiration	71.01	-2.68	-11.67
Water	71.47	-2.56	-11.10
Light	88.22	-2.09	6.55
Colour fastness Test		B 3 – Punica granatum	
Values	L*	a*	b*
Reference value:	65.34	17.98	4.35
Washing	74.55	13.15	2.62
Acid perspiration	68.30	16.72	7.70
Alkaline perspiration	66.64	17.37	5.07
Water	67.18	17.10	4.25
Light	85.05	5.77	5.96
Colour fastness Test		B 4 – Punica granatum	
Values	L*	a*	b*
Reference value:	64.19	18.48	4.96
Washing	75.25	10.64	7.22
Acid perspiration	68.88	16.74	8.22
Alkaline perspiration	65.81	17.75	5.85
Water	64.24	18.03	5.32
Light	82.14	6.12	6.43
Colour fastness Test		B 9 – Punica granatum	
Values	L*	a*	b*
Reference value:	55.09	21.84	8.89
Washing	66.10	19.37	8.59
Acid perspiration	58.55	19.37	8.59
Alkaline perspiration	57.80	20.14	6.98
Water	56.24	20.36	6.77
Light	80.45	7.79	8.33

Analysing the data obtained it can be seen that all the samples have been losing saturation, samples luminosity has increased and the shade was altered. The data obtained through visual assessment was confirmed: the most significant fading was observed in the case of samples submitted to washing and to artificial light for denim garments dyed with natural indigo, followed by pomegranate. The best results obtained were noticed in the case of using walnut shells.

The colour degradation has reached the lower limit (grade 1) according to the 9 grade scale after 5 consecutive washings for the denim samples dyed with extracts of walnut shells and pomegranate and after 7 consecutive washings for the denim samples dyed with natural indigo.

CONCLUSION

Over the past few years natural textiles have been developed out of a growing awareness of the environmental, health-related and social problems caused by the conventional production of textiles. Many producers are also realising that low consumption and more careful and efficient use of water, energy and raw materials bring benefits to their performance. There is clear evidence that opportunities exist for optimizing the use of natural resources, while simultaneously creating opportunities for cost savings and increased competitiveness. Textile

industry is continuously searching for new technologies in order to accomplish the consumer's demands. In recent years, there has been a revival of the use of dyes and colors of natural origin for coloring textile products. This increasing demand for the material with natural origin is because of the health hazards attributed to some of the synthetic dyes.

Natural dyes are subjected to more destructive agents who can fade significantly the color of a naturally dyed product. Considering the low affinity for natural dyes specific for cotton fibers used within traditional denim garments, the purpose of this study was to assess the fastness properties of the preliminary samples obtained by MODAZEN INC within Crosstexnet EraNet Project acronym VEGDENIM.

Laboratory tests were performed, according to specific standardized methods. The visual assessment of the samples subjected to different treatments was confirmed instrumental results. All samples highlight a change in colour in the sense of fading, which has occurred to the highest extent at exposure to artificial light and washing. Slightly fading has been observed also for the other performed tests, but to a much smaller extent. As a conclusion generated from the information gathered so far: colour fastnesses of denim naturally dyed samples are generally poor. Lower limit of color change (grade 1) is reached after 5 to 7 consecutive washings.

From the sustainability point of view it is desirable to use natural dyes to a greater extent. An intensified use of renewable raw materials represents a substantial contribution to sustainable development and reduced environmental impact.

There is clear evidence that opportunities exist for optimizing the use of natural resources, while simultaneously creating opportunities for cost savings and increased competitiveness.

As a conclusion generated from the information gathered so far: colour fastnesses of denim naturally dyed samples are generally moderate. Optimization of the dyeing procedure is necessary.

Acknowledgment

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"SYNTAN" AND "SYNTHOL" – A RESPONSE TO CURRENT ISSUES

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In this paperwork are presented some solutions needed for both, professionals and beginners in the "secrets" of retanning leather. Modern management of the leather business involves a large number of skills and guidelines, many of them involving skills related to the problem, solving rational and logical thinking. Also, the paper presents real solutions -syntans lineup, because leather and fur skins items have restrictions on the content of some chemicals considered toxic under the regulations stipulated in the various product standards or technical specifications. Importance of the retanning operations has increased recently because of liming and tanning operations, which are more streamlined. Today, everything is done through a standard process, and the specific properties of different types of leather are adjusted during retanning and fat liquoring. To obtain the desired character of the finished leather product combinations are used, each recipe so perfectly combined tend to find the optimum, to match the desired leather sample.

Keywords: syntan, synthol, retanning.

INTRODUCTION

Decision on Retanning

While defining decisions, Drucker suggests that they are judgments, then he suggests that decisions are choices between alternatives. We can observe in his analysis that decisions are considered processes (judging is a process), or they are considered the results of the processes (the chosen alternative).

Decisions on retanning are several criteria used for decision categorization:

- Considering the organizational area which decisions affect (IPPC Bureau, 2013), decisions can be identified which concern people (human resources); money (budgeting); buying and selling (marketing); how to do things (operations); or how to do things in the future (strategy and planning);
- Considering their reiterative character, decisions on retanning can be categirized: routine decisions (decisions that need to be made on a recurring basis); non-routine decisions (unique, random, non-recurring decision situations);
- Considering the period of time these decisions affect, there are: operational decisions are concerned with the day-to-day running of the business; strategic decisions are those concerned with organizational policy and direction over a longer time period.

Decision Making in Models Retanning Process

The rational model is the most used model for decisions on Retanning making.

Several models have been developed in order to solve rational problems. Risk problem solving and uncertainty problem solving are a part of this paradigm.

Another model identified is normative model. In this view decision making is constrained by managers' limited ability to process information ("bounded rationality") and their use of shortcuts and rules of thumb based on prior experience with problems that seem similar to the current situation. Given these constraints, in real life managers don't actually optimize as much as they satisfy that is, they choose a solution that is just good enough to solve the problem and get on with it. It's a satisfactory solution, not necessarily the best or optimal solution (if there even is such a thing).

The third identified model is the bureaucratic model (Aydin *et al.*, 2012). In the bureaucratic model, decision on retanning makers interpret rules to formulate decision. These rules form part of an organizational master plan. While not suitable for highly dynamic environments, the bureaucratic model of decision on retanning making can be successfully applied in organization where the decision environment is mostly routine or predictable.

Judgment decision making, the fourth used model, deals with known processes and logically structured decision steps,judgment decision making deals with intuition and instinct.This is a characteristic of naturalistic decision making.

Decison Making Complexity

From the contextual model, it can be seen that a number of tangible and intangible factors affect a decision outcome and the decision making in retanning process. The key element of this model is the explicit acknowledgement of the effects of context over both the decision making process and the information that is used to produce decision outcomes. Informational factors consist of data that is processed in such a way that it increases the knowledge of the person using it. Contextual factors provide the lens or environment in which information is examined. While not an explicit consideration in a decision, a contextual factor shapes both the way the decision is made and the way in which the information is used (figure 1).

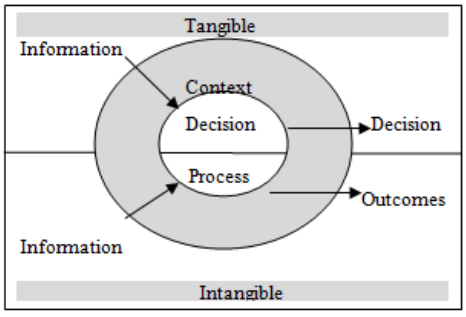

Figure 1. Contextual decision making model (Gully and Stainer, 2006)

Decision making becomes more than a rational model-together with its several technological tools. The truth is that when a person makes a decision, it is influenced by his personal background-we would call this category the personal factors, but also by organizational factors. The modern management of leather business involves many skills and orientations, many of them assuming abilities linked on rational resolving of problems and logical thinking! Situational management is a trial in underlining the

importance of the management's flexibility in function of a biggest number of working situations possible, but it's not telling managers how to act to be more efficient. It seems that there is a serial of managerial actions applicable to any situation (listening, showing importance to all people, use of all resources), since other actions are specific to some unique situations.

This means that the leather industry managers should have general managerial knowledge, but to understand also some specific aspects of the organization and of the working situation that these aspects are placed.

EXPERIMENTAL

Making Samples Retanning

In this study were randomly selected skins sheep, goats and cattle of different sources, from Romania, France, Egypt, Tunisia, USA, Nigeria, India and Brazil. Importance of the retanning has increased in recent years because of liming and tanning operations were more streamlined. Today, everything is done through a standard process (Albu *et al.*, 2011), and the specific properties of different types are adjusted during retanning and anointing. Putting together all this information to achieve the objectives samples grafted on the principle of sustainable production can design different manufacturing recipes (COTANCE *et al.*, 2012). To obtain the desired character of the finished leather product combinations are used, each recipe so perfectly combined tend to find each target to achieve the desired skin sample. Before starting we need to know exactly what you want to achieve through the process and what optical aspects and technical specifications must be respected. The samples were carried out both in the in-pilot station tanneries which have signed one accept the collaboration.

We took into account the achievement of three objectives:

I. Making skins to produce footwear children items, leather and upholstery (furniture and car) by obtaining support type wet-white;

II. Simplifying retanning with vegetable leather by sustainable techniques;

III. Develop range of manufacturing automotive leather upholstery and furniture with low formaldehyde free.

To obtain the wet white support for baby shoe upper, leather goods and upholstery (car and furniture), we have started from the hypothesis that the wet white tanned leathers (Chrome free) will give much different articles comparing with the ones obtained from wet blue (Chrome tanning).

Most of the wet white tanned articles are treated with gluter-aldehyde after pickling. The shrinkage temperature are lower than the ones from the wet blue, this being the reason why in the wet white tanning, syntans are also used. Used of syntans are also positively influencing the setting out and shaving operations after the tanning. The leathers will not be "burned" during the shaving operation.

Wet white tanned leather that will be stocked for a longer time should be treated with syntans based on gluter-aldehyde or modified formaldehyde, due to the fact that long time stockade changes the structure and the leather properties. This is not happening with the wet blue stock, where only an acidification (due to the sulfuric acid released) can be observed. The collagen fibers should be lubricated in such a way that the leather main properties: milling, elasticity, softness, be granted. Fat liquoring is also influencing other leather properties such as: elongation, tear strength or fogging value.

Different chemical products have been used, whose main characteristics are shown in the tables 1 and 2.

Table 1. The oils used and their essential characteristics

No.	Product name	Chemical base	Electrical load	Active substance %	Ph (10% sol)	Stability in formic acid	Stability in NaCl	Stability in Crom	Stability in pickle	Stability In mimosa
1.	Synthol CP 996	Vegetable sulphited oil and synthetic oil based on paraffins	anionic	50	6,5	>2h	>2h	>2h	>2h	>2h
2.	Synthol FL 327	Polymers emulsions and synthetics oils		47	5,5	+/- 30 min	1 h	1 h	0 min	>2h
3.	Synthol PD 990	Synthetic oils, esters phosphates and succinic acid		48	6,5	1 h	1 h	1 h	< 15 min	0 min
4.	Synthol WP	Synthetic phosphated oils and esters of succinic acid		45	7,0	min	0 min	0 min	0 min	1 h
5.	Sulphirol CF 177	Fish Oil particular		65	7,0	>2h	>2h	>2h	>2h	>2h
6.	Synthol CS 606	Polymers natural phosphates and synthetics		37	7,0	>30 min	0	>30 min	0 min	1 h

Table 2. The syntans used and their essential characteristics

No.	Product name	Chemical nature	Active substance	Aspect	pH (10% sol)
1.	Syntan SF 156	Phenol sulfonic acid condensed	> 95%	white	6,0-7,0
2.	Syntan SG	Phenol sulfonic acid condensed	> 93%	powder	4,5-5,5

For realizing the second objective of simplifying of retanning of vegetable tanned leathers by sustainable techniques, it started with hypothesis that syntans, resins and the polyacrylates or agents additional or alternative used instead of chrome and vegetable tannins during the processes. There is a big variety of syntans, more or less bio-degradable. The ones with low free phenol, having a minor impact on the environment are commercial availed.

To realize the third objective, of enlarging the range of articles with low free formaldehyde, we started with the premise that formaldehyde is on RSL list.

RESULTS AND CONCLUSIONS

- Mostly the wet blue support used for waterproofing articles cannot be controlled by its producer. We can somehow influence the quantities of salts and soaking agents contained, by repeated washings performed before starting the re-

tanning process, in order to eliminate the excess of these substances that have a negative influence on the waterproofing effect. When the tanning quality is doubtful a re-chroming should be applied prior the re-tanning process.

- Washings remain an important factor along the entire re-tanning process, the one after the neutralizing being crucial in obtaining good waterproofing effects. To easy the penetration of the waterproofing agents into the leather, a small quantity from these products it's added together with a polymer, before the addition of the acrylic resin. We should note that big pH variations should be avoided all along the re-tanning process. This can be done by using low temperature bath (35-40°C) and adding the formic acid necessary for the fixation, slowly, in a long time. This method avoids the formation of some deposits in re-tanning agents in the external layers of the leather, which would negatively influence the waterproofing properties of the crust.
- Top dyeing is preferable to be done in a new bath, between the re-tanning and fat liquoring processes, method which allows obtaining vivid and intense colors and without affecting the waterproofing properties.
- Washings between the different re-tanning processes have a positive effect on waterproofing. Dyestuffs should not be added into the fat liquoring bath. The technological time of the fat liquoring should be well monitored, the waterproofing leather properties not being in direct proportion with the duration of the fat liquoring process. For the fixation of the waterproofing agents used, 2% of Chrome sulfate is enough. Bigger quantities will not be absorbed and will not increase the waterproofing but will be present into the exhausted bath.
- The re-tanning agents used are non-astringent and have a relaxing action on the leather fibers, which allows a good dyestuff penetration and repartition, as well as of the other re-tanning agents or fat liquors.
- The crust leather obtained is very soft and has a "spongy" feel. After milling a very fine and homogeneous grain is obtained on the entire leather area.
- Re-chroming and neutralizing can be performed into the same bath. This is bringing a better Chrome fixation. Acidity should be neutralized but a too strong neutralizing can bring looseness problems. That's why the neutralizing process can be done in two steps, in order to avoid a sudden pH increase. It has been used sodium formate and a neutralizing agent for the first 30 minutes, adjusting than the pH by sodium bicarbonate, up to a value of 5.8-6.0, stopping the drum after and leaving the leathers into the neutralizing bath. Use of ammonium bicarbonate is not recommended because of the bad "fogging" values of it!
- The fat liquor mix used in the shown recipes slows down the wet- white leather drying and facilitates their wetting back.
- The 2% phenolic syntans used together with the sodium bicarbonate in the neutralizing bath confers a good filling to the sheep and goatskins. For the softness they can be used into the re-tanning bath, having their own tanning properties and bringing a medium softness to the crust, desired article.
- Another question asked was: "should we perform a pre-fat liquoring and to re-tan after it or we should to re-tan first after the neutralizing and to perform a single fat liquoring, after the re-tanning? These two options have being performed and compared. In the first way, pre-fat liquoring brought a drier crust and showing more looseness. In the second way, we have re-tan, fat liquored and again re-tan and fat liquor, obtaining better results. We have concluded that re-tanning and fat liquoring should be applied in

separate steps. The chemical products are better absorbed and the obtained crust has a smaller shrinkage tendency.

- The resulting crust is fuller and with a more pleasant, waxy touch.
- The grain of the crust is natural, smooth and uniform.
- Are obtained excellent soft leathers for garment and furniture upholstery.
- Excellent lubrication of the leather fibers that reduce the friction between the leather and the drum walls.
- No foam is forming in the drum and a better setting out effect is obtained.
- The chemical products used in the shown recipes are entirely exhausted.
- The leather flexibility and the fat liquors fixation into the leather are increased.
- The chemical products used disperse in an efficient way the natural fat of the leathers and promote the rapid penetration of the tannins.
- The chemical products used bring brilliant and intense dyeings.

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*** http://www.ctc.fr/documentation/index.php3.

THE CUSTOMIZED FOOT WEAR FOR ELDERLY – ASSISTIVE PRODUCT ACTING AS A FACILITATOR FOR REDUCING DISABILITY WHILE ENHANCING THE QUALITY OF LIFE FOR OLD PEOPLE

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"The 2012 Aging Report", an extensive document of analysis and prognosis, recently published by EU, estimates that in 2060 over 30% of the population of the European countries will be older than 65. The demographic ageing enhances the importance of research in the field of independent living and social integration of the elderly, important aspects for both the old people and the society. It has been proven that up to 40-50% of the old people have lost their autonomy and need home long-term care because of the walking impairments. Walking disorders in elderly are mostly caused by: the impaired capacity to integrate the proprioceptive stimuli, the associated health conditions and the degenerative morphologic and functional alterations of the foot. In 2003, ISO 9999, taking over the terminology of the ICF (The International Classification of Functioning, Disability and Health, WHO 2001) defined the concept of *assistive product.* The authors are analysing all the possible biomechanical changes of the old foot in order to design customized footwear. The customized footwear which fits the morphologic and functional modifications of the old foot might become an assistive product acting as a facilitator towards diminishing the age-related disability.

Keywords: ageing foot, assistive product, special shoe

INTRODUCTION

In the last decades, due to increased life expectancy and the increasingly complex problems facing national economies, aging became a very important social issue (Muresan, 2012).

"The 2012 Aging Report", a comprehensive document of analysis and prognosis recently published by the European Union, has estimated that in 2060 more than 30% of the EU population will be aged over 65 years.

According to "The 2012 Ageing Report" health expenditure in EU countries (percentage of GDP ascribed to health per capita in EU) also increase with age, especially over 55 years for men and 60 years for women (Ageing Report 2012).

Aging emphasizes the importance of research on maintaining the autonomy and social integration of the elderly. These issues are important both for the individual and for society.

It was found that 40-50% of the elderly who have lost their autonomy and require home care present walking difficulties (Sbenghe, 2012). Walking difficulties in elderly are consecutive: disturbance of the capacity to integrate proprioceptive information, various pathological processes associated, and/or morpho-functional impairments of the foot.

There are studies (Menz, 2012) who claim that 24% of persons over the age 65 complain of foot pain, associated with difficultiesin performing normal daily activities, balance and walking disorders, increased risk of falls and reduced quality of life.

In the elderly, poor performance is caused by several factors: degradation of the structure of different tissues, accumulation of various pathological processes (cardiovascular, locomotor, respiratory, metabolic, neurological, etc.), or simply, the phenomenon of "deconditioning" or "not being used".

With the aging advance, there are a number of biological changes in the musculoskeletal system such as: decrease in muscle cell volume, increase of interstitial collagen and fat, lipofuscin deposition, resulting in decreased strength, endurance and speed of muscle contraction; cartilage degeneration and osteoporosis, leading to deformities and fragility, with subsequent mobility and postural disorders.

Walking disorders in the elderly, generated by disturbance of the capacity to integrate proprioceptive information (impairment of body balance), various pathological processes associated, and/or morphofunctional impairments of the foot, may frequently cause falls with serious consequences (osteoporotic spine and hip fractures, luxations, etc.) (Sbenghe, 2012).

Generally, pathological walking is classified into two categories:

- painless walking(aspect and rhythm changed),of osteogenic, arthrogenic, myogenic, neurogenic causes, etc.
- painful walking, caused by damage of foot structures: bones, joints, muscles, skin, soft tissue,
- peripheral vascular system, peripheral nervous system, etc. (Sbenghe, 2012).

The most common morphological and functional changes of the foot, found in the elderly are (Moyer, 2012):

- increase of the size foot(especially the length and the width)
- loss of the plantar soft connective tissue, which is designed to absorb the impact of walking, especially in the metatarsal phalangeal joints, thus becoming painful, with the incidence of indurations, callosities at this level,
- weakening of ligaments, fasces and tendons, frequently accompanied by tendinitis, plantar fasciitis, muscle stretching,
- flattening of the plantar vault(flat foot)
- ankle, subtalar and metatarsal-phalangeal joints arthritis(especially of the hallux), causing derivations and deformations such as: flat foot or varus foot, hallux valgus, hallux rigidus, toes "in hammer", etc.
- osteoporosis is more common in women, increasing vulnerability to fractures,
- ankle and foot swelling, often due to venous insufficiency or peripheral circulation disorder,
- thinning skin, becoming flaccid and dry, often associated with keratosis
- thickening and deformation of the nails up to “fingers in claw”(onicogrifosis).

Using the concepts and the taxonomy of The International Classification of Functioning, Disability and Health (ICF) aproved for use by the World Health Assembly in 2001, the impairment of the foot structures along with the *neuromusculoskeletal and movement-related functions* may result in disabilities with activity limitations and participation restrictions which can vary between mild mobility limitation- *walking and moving (d450-d469), moving around using equipment, moving around using transportation (d470-d499)* and complete limitation of the activities, including domestic life (d 610-d 660) and self-care (d 510-d 599) and complete participation restrictions to social life based on *environmental factors* which may act as

barriers or, on the contrary as *facilitators*, such as: health care services for treatment (medical or surgical) and rehabilitation programmes, provision of medical devices (orthopaedic footwear, orthesis, walking stick, crutches, etc.), assistive technology (including home arrangement and access ways), other adpted equipments, social insurance services, etc. according to personal needs *(ICF,WHO, 2001*).

According to ICF "***disability*** *is an umbrella term for impairments, activity limitations and participation restrictions, denoting a negative aspect of the interaction between a person's health condition and the individual's contextual factors: personal and environment factors"(1)*. This concept was also assumed by the Romanian legislation (Law of Social Assistance, no.292/ 2011. Art.6, al.h) (2).

A variety of conceptual models16 has been proposed to understand and explain disability and functioning. These may be expressed in a dialectic of "medical model" versus "social model" (ICF, WHO 2001).

The medical model views disability as a problem of the person, directly caused by disease, trauma or other health condition, which requires medical care provided in the form of individual treatment by professionals. Management of the disability is aimed at cure or the individual's adjustment and behaviour change (ICF, WHO 2001).

The social model of disability, on the other hand, sees the issue mainly as a socially created problem, and basically as a matter of the full integration of individuals into society. Disability is not an attribute of an individual, but rather a complex collection of conditions, many of which are created by the social environment. Hence the management of the problem requires social action, and it is the collective responsibility of society at large to make the environmental modifications necessary for the full participation of people with disabilities in all areas of social life. The issue is therefore an attitudinal or ideological one requiring social change, which at the political level becomes a question of human rights (ICF, WHO 2001).

ICF is based on an integration of these two opposing models.

Disability denotes a negative aspect of the interaction between a person's health condition and the individual's contextual factors: personal and environment factors (1).

The participation restrictions replaced the term "handicap" used in the 1980 version of ICIDH and refers to the difficulties that an individual may encounter when implying in existential conditions, compared to what is expected (in a specific cultural and social environment) from a person who hasn't any disability.

Contextual factors are the factors that together constitute the complete context of an individual's life, general framework and individual life conditions. There are two components of contextual factors: Environmental Factors and Personal Factors.

Environmental factors constitute a component of ICF, and refer to all aspects of the external or extrinsic world that form the context of an individual's life and, as such, have an impact on that person's functioning. Environmental factors include the physical world and its features, the human-made physical world, other people in different relationships and roles, attitudes and values, social systems and services, and policies, rules and laws. (1) They can influence in a positive or negative way, the performance of an individual as a member of society, the individual's capacity to fulfil actions and tasks, or the structures and the functions of the body. The contextual factors may limit functioning ("***obstacles***") or, on the contrary, may prevent an impairment to become a cause for participation restriction ("***facilitators***").

In the ICF the *environmental factors* are presented over the course of five chapters: products and technology, natural environment and human-made changes to environment, support and relationships, attitudes, services, systems and policies.

The first chapter deals with products and technology defined as natural or human-made products or systems of products, equipment and technology in an individual's immediate environment that are gathered, created, produced or manufactured.

The international standardization system SR EN ISO 9999: 2003 classification of technical aids defines these as "any product, instrument, equipment or technical system used by a disabled person, especially produced or generally available, preventing, compensating, monitoring, relieving or neutralizing disability".

In 2007, ISO 9999 assumed the terminology of ICF (ICF, WHO, 2001). The term *technical aid* was replaced by *assistive product* defined as "any product (including devices, equipments, instruments, technologies and software) produced or generally available, preventing, compensating, monitoring, relieving or neutralizing disability, activity limitation and participation restriction".

Section e 120 of the ICF is dedicated to the products and technologies for mobility and indoor and outdoor personal transportation, including : equipment, products and technologies used by people in activities of moving inside and outside buildings, including those adapted or specially designed, located in, on or near the person using them.

Customized footwear adapted to the special needs of the elderly might be an "assistive product" acting as a facilitator for which prevents the disabilities induced by aging.

An environment which offers facilitating means can enhance the individual's performance. "Society may hinder an individual's performance because either it creates barriers (e.g. inaccessible buildings) or it does not provide facilitators (e.g. unavailability of assistive devices)" as the ICF states.

The customized footwear, based on innovative technologies, fitted to the structural and functional modifications of the old feet and the associated pathology , may compensate the locomotor dysfunction and the disabilities, that may result in mobility limitation, decreased autonomy, participation restrictions to social life and the overall decreased quality of life.

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RESEARCH ON DETERMINING THE EXPRESSION OF HARDNESS VARIATION OF MATERIALS USED IN SHOE HEEL MANUFACTURING DEPENDING ON THE MEDIO-LATERAL COMPONENT, Fy, OF THE GROUND REACTION FORCE

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This study was aimed at obtaining an empirical relationship in order to use hardness of materials in constructing a custom heel depending on the medio-lateral component, Fy, of the ground reaction force. Custom heeled orthopedic footwear is designed for people with no structural or functional abnormalities in the lower limb, but who value comfort in wearing shoes to the detriment of other aspects related to heel height or shape. The three components of the ground reaction force, namely: the vertical component, Fz, the antero-posterior component, Fx, and the medio-lateral component, Fy, upon ground contact, were measured with AMTI's AccuGait force plate, using the NetForce component, and they were analyzed using the BioAnalysis module. Tests were conducted to analyze materials with various hardness values used in the construction of a custom heel. Thus, after determining the relation, depending on the desired value of the medio-lateral component, Fy, of the ground reaction force, the hardness required for the construction of the custom heel is obtained.

Keywords: hardness, custom heel, ground reaction

INTRODUCTION

Manufacturing custom heeled shoes in order to reduce the medio-lateral component of the ground reaction force, involves the use of materials with different hardness, depending on gait style, walking surfaces and type of shoe, so as to provide the required comfort during walking. Viscoelastic materials for the heel and sole have been widely used since the 1980s, in order to ameliorate or prevent certain diseases of the foot joints, plantar fasciitis, sprains, stress fractures, etc., due to the property of reducing the size of the impact forces upon heel contact with the ground (Whittle, 1996).

Previous studies have shown that attenuation of ground reaction force is a constant concern in footwear manufacturing (Clarke *et al.*, 1983; Logan *et al.*, 2010; Cavanagh *et al.*, 1979).

Experiments performed yielded a database that was used in manufacturing custom heel shoes with the aim to reduce the medio-lateral component of the ground reaction force. The proposed solutions have been based on the development of custom heels of different materials with different hardness for different types of shoes. The three components of the ground reaction force, namely: the vertical component, Fz, the antero-posterior component, Fx, and the medio-lateral component, Fy, upon ground contact, were measured with AMTI's AccuGait force plate, using the NetForce component, and they were analyzed using the BioAnalysis module (Vasilescu *et al.*, 2011; 2012; 2013).

For the design of custom heeled orthopedic footwear in order to reduce the medio-lateral component of the ground reaction force, the possibility of obtaining an empirical relationship that allows the determination of hardness of materials used in heel construction depending on the medio-lateral force, Fy, was analyzed.

METHOD

In order to obtain an unknown function that describes a data set exhibiting a curvilinear behavior, the most common method is non-linear regression. This method involves the generation of new variables in that data set. Thus, once these variables are set, the unknown function (with curvilinear appearance) in the initial state can be expressed as a linear function by the new variables (Baker, 2008; Geaghan, 2012; Seber and Wild, 1989; Meade and Islam, 1995; Schittkowski, 2002; Bethea *et al.*, 1985; Oosterbaan *et al.*, 1990).

To illustrate the above, we may use two non-linear equations encountered in technique (Baker, 2008):

Equation	Interpretation	Linearized form
$Y = Ae^{bX}u$	Y function has a variation (increase/decrease) with a rate dependent on b	$ln(Y) = ln(A) + bX + ln(u)$
$Y = AX^{b}u$	Elasticity of the Y function in relation to X is a b constant	$ln(Y) = ln(A) + b \cdot ln(X) + ln(u)$

In the first example, the exponential growth depends on the b term while the u term models the error (deviation).

Using logarithms on both sides of the equation yields:

$$ln(Y) = ln(A) + bX + ln(u) \quad (1)$$

It is noticed that, although the equation contains logarithms, the terms are linearly dependent on each other. In this form, we may write – by changing the variable:

$$y = a + bX + eroare \quad (2)$$

Thus, by creating a new variable, the natural logarithm of Y, we can use the method of least squares to obtain a regression relationship.

We can proceed similarly with the second equation:

$$Y = AX^{b}u \quad (3)$$

It is specific to processes with constant elasticity (the term elasticity is used broadly, without necessarily referring to mechanical elasticity). Proceeding to apply logarithms to the two sides of the equation, we get a linearized form:

$$ln(Y) = ln(A) + b \cdot ln(X) + ln(u) \quad (4)$$

The above examples are a way of approaching this problem: by empirically estimating the shape (appearance) of the desired equation we can then linearize the said equation in order to apply the method of least squares. There is a whole series of types of equations with various shapes which may be used as initial estimations. The initial equation is usually chosen by evaluating the generic shape described by the set of experimental data; this practice is, however, a simplistic one and does not reflect the complexity of this mathematical instrument.

Thus, by understanding the nature of the physical phenomenon, a shape of the unknown equation can be drawn. Then, by iterative variation of its various constants

and parameters, we can obtain a physical-mathematical model that fits experimental data. It is noteworthy that the importance of choosing the shape of the empirical equation may be higher than the otherwise intuitive principle of choosing an equation that leads to a minimum deviation.

RESULTS AND DISCUSSIONS

The utility of a well constructed equation is that, by adding new experimental data, eventually, a theoretical depth of the phenomenon in question can be reached, while by choosing a polynomial equation of degree n, this is more difficult. Also, by using a carefully constructed equation, the limits or restrictions useful in defining the studied phenomenon can be drawn.

It is noted that there is compatibility between the analytical function shaped like a third-order polynomial equation and the graph based on the experimental data set. The polynomial function may have a higher degree, however, it introduces an increasingly higher number of constants that are only desirable if they lead to more accurate approximations.

The dependence of medio-lateral component, Fy, on hardness can be expressed by the following equation (5):

$$y = 0.0005x^3 - 0.1018x^2 + 6.6289x - 130.85 \quad (5)$$

Figure 1 presents the evolution of the medio-lateral component, Fy, depending on hardness.

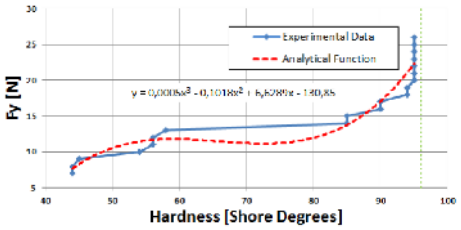

Figure 1. Evolution of the medio-lateral component depending on hardness

The dependence of hardness on the medio-lateral component, Fy, can be expressed by the following equation:

$$Hardness = -4 \cdot 10^{-5} x^5 + 0.0073x^4 - 0.3949x^3 + 8.6715x^2 - 77.032x + 278.1 \quad (6)$$

It is noted that the dependence is of the 5th order, with 6 empirical terms - virtually representing, in turn, functions related to the physical process. Although this relationship has a less intuitive physical interpretation, it fulfills a very precise purpose because it offers the possibility of linking the two physical parameters.

Thus, by entering the desired value of the medio-lateral component, Fy, of the ground reaction force, the hardness required for manufacturing a custom heel is obtained.

The evolution of hardness depending on the medio-lateral component is shown in Figure 2.

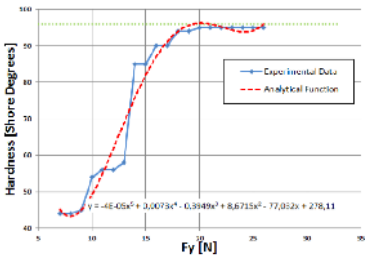

Figure 2. Evolution of hardness depending on the medio-lateral component, Fy

Another approach is that of using an equation similar to the Boltzmann sigmoidal equation:

$$Y = LowerLimit + (UpperLimit - LowerLimit) / \{1 + \exp[(a - X) / IntermediarySlope]\} \quad (7)$$

In this case we can express the physical process by introducing values obtained for the four variables of the function, using the following equation (hardness depending on Fy) (8):

$$Hardness = 45.9 + \frac{48.87}{1 + e^{(13.36-x)/1.138}} \quad (8)$$

Figure 3 shows correlation between the empiric function and experimental results.

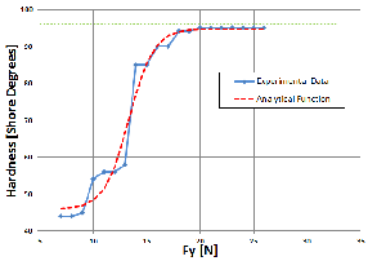

Figure 3. Evolution of hardness depending on the medio-lateral component, Fy

A similar shape may be obtained using a decimal logarithm:

$$Y = LowerLimit + (UpperLimit - LowerLimit) / \{1 + 10^{[(Log(a)-X)]}\} \quad (9)$$

It is noted that this new formula has fewer variables that can be stored, which leads to a simpler mathematical model. Although experimental data fit is not as good as in the case of the sigmoidal, the simplicity of the equation may be helpful in further research. Thus, the constants obtained through regression lead to the final shape (10):

$$Hardness = 49.36 + \frac{44}{1 + 10^{13.5-x}} \quad (10)$$

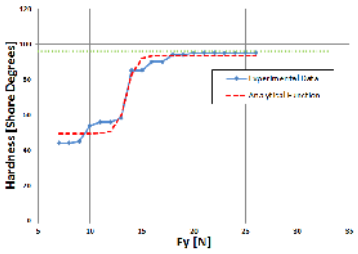

Figure 4. Evolution of hardness depending on the medio-lateral component, Fy

The figure above illustrates the correlation between experimental data and the empirical logarithmic relationship derived by nonlinear regression.

A general conclusion is that all three models of empirical equations have a horizontal asymptote corresponding entirely with experimental data. This, coupled with the step-like shape of the data set, leads to the conclusion that each of the models fulfills its purpose to describe the physical model (to the extent it is studied).

Only by further experimental analysis we can deduce which the equations found is correct one. This can be determined only by isolating the different terms in each equation and their correlation with physical parameters of the experiment.

CONCLUSIONS

The aim of this study is to determine the variation of the hardness of the material used to manufacture the shoe heel, depending on the medio-lateral component, Fy, of the ground reaction force.

It was shown that there is the possibility of changing the distribution of ground reaction force components by heel construction and by using materials of appropriate hardness.

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IV.
ENVIRONMENT

EVALUATION OF LEATHER BIODEGRADABILITY

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This paper presents a study regarding the biodegradability of tree types of finished leathers tanned with different tanning agents: based on Chromium (III), based on Ti-Al, based on Ti-Zr. For assessment of leather biodegradation, EN ISO 20200:2005 was used as method. Physical-chemical analyses were performed on leathers at initial state, after 90, 120 and 220 days of composting. The conclusion of the study is that all types of tanned leather studied undergo the biodegradation process but at different rates. A hierarchy was established for leathers taken in this study, as follows: leather tanned with Ti-Al, leather tanned with Ti-Zr, leather tanned with chromium, where chromium (III) tanned leather has the lowest rate of biodegradability.

Keywords: leather tanned with inorganic salts, composting, biodegradation.

INTRODUCTION

Sustainable disposal of chromium tanned leather wastes is essential under current legislation and thus an appreciation of the extent of leather decomposition is essential.

The microbial decomposition of tanned leathers is poorly understood with relatively few reported studies existing. Leather, containing high levels of nitrogen (ca. 16%) is generally considered a high quality resource capable of degrading readily. However, the tanning process, whereby tanning agents are incorporated into the collagen matrix, results in the reduction of substrate quality and reduced microbial decomposition.

Relatively little is known about the toxicity of Cr(III) towards microorganisms. Generally, heavy metal toxicity affects bacterial growth, morphology, and biochemistry (Aftab, 2006; Bhat *et al.*, 1998; Pillai and Archana, 2012) and is usually via one of three mechanisms: (1) blocking of essential functional groups, (2) displacement of essential ions, (3) modification of active conformation of biological molecules.

In the past years, few determinations of leather biodegradability have been made using compost similar to the one used for plastics and which accelerate the natural degradation process (Thanikaivelan *et al.*, 2004; Pantazi *et al.*, 2014) or under natural conditions (Bacardit *et al.*, 2011; Chirila *et al.*, 2014).

In this study, investigations into the microbial decomposition of leather, under a variety of simulated environmental conditions, were performed in accordance with standard EN ISO 20200:2005 - Plastics - Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test. The method determines the degree of leather disintegration at laboratory scale under conditions simulating an intensive aerobic composting process.

The paper presents a comparative study regarding biodegradation in composting environment of various leathers, such as chrome, Ti-Al and Ti-Zr tanned leathers. Modification of some leather characteristics was monitored for 220 days and differences are discussed.

EXPERIMENTAL

Materials

Finished bovine leathers tanned with different tanning agents: finished bovine leathers tanned with tanning agents based on titanium and zirconium (P_{Ti-Zr}), (Crudu *et al.*, 2012; Adiguzel Zengin *et al.*, 2012; Mutlu *et al.*, 2014), finished bovine leathers tanned with tanning agents based on titanium and aluminum ($P_{Ti–Al}$), (Crudu *et al.*, 2014; Deselnicu *et al.*, 2014, Mutlu *et al.*, 2014), finished bovine leathers tanned with tanning agents based on chromium (III) (P_{Cr}). The chemicals used in the operations were those normally used in the leather industry.

Methods

For assessment of leather biodegradability Standard EN ISO 20200:2005 - Plastics - Determination of the degree of disintegration of plastic materials was used under simulated composting conditions in a laboratory-scale test.

For compost, the following parameters have been considered: temperature: 15-60°C; humidity: 50-60%; oxygen: 15-20%; C/N: 20-30/1.

Four leather samples of each type of tanned leathers were placed in the boxes with compost and one piece of each type of leather was analyzed after 60, 90 and 220 days for the characteristics mentioned below.

For determination of physical-chemical characteristics of leather the following standards were used:

- SR EN ISO 3380:2003 - Leather - physical and mechanical tests - determination of shrinkage temperature up to 100 degrees C;
- SR EN ISO 4045:2008 - Leather - chemical tests - determination of pH.
- SR EN ISO 4684:2006 - Leather - chemical tests - determination of volatile matter;
- SR EN-ISO 4048:2008 - Leather - Chemical tests - Determination of matter soluble in dichloromethane and free fatty acid content;
- SR EN ISO 4047:2002 - Leather - determination of sulphated total ash and sulphated water-insoluble ash;
- SR ISO 5397:1997 - Leather - determination of nitrogen content and "hide substance" - titrimetric method.

The chemicals used for analytical tests were of laboratory grade.

RESULTS AND DISCUSSION

Composition of animal skin consists in: water ca. 65%, proteins ca. 33%, mineral matter ca. 0.5%, fatty substances 2-6% (cattle, calf). Proteins are formed by globular proteins ca. 3.5% and fibrous proteins from which collagen represents ca. 98%. During leather manufacture the collagen interacts with organic and inorganic substances which combine chemically with collagen or are physically deposited within the interfibrillar spaces. The main components of the tanned leather are: "hide substance", fatty matter, humidity, water-soluble organic substances, water-insoluble organic substances, ash. Those components which are likely to be degraded have been analyzed in this study: volatile matter, matter soluble in dichloromethane, sulphated total ash, shrinkage temperature, nitrogen content and pH. All these physical-chemical analyses were performed on leather samples in the initial state, after 90, 120 and 220 days of composting process. Figures 1-5 present the evolution of these components over time.

Measurement of the Shrinkage Temperature

Shrinkage temperature of tanned leather is an indicator of the stability of collagen. It was measured to determine the degree of deterioration in collagen. The shrinkage temperature decreases over time for all type of leathers (Figure 1). Decreasing rate was 12% for chrome tanned leather (P_{Cr}), 17% for Ti-Zr tanned leather ($P_{Ti\text{-}Zr}$) and 18% for Ti-Al tanned leather ($P_{Ti\text{-}Al}$). A detanning process is initiated during biodegradation.

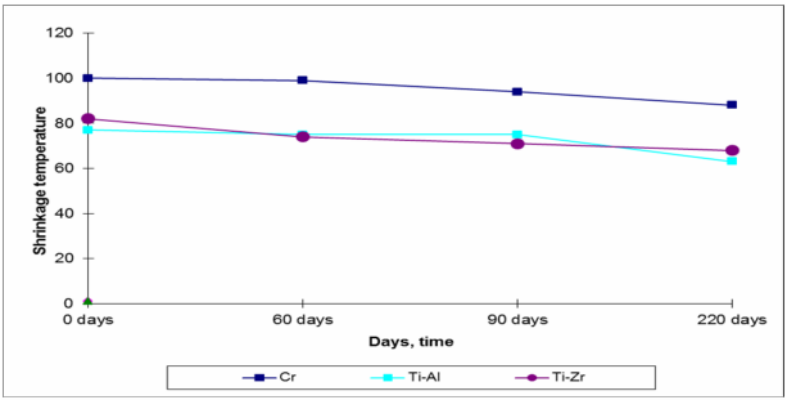

Figure 1. Modification of shrinkage temperature

Determination of pH

pH was determined in water extract and increased over time for all type of leathers during biodegradation process (Figure 2). There is a relation between the pH value and shrinkage temperature. It can be said that loss of stability of the collagen molecule can occur as a result of hydrolytic degradation which occurs when tanned leather is held under warm moist conditions at acidic pH level (Haines, 1987). This type of chemical degradation leads to a progressive loss of strength and a fall in shrinkage temperature.

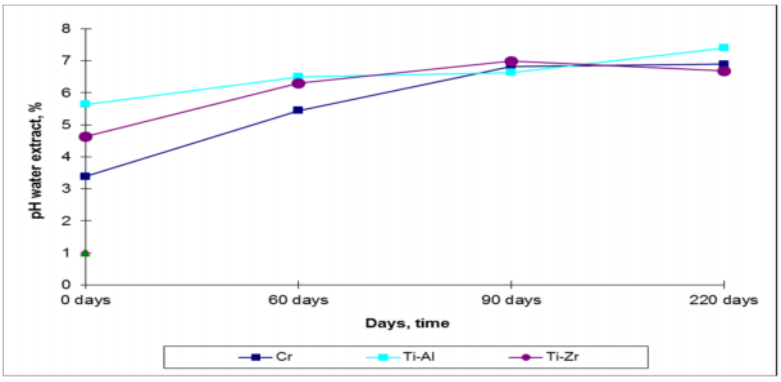

Figure 2. Modification of pH

Determination of Volatile Matter

Volatile matter consists mainly in humidity, but also other volatile matter appears as a result of reaction of peroxide radicals with organic constitutes of leather, and dyes, tanning agents and fatliquors, breaking bonds between the said products and collagen. For the first 90 days evolution of volatile matter is comparable for all types of leather; after 220 days degradation processes intensified and volatile matters content increased by 273.7% for Ti-Al tanned leather (P_{Ti-Al}), by 122.63% for Ti-Zr tanned leather (P_{Ti-Zr}) and by 7.96% for chrome tanned leather (P_{Cr}) (Figure 3).

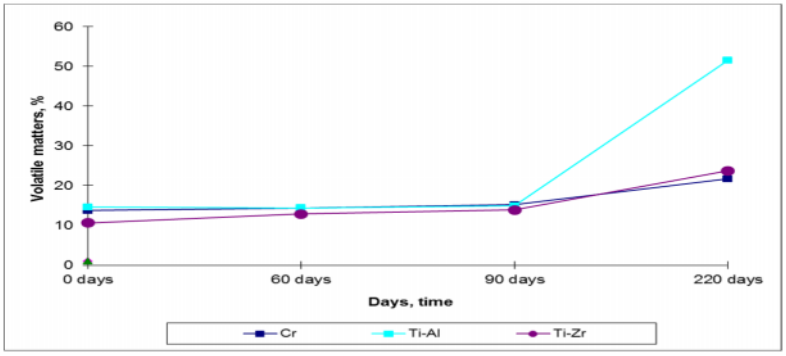

Figure 3. Modification of volatile matters

Determination of Matter Soluble in Dichloromethane

The content of matter soluble in dichloromethane decreased by 60-90% for all types of leather after 60 days of composting, due to biodegradation process and breaking of chemical bonds of fatliquors to collagen (Figure 4):

a) after 90 days matter soluble in dichloromethane decreased by 59.2% for Ti-Al tanned leather (P_{Ti-Al}), by 68.87% for Ti-Zr tanned leather (P_{Ti-Zr}) and by 94.60% for chrome tanned leather (P_{Cr});

b) after 220 days matter soluble in dichloromethane decreased by 80.69% for Ti-Al tanned leather (P_{Ti-Al}), by 99.95% for Ti-Zr tanned leather (P_{Ti-Zr}) and by 99.51% for chrome tanned leather (P_{Cr}).

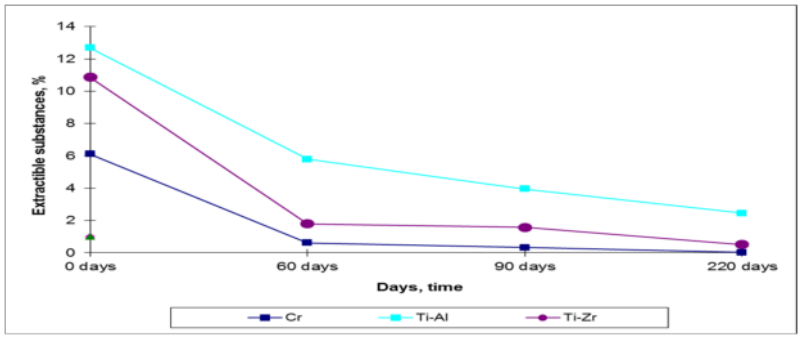

Figure 4. Modification of matter soluble in dichloromethane

Determination of Total Ash

Total ash consists of inorganic salts and tanning agents oxides from leather. Total ash content (Figure 5) increased over time for all types of leather due to the mineralization processes produced during composting:

a) after 90 days total ash increases by 59.2% for Ti-Al tanned leather (P_{Ti-Al}), by 64.93% for Ti-Zr tanned leather (P_{Ti-Zr}) and by 26.47% for chrome tanned leather (P_{Cr});

b) after 220 days total ash increases by 162.69% for Ti-Al tanned leather (P_{Ti-Al}), by 82.59% for Ti-Zr tanned leather (P_{Ti-Zr}) and by 53.48% for chrome tanned leather (P_{Cr}).

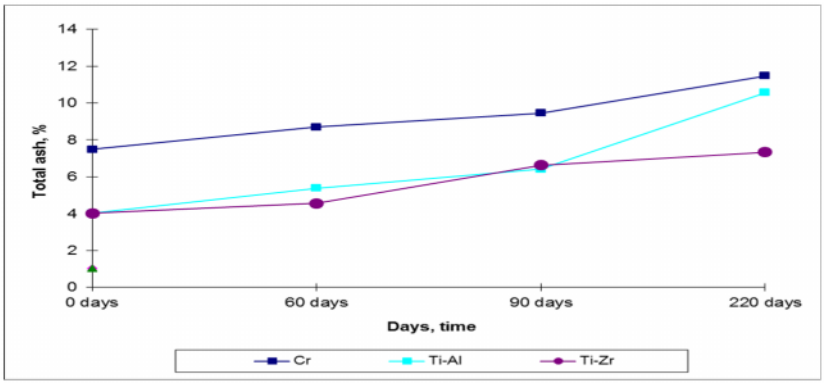

Figure 5. Modification of total ash

Determination of Kjeldahl Nitrogen

Nitrogen content of leather (Figure 4) decreases slowly for the first 90 days of composting; after that, after 220 days, an important reduction is observed due to the biodegradation process.

a) after 90 days nitrogen content decreases by 18.22% for Ti-Al tanned leather (P_{Ti-Al}), by 26.56% for Ti-Zr tanned leather (P_{Ti-Zr}) and by 0.3% for chrome tanned leather (P_{Cr});

b) after 220 days nitrogen content decreases by 5.82% for Ti-Al tanned leather ($_{Ti-Al}$), by 35.05% for Ti-Zr tanned leather (P_{Ti-Zr}) and by 3.07% for chrome tanned leather (P_{Cr}).

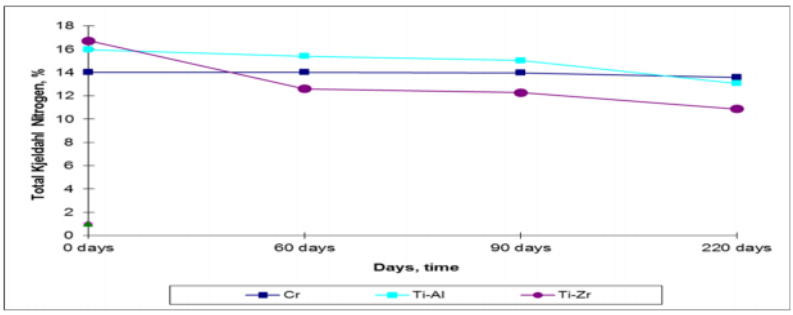

Figure 4. Modification of Kjieldal nitrogen

CONCLUSIONS

The paper presents a comparative study regarding biodegradation of various leathers, such as chrome, Ti-Al and Ti-Zr tanned leathers in composting environment, in accordance with Standard EN ISO 20200:2005 - Plastics - Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test. Composting causes a physical degradation on leathers, a chemical degradation with dehydration, a partial scission of protein chain of the collagen, detanning and loss in oils due to volatilization and/or decomposition.

A significant evolution of the properties of the leather tanned can be observed after 60 days of composting. All types of tanned leather studied undergo biodegradation processes, but at different rates. Leather tanned with Ti-Al showed a higher rate of biodegradation than leather tanned with Ti-Zr and chrome tanned leather.

Acknowledgements

This work was supported by the European Fund for Regional Development and the Romanian Government in the framework of Sectoral Operational Programme under the project INNOVA-LEATHER: «Innovative technologies for leather sector increasing technological competitiveness by RDI, quality of life and environmental protection» – contract POS CCE-AXIS 2-O 2.1.2 nr. 242/20.09.2010 ID 638 COD SMIS – CSNR 12579.

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ENVIRONMENTAL ASPECTS FOR LEATHER FROM A LIFE – CYCLE PERSPECTIVE. PART I: METHODOLOGY

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The goal of this paper is to quantify the environmental impact of new pre-tanning technology with Ti-Al tanning agents, developed during the execution phase of INNOVA-LEATHER project, as well as the thereof assertion of their improved environmental performance when compared against commercial chromium (III) tanning, currently applicable for the production of eighty five per cent of the total volume of finished leathers by the tanning industry worldwide. LCA study was performed using the GaBi 6.0. software and databases in accord with the ISO standard 14044:2006: Environmental management - Life cycle assessment - Requirements and guidelines. Part I of this paper presents the methodology and data collection activities.

Keywords: leather, LCA, chrome tanning, Ti-Al tanning, carbon footprint

INTRODUCTION

As a central objective of the Europe 2020 strategy, the EU as a whole aims to reduce GHG emissions (including emissions from international aviation) by 20 % compared to 1990 levels. By 2020, the national targets will collectively deliver a reduction of around 10 % in total EU emissions from the non-EU ETS sectors, and a 21 % reduction in emissions for the sectors covered by the EU ETS (both compared to 2005 levels). This will accomplish the overall emission reduction goal of a 20 % cut below 1990 levels by 2020, as stated by EUROSTAT in 2013.

Leather industry falls into the category of industries of medium polluting the environment. Over time, four main problems were identified, whose resolution has a significant impact on the economic efficiency of leather processing at industrial level and on the environment: (i) Industrial water consumption; (ii) Cumulative energy consumption (equivalent consumption of oil and/or coal); (iii) Pollution reduction; (iv) The necessary active chemical compounds used in the process *(*auxiliary chemical substances and their adjuvants).

In the last decade, the entire philosophy of development of leather processing and related sectors (especially the production of chemical auxiliaries) was centered on solving the third problem, namely the reduction of pollution.

Chrome tanning is the most common type of tanning in the world. Chrome waste from leather processing poses a significant disposal problem. It occurs in three forms: liquid waste, solid tanned waste and sludge. In most countries, regulations governing chrome discharge from tanneries are stringent. Today, all tanneries must thoroughly check their waste streams. Chrome discharge into those streams is one of the components that have to be strictly controlled.

Many researches were developed to find alternative free of chrome (FOC) tanning technologies. FOC tanning technologies include the use of tanning agents based of titanium-aluminum (Adiguzel Zengin *et al.*, 2012; Mutlu *et al.*, 2014, Crudu *et al.*,

2014; Deselnicu, V. *et al.*, 2012; 2014), which in combination with other retanning agents of vegetable or synthetic origin, allow obtaining quality leathers that may be used by footwear and upholstery industries.

Interest has been developed in estimating the total amount of GHG produced during the various stages in the life cycle of products. The outcome of these calculations, are referred to as Product Carbon Footprints (PCFs). The Carbon footprint of a product is defined as the "weighted sum of greenhouse gas emissions and greenhouse gas removals of a process, a system of processes or a product system, expressed in CO_2 equivalents" referred to a product system.

In case of finished leather, the carbon footprint is expressed as: Kg of CO2eq/m^2 of finished leather (Brugnoli *et. al*., 2012).

The goal of this paper is to quantify the environmental impact of new pre-tanning agents and technologies developed during the execution phase of INNOVA-LEATHER project, as well as the thereof assertion of their improved environmental performance when compared against commercial chromium (III) tanning.

The developed LCA study is a cradle-to-gate approach, evaluating the environmental impact of the finished leather starting with the slaughtering of the cattle, preservation of the raw cattle hides (by treatment with salt), and tanning of the raw salted hides through all core processes until finished leather, taking into consideration the impact of electricity production, water, chemical substances, natural gas production etc., as well as wastes and waste water treatment, water pollutants and air emissions. The agricultural phase is not included in the system boundaries, and cattle husbandry phases are taken into consideration as bringing a zero impact.

METHODOLOGY

The study has been conducted in Romania, since raw material resourced only from Romanian cattle livestock, slaughtered and flayed in Romania, whereas the establishment of the slaughterhouse, tannery and the investigating Institute - ICPI is in Bucharest (Romania). All core processes operations (mechanical, chemical) took place in the tannery.

The study follows the indications of Standard ISO 14044:2006: *Environmental management - Life cycle assessment - Requirements and guidelines.* The GaBi software 6.0. and databases were used in this study.

Product Systems and Boundaries of the LCA Study

Two product systems (technologies) were studied and compared, namely:

S1: Chrome tanned finished box calf upper full grain embossed upper leather sides (1.2 / 1.4 mm), destined for the manufacture of classic men's footwear.

S2: Ti-Al tanned finished box calf upper full grain embossed upper leather sides (1.2/ 1.4 mm), destined for the manufacture of classic men's footwear.

The methodological approach adapted comprises in a step-by-step approach for the identification and quantification of all consumptions and emissions of all core tannery processes for the two product systems. Upstream and downstream processes specific and background data were collected, but the environmental impacts generated were considered to be outside the boundaries of the systems.

The Functional Unit chosen as the reference for all quantified environmental impact values were one square meter (m^2) of surface area of the finished leather.

In turn, the reference flows selected for both systems were the functional unit defined as above mass equivalents of the wet salted skins, intermediate products (pelts, wet stabilized, crust, semi-finished leathers) and finished leathers, respectively.

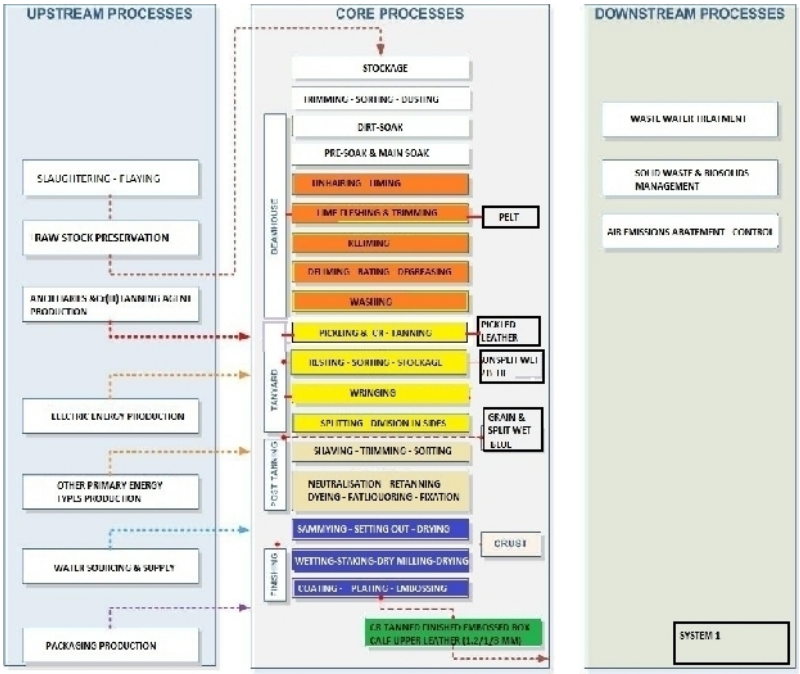

Figure 1. SYSTEM 1 (Chrome Tanning Technology) – Boundaries and outline of processes

System boundaries serve to identify the processes to include in the LCA study, as well as which data can be excluded. The system boundaries for the two technologies under study are described in Figure 1. The boundaries include Upstream processes, which take place before the tanning phases (Core processes), and Downstream processes, including phases taking place after the tanning phases.

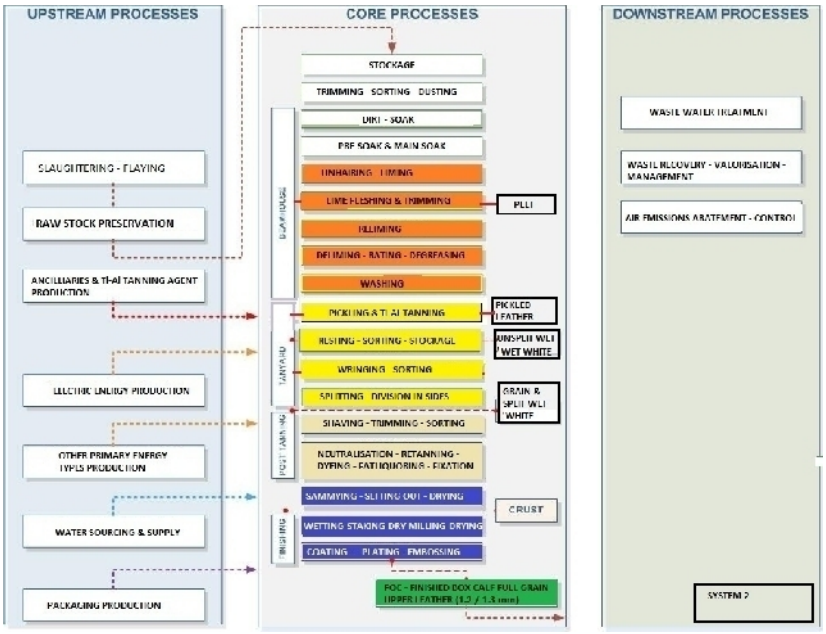

Figure 2. SYSTEM 2 (Ti-Al Tanning Technology) – Boundaries and outline of processes

Data Quality

(1) Upstream processes: Site specific data from the Slaughterhouse; GaBi databases data for the Electricity, natural gas production, water, chemical substances.

(2) Core Processes: Site specific data, GaBi databases data for the Electricity, natural gas production, water, chemical substances.
Chemical substances core inventory and cut off criteria: All chemical technologies used for the production of the two technologies have been listed, together with their specific consumption in kg.
Downstream processes: Site specific data, GaBi databases data for the Electricity, natural gas production, water, chemical substances etc. Pollutant parameters for waste water were established and measured in the final mixed waste waters: chlorides, sulphates.
Air emissions were measured over 24 hours and allocation employed for the objects of the study.

Allocation Principles

Allocation permits the attribution of a correct quantity value to an input, output and concomitantly related impact to the quantity of the specific products defined with the functional unit.

For every phase allocation coefficients have been calculated from the total produced using each of the surveyed and compared technologies). Consumptions of process

water, effluent and solid waste generated, chemicals were measured directly for each aggregated phase and process per mass or surface area unit.

The machines, equipment and buildings used for the production of the two articles were not taken into consideration for the calculation of the environmental footprint and other LCA impact categories.

The data used for the LCA impact evaluation was taken from GaBi software databases, representing Romanian (or European average data where applicable) for the inputs and outputs (water, chemical substances, transportation, waste treatment, etc).

CONCLUSIONS

In the first part of the study was presented the objective, the methodology and the data collection for the LCA study in order to quantify the environmental impact of new pre-tanning agents and technologies developed during the execution phase of INNOVA-LEATHER project, as well as the thereof assertion of their improved environmental performance when compared against commercial chromium (III) tanning.

Acknowledgements

This work was supported by the European Fund for Regional Development and the Romanian Government in the framework of Sectoral Operational Programme under the project INNOVA-LEATHER: "Innovative technologies for leather sector increasing technological competitiveness by RDI, quality of life and environmental protection" – contract POS CCE-AXIS 2-O 2.1.2 nr. 242/20.09.2010 ID 638 COD SMIS – CSNR 12579.

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ENVIRONMENTAL ASPECTS FROM A LIFE –CYCLE PERSPECTIVE FOR TWO LEATHER TANNING SYSTEMS. PART II: IMPACT ASSESSMENT

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The goal of this paper is to quantify the environmental impact of new pre-tanning technologies developed during the execution phase of INNOVA-LEATHER project, as well as the thereof assertion of their improved environmental performance when compared against commercial chromium (III) tanning, currently applicable for the production of eighty five per cent of the total volume of finished leathers by the tanning industry worldwide. The environmental impact assessment was made based on the LCA impact categories indicators: Global Warming Potential (GWP), Ozone depletion potential (ODP), Acidification potential (AP), Eutrophication potential (EP), Abiotic depletion potential (fossil) (ADP), and Photochemical ozone creation potential (POCP). The results indicate that significant environmental impacts were caused by chrome tanning technology; carbon footprint – GWP for Chrome tanning technology was 11,4848 kg CO_2 equiv. and for Ti-Al tanning technology was 9,7250 kg CO_2 equiv. Regarding tanning process, carbon footprint – GWP for Chrome tanning process was 3,0766 kg CO_2 equiv. and for Ti-Al tanning process was 1,1752 kg CO_2 equiv., being about three times less.

Keywords: leather, LCA, chrome tanning, Ti-Al tanning, carbon footprint

INTRODUCTION

It is recognized that at the moment the LCA – Carbon Footprint topic is primarily of interest to tanners in industrialized, especially EU countries; however it is felt that also those in BRIC and even Least Developed Countries should be aware of the current environmental impact assessment and protection trends, and be ready to apply them at appropriate time as needed. It is hoped that in the meantime better standardized methodologies and probably some blueprints will also be made available (Brugnoli *et al*., 2012).

The goal of this paper is to quantify the environmental impact of new pre-tanning technologies developed during the execution phase of INNOVA-LEATHER project, as well as the thereof assertion of their improved environmental performance when compared against commercial chromium (III) tanning (Adiguzel Zengin *et al.*, 2012; Mutlu *et al.*, 2014; Crudu *et al.*, 2014; Deselnicu, V. *et al.*, 2012; 2014).

The LCA study is a cradle – to – gate approach, evaluating the environmental impact of the finished leather starting with the slaughtering of the cattle, preservation of the raw cattle hides (by treatment with salt), and tanning of the raw salted hides through all core processes until finished leather, taking into consideration the impact of electricity production, water, chemical substances, natural gas production etc., as well as wastes and waste water treatment, water pollutants and air emissions. The agricultural phase is not included in the system boundaries, and cattle husbandry phases are taken into consideration as bringing a zero impact.

Method

The LCA study was performed using the GaBi software 6.0. and its databases in accord with ISO 14044:2006 Standard: Environmental management - Life cycle assessment - Requirements and guidelines.

The methodology was presented in the first part of this study (Deselnicu D.C. *et al.*, 2014). The environmental impact assessment was made based on the LCA impact categories indicators, which quantify the global environmental impact of the studied technologies, and allow the technologies comparison according to their environmental performance.

Typically, the commonly used and accepted impact categories for LCA studies are the following:

Global warming potential, GWP, unit: [kg CO2-eq.] – commonly known as climate change indicator;

Ozone depletion potential, ODP, unit: [kg R11-eq.] – measuring ozone hole in higher atmosphere;

Acidification potential, AP, unit: [kg So2-eq.] - environmental effect by the acid rain/ forest dieback;

Eutrophication potential, EP, unit, [kg PO43-eq.] – measuring over-fertilization of soil and water;

Abiotic depletion potential (fossil), ADP, unit: [MJ.] – indicating non renewable resources, e.g. coal, crude oil, natural gas;

Photochemical ozone creation potential, POCP, unit: [kg C2H4-eq] – indicator for ozone creation in lower atmosphere.

All these indicators were calculated and assessed during the study, and will be further discussed.

RESULTS - LIFE CYCLE ASSESSMENT IMPACT

The quantification of the total impact categories for the two investigated technologies is presented in Tables 1 and 2, and in Figures 1 to 3.

Table 1. Total assessed impact of LCA impact indicators for Chrome and Ti-Al leather production technologies

	TOTAL ASSESSED IMPACT									
Catego-ries	Global Warming Potential (GWP 100 years)		Ozone Depletion Potential (ODP)		Acidification Potential (AP)		Eutrophication Potential (EP)		Photochemical Ozone Creation Potential (POCP)	
Unit	kg CO2 equiv.		kg R11 equiv.		kg SO2 equiv.		kg PO43-equiv.		kg C2H4 equiv.	
Techno-logy	Cr	Ti-Al	Cr	Ti-Al	Cr	Ti-Al	Cr	Ti-Al	Cr	Ti-Al
Quantity	11.4848	9.7250	.00001	.00000	0.0883	0.0802	0.0171	0.0176	0.1513	0.1535

The most important and commonly used impact indicator is the Global Warming Potential (GWP), known also as Carbon footprint. As can be seen from Table 1 and Figure 1, the Ti-Al tanned technology resulted in a smaller carbon footprint – Global Warming Potential (9,7250 kg CO2 equiv.) than the Chrome tanned one (11,4848 kg CO2 equiv.).

The LCA impact indicators of the two compared technologies show the higher environmental impact of the classic Chrome leather production technology as compared to the Ti-Al leather production technology.

The Ti-Al leather production technology also resulted in significantly smaller Ozone Depletion Potential (ODP), Acidification Potential (AP) and Marine Aquatic Ecotoxicity Potential (MAETP) than the classic Chrome leather production technology:

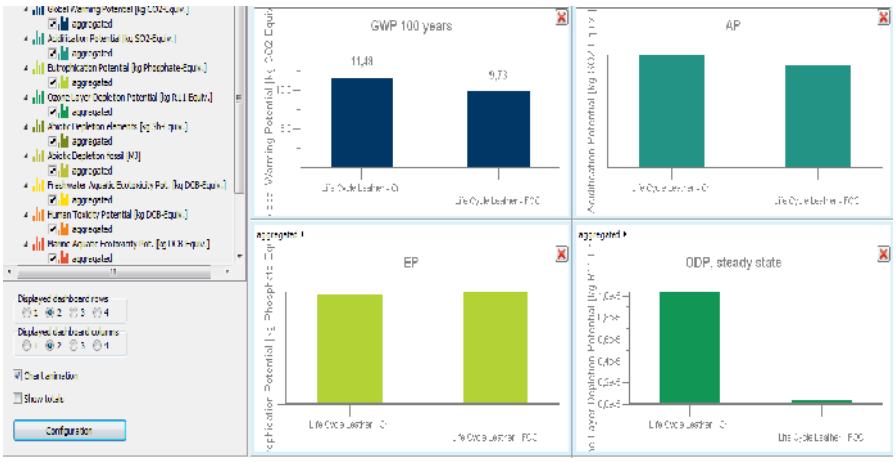

Figure 1. LCA impact indicators comparing the two technologies: Global Warming Potential (GWP), Acidification Potential (AP), Eutrophication Potential (EP) and Ozone Depletion Potential (ODP)

The Eutrophication Potential (EP) and the Photochemical Ozone Creation Potential (POCP) indicators for Ti-Al leather production technology show a slight increase as compared to the ones for Chrome leather production technology, but the values are comparable:

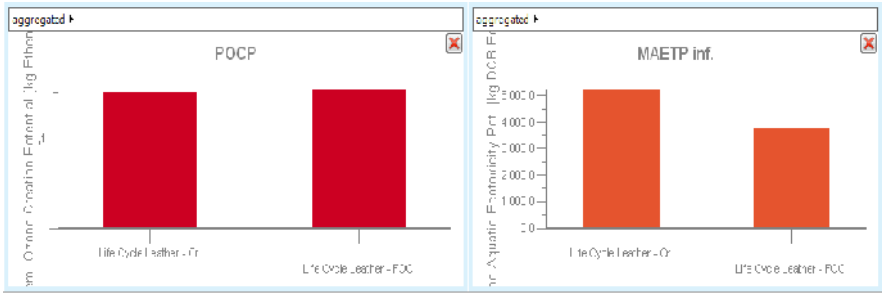

Figure 2. LCA impact indicators comparing the two technologies: Photochemical Ozone Creation Potential (POCP), Marine Aquatic Ecotoxicity Potential (MAETP)

Table 2. Total assessed impact of LCA impact category per technology phases / processes for the two compared technologies

Categories/ Unit	Global Warming Potential (GWP 100 years) – kg CO2 equiv.		Ozone Depletion Potential (ODP) kg R11 equiv.		Acidification Potential (AP) kg SO2 equiv.		Eutrophication Potential (EP) kg PO4 equiv.		Photochemical Ozone Creation Potential (POCP) kg C2H4 equiv.	
Technology	Cr	Ti-Al	Cr	Ti-Al	Cr	Ti-Al	Cr	Ti-Al	Cr	Ti-Al
Slaughter-house	1.5706	1.5706	.00000	0.0	.0114	0.0114	0.0005	0.0005	0.0006	0.0006
Beamhouse	0.9357	0.8835	.00000	0.0	.0273	0.0247	0.0020	0.0020	0.0013	0.0012
Tanning	3.0766	1.1752	.00000	0.0	.0200	0.0112	0.0035	0.0018	0.0014	0.0008
Post-tanning	1.1805	1.3278	.00001	0.0	.0135	0.0162	0.0027	0.0032	0.0014	0.0018
Finishing	1.0204	1.0341	.00000	0.0	.0109	0.0109	0.0008	0.0008	0.1464	0.1490
Wastewater treatment	0.2458	0.2458	.00000	0.0	.0040	0.0040	0.0036	0.0056	0.0002	0.0002

Figure 3 shows the LCA impact indicators comparing the two technologies on human toxicity: Human tox (cancer) indicator is zero for the new production technology, and Human tox (noncancer) indicator is smaller than the one of the Chrome leather production technology.

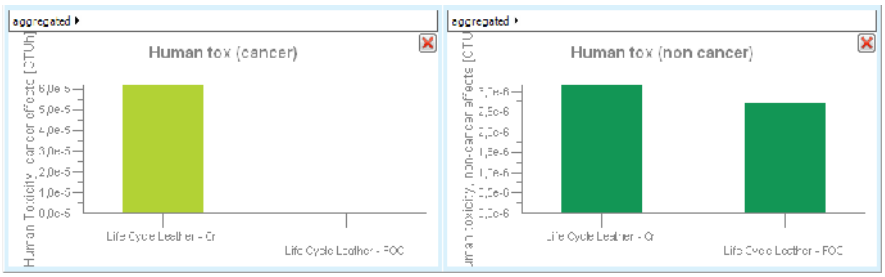

Figure 3. LCA impact indicators comparing the two technologies Human tox (cancer) and Human tox (noncancer)

If we analyses the impact indicators of the main processes for leather production (Table 2), the *Chrome technology has a higher environmental impact than the Titanium – Aluminum technology in almost all the processing phases*, except for the Post-tanning and Finishing phase.

In the Tanning phase, where the two leather production technologies differ – one is using the classical Chrome based tanning agents, and the other is using the newly developed Ti-Al tanning agents, there is the most significant difference in terms of Carbon footprint: the new INNOVA – LEATHER leather production technology *is generating three times less environmental impact* than the classical Cr-based tanning technology.

CONCLUSIONS

The goal of this study was to quantify the environmental impact of new tanning agents and related technologies for leather production, developed during the execution

phase of INNOVA-LEATHER project, as well as the assertion of their improved environmental performance when compared against chromium (III) tanning.

The LCA study was performed with GaBi software 6.0. and databases in accord with Standard ISO 14044:2006: Environmental management - Life cycle assessment - Requirements and guidelines.

The main conclusion of the study is that the new overall tanning technology developed in INNOVA-LEATHER project generates a 15% lower environmental impact measured as Carbon footprint (Global Warming Potential indicator) than the chrome tanning technology. The other calculated impact indicators have comparable values between the two technologies.

In terms of the investigated Life Cycle process phases, the Tanning phase brings the most significant difference in terms of Carbon footprint: the new INNOVA – LEATHER technology is generating almost three times lower environmental impact than the Cr-based tanning technology in the tanning phase.

Out of the five main LCA impact indicators that were investigated in this study, three of them (namely Global Warming Potential – GWP, Ozone Depletion Potential – ODP and Acidification Potential – AP) have significantly lower total values for the new Ti-Al based technology than the classic Chrome leather production technology. Other three secondary impact indicators (Human toxicity cancer effects and non-cancer effects – Human tox cancer and Human tox non-cancer, and Marine Aquatic Ecotoxicity Potential - MAETP) also remarkably low values (sometimes negligible) for the new technology.

This demonstrates that the new tanning agents based on Ti-Al developed in the INNNOVA – LEATHER Project generate a significantly smaller environmental impact than chrome tanning agents and related technologies, recommending it for increased industrial use for a more sustainable and eco-efficient leather production, as new and innovative low carbon technologies help to reduce greenhouse gas emissions and create new employment and growth.

Acknowledgements

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INFLUENCE OF LAND USE ON MICROBIOLOGICAL ACTIVITY OF SANDY SOILS

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Major interrelated factors affecting microbiological diversity in soil include soil forming processes, physico-chemical properties of soil, soil particle size distribution, vegetation, and land use type. In the south-east Romanian Plain important land use changes occur in the last two decades. The aim of the study is to analyze the influence of the land use on the microbiological properties of sandy soils. We examined five sites, representing four different land-use types (cultivated land, vineyard, acacia forest, and pasture), in the south-east Romanian Plain. The soil profiles were described in the field and sampled (from each genetic horizon), after the removal of forest litter, for particle size distribution, pH, $CaCO_3$ content, organic matter content, V8.3% analyses, conforming to RISSA Methodology-1987. For microbiological analyzes three indices were determined: number of heterotrophic bacteria, number of microscopic fungi and soil respiration. Soil respiration, as a global indicator of soil microbial activity, has the highest values for pasture, while lowest values for bacterial and fungal microflora were recorded under vineyard use, reflecting soil life response to anthropic interventions.

Keywords: sandy soils, microbiological activity, land use.

INTRODUCTION

Microbial communities can provide a measure of soil quality because of their capacity to respond sensitively to changes and environmental stress (Cornea *et al.*, 2011). The modifications of microbiological parameters can precede detectable changes in soil or plant properties, which can be an early sign of improving soil quality or, by contrary, an early warning of soil deterioration (Winding *et al.*, 2005).

Major factors affecting microbiological diversity in soil include soil forming processes, physical and chemical properties of soil, soil particle size distribution, vegetation, and land use type. Many publications emphasize the influence of the land use on the structure of soil microbial communities (Nusslein and Tiedje, 1999; McCaig *et al.*, 2001; Webster *et al.*, 2002; Clegg *et al.*, 2003, cited by Grantina *et al.*, 2011).

In the south-east Romanian Plain important land use changes occur in the last two decades (Pravalie *et al.*, 2013), which led to serious problems such as erosion and structure degradation, groundwater contamination, insufficient water holding capacity, increasing susceptibility to pests, reduced soil fertility.

The paper presents the results concerning the quantitative estimations of densities in heterotrophic bacteria, fungi and their global physiological activities in five sites, representing four different land-use types (cultivated land, vineyard, acacia forest, and pasture) of sandy soils.

MATERIALS AND METHODS

Study Area

Study area is located in the southwestern part of the Romanian Plain (Băileşti Plain). The landscape is presented as a series of sand dunes which cover the terraces and meadow of Danube River. Parental materials of the soils consist of carbonated and

uncarbonate sands deposited as dunes and interdunes. The climate is characterized by hot and dry summers with few precipitations, with mean annual temperatures exceeding 11°C and mean annual values of precipitation between 525 and 550 mm.

Natural vegetation is characterized by forest-steppe vegetation, with xerophyte oak as representative species, severly restricted due to extention of arable lands (The Geography of Romania, Volume V, 2005).

Figure 1. Localization of the study area

Large areas have been planted with acacia (*Robinia pseudoacacia*) in the first half of the twentieth century, and with vineyards starting with 1961, for the stabilization of the sandy soils, but some forest areas have been cleared during second half of the twentieth century in order to expand the agricultural areas (Nuță, 2005).

Soil Sampling and Laboratory Analysis

We examined five sites, representing four different land-use types. First two soil profiles, P1 and P2 are Eutric Arenosols, under pasture. P1 is formed on a sand dune deposit that cover the Danube terrace (location on the background picture of Figure 3) and P2 is formed on a sand dune deposit that cover the Danube meadow sediments (location on the background picture of Figure 4). P3 is an Aric Arenosol, under vineyard, formed on a stabilized sand dune deposit that was ploughed to a depth of 40 cm. P4 is an Arenic Chernozem, under arable (wheat stubble), formed on interdune and P5 is an Eutric Arenosol, under accacia forest, formed on a stabilized sand dune deposit (location of the P3 to P5 soil profiles on Figure 1).

The soil diagnostics were based on the concept of elementary pedogenetic processes, in agreement with the World Reference Base for Soil Resources (IUSS - FAO, 2014).

The soil profiles were morphologically described, and disturbed soil samples were collected from diagnostic horizons within the first 40 cm of the soil profiles. Physical and chemical determinations were made: particle size distribution, pH, calcium carbonate ($CaCO_3$) content, organic matter content analyses, base saturation degree ($V_{8.3}$ %), conforming to RISSA Soil Survey Methodology-1987.

Microbiological analyses of soil samples were performed by plating soil decimal dilutions on specific solid culture media, Topping for heterotrophic bacteria and Czapek for fungi (Papacostea, 1976). After incubation, the developed colonies were counted and the densities of microbial structures were reported to gram of dry soil. Global physiological activities of microbial communities were determined by the substrate induced respiration method (Ştefanic, 1991).

RESULTS AND DISCUSSION

Physical and Chemical Characterization

The soils analyzed inherited the *textural characteristics* from the parent material and have coarse texture, with up to 70 % sand content (coarse and fine sand, between 2-0.02 mm) (table 1).

The colloidal clay (< 0.002 mm) has the lowest values for P3 - vineyard (between 4.7 and 4.2%) and P5 - forest (between 5.9 and 4.4%), while P2 - pasture has the highest clay content (17.9% in At and decrease to 10.2% in Am2 horizon). Also, P2 profile has the lowest coarse sand content (0.2-2 mm), between 10.0 and 11.6%, while whithin P3 soil profile the coarse sand values exceed 60%.

The *calcium carbonate* has been leached from the soil profiles.

Table 1. Physical and chemical properties of studied soils

Horizon	Depth (cm)	Texture	Granulometrical fractions (% g/g)				Carbo-nates (%)	V8.3 (%)	pH	SOM (%)
			Clay <0.002 mm	Silt 0.002-0.02 mm	Fine sand 0.02-0.2 mm	Coarse sand 0.2-2.0 mm				
P1 – Eutric Arenosol, pasture, N: 43°50’55,07”, E: 23°0’8,24”, Alt: 32 m										
At	0-12	LS	8.5	4.3	54.3	32.9	-	70.0	5.72	2.52
Am	12-28	LS	7.8	4.8	51.1	36.3	-	69.2	5.93	0.96
AB	28-35	LS	10.7	3.9	53.1	32.3	-	83.5	6.64	0.78
P2 - Eutric Arenosol, pasture, N: 43°49’13,55”, E: 22°58’49,08”, Alt: 31 m										
At	0-12	SL	17.9	11.3	60.7	10.0	-	100	7.73	4.38
Am1	12-24	SL	14.7	11.3	62.3	11.7	-	100	7.74	2.34
Am2	24-37	LS	10.9	10.2	67.3	11.6	-	100	7.87	1.50
P3 - Aric Arenosol, vineyard, N: 43°51’21,55”, E: 23°1’21,27”, Alt: 36 m										
Ao1d	0-12	CS	4.5	0.9	33.1	61.6	-	100	7.94	0.30
Ao2d	25	CS	4.7	0.7	30.3	64.3	-	100	8.16	0.36
(A+C)d	25-40	CS	4.2	0.9	30.2	64.7	-	100	8.08	0.30
P4 - Arenic Chernozem, arable, N: 43°51´27,14”, E: 23°1´22,30”, Alt: 34 m										
Ap	0-15	SL	16.5	12.8	50.3	20.4	-	77.3	6.28	2.46
Am	15-26	SL	16.2	12.4	51.8	19.5	-	76.6	6.19	2.22
AB	26-43	SL	15.7	10.9	56.5	16.9	-	85.2	6.64	2.04
P5 – Eutric Arenosol, accacia forest, N: 43°51’44,75”, E: 23°1’10,13”, Alt: 42 m										
Ao	0-10	LS	5.9	1.5	45.5	47.1	-	71.4	6.29	1.44
AC	10-27	MS	4.9	1.3	46.2	47.7	-	85.3	6.7	0.54
C1	27-42	CS	4.4	0.2	32.4	63.0	-	100	7.56	0.36

LS – loamy sand, SL – sandy loam; CS – coarse sand; MS – medium sand

The *base saturation degree* ($V_{8.3}$ %) correlates with soil reaction, and has maximum value of 100% for P2 - pasture, due to annual Danube overflows), and for P3 - vineyard profile, due to chemical treatments applied (table 1).

P1 - pasture has the mezobasic values of the base saturation within the first two horizons (70.0% in At and 69.2% in Am) and eubasic values in AB horizon (83.5%), while P4 - arable and P5 - forest are eubasic within the soil profile.

Soil reaction (pH) is slightly acid for P1 - pasture (except the surface horizon which is moderate acid - 5.72), with 5.93 in Am and 6.64 in AB, and P4 - arable, with pH values between 6.28 in Ap and 6.64 in AB horizon.

P2 - pasture and P3 - vineyard have slightly alkaline reaction, with values varying between 7.73 and 7.87 for P1 and between 7.94 and 8.16 to P3.

P5 - forest has slightly acid reaction within the first two horizons (6.29 and 6.7 respectively) and slightly alkaline reaction in C1 horizon (7.56).

The soil organic matter content (SOM) varies from medium to low for the soil profiles under pasture and arable land use. The SOM values decrease from 2.52% in At to 0.78% in AB within the P1 - pasture, and from 4.38% in At to 1.50% in Am2 within the P2 - pasture, while the values determined for P4 - arable are more closed and vary between 2.46% in Ap to 2.04% in AB horizon.

The SOM content is very low for the P3 - vineyard, with values between 0.36% in Ao2d and 0.30% in Ao1d and (A+C)d.

P5 - forest has low values of SOM within Ao horizon (1.44%) and very low values within AC (0.54%) and C1 (0.36%) horizons.

Microbiological Characterization

Mostly low values of potential level of *soil respiration* have been determined in analyzed soil profiles, corresponding to a reduced activity of microflora.

Surface horizons of the five profiles (correspond to depths of about 0-40 cm profile) show values exceeding 30mg CO_2/100g soil, considered average values for analyzed parameter.

The highest level of soil respiration was determined for P2 - pasture (85.89 mg CO_2/100g soil in At, 54.90 mg CO_2/100g soil in Am and 44.27 mg CO_2/100g soil in AC), while the lower values were found for P3 - vineyard (29.13 mg CO_2/100g soil in Ao1d, 39.08 mg CO_2/100g soil in Ao2d and 36.95 mg CO_2/100g soil in (A+C)d).

Bacterial microflora is best developed in P2 - pasture (38.60 viable cells / g dry soil in At) and P5 - forest (33.66 viable cells / g dry soil in Ao) profiles and average developed in P1 - pasture, P3 - vineyard and P4 - arable profiles (between 19.83 and 14.80 viable cells / g dry soil in P1, 13.80 and 10.69 viable cells / g dry soil in P3 and between 15.70 and 12.38 viable cells / g dry soil in P4).

The analysis of the *fungal microflora* on soil profile highlights an average load for P2 - pasture (between 88.61 and 54.79 cfu / g dry soil), P1 - pasture (between 56.99 and 47.90 cfu / g dry soil), P4 - arable (between 58.63 and 52.67 cfu / g dry soil), and P5 - forest (between 60.42 and 47.87 cfu / g dry soil), while the lowest level of fungi in soil has been determined for P3 - vineyard (50.39 cfu / g dry soil in Ao_1d horizon).

The organic horizons of P5 - forest, Ol and Of+h, respectively, have an intense activity of microflora, with high number of bacteria (22.37 viable cells / g dry soil in Ol and 47.12 viable cells / g dry soil in Of+h) and an average number of fungi (61.72 cfu / g dry soil in Ol and 78.31 cfu / g dry soil in Of+h).

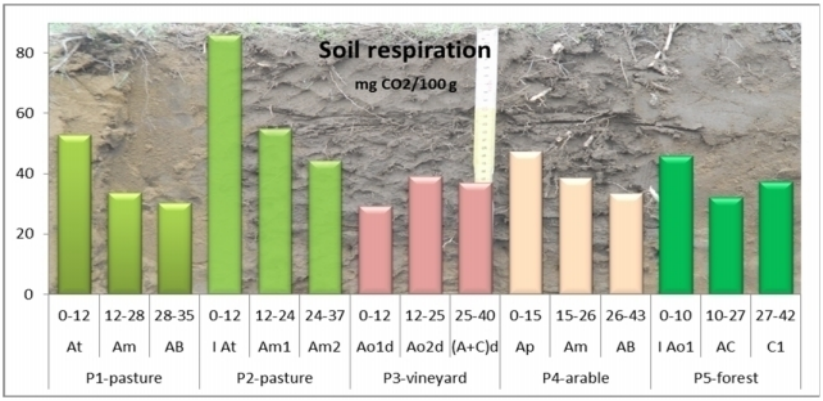

Figure 2. Soil respiration potential level within the first 40 cm of the soil profiles

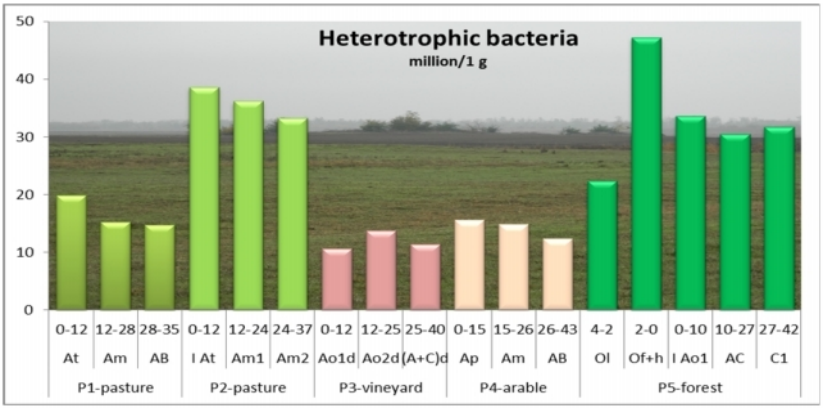

Figure 4. Total number of bacteria within the first 40 cm of the soil profiles

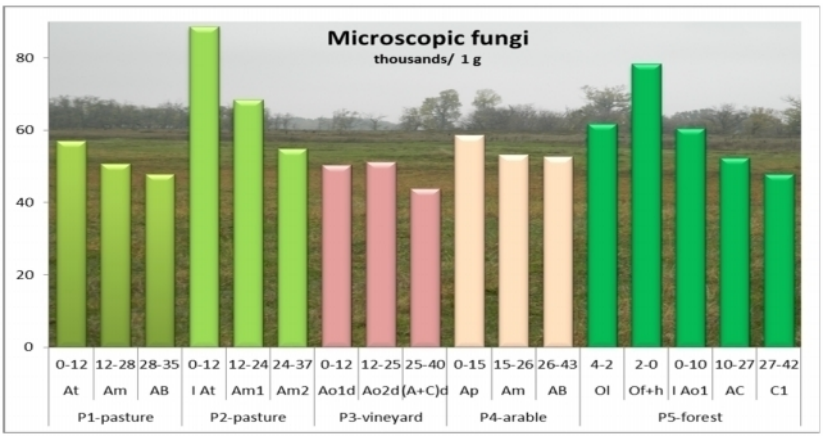

Figure 3. Total number of fungi within the first 40 cm of the soil profiles

DISCUSSIONS AND CONCLUSIONS

Physical and chemical properties of analysed soils (sandy texture, with high percentage of coarse sand, slightly acid reaction, low soil organic matter content due to intense mineralization of organic residues) create conditions for a reduced activity of microflora.

Soil respiration, as a global indicator of soil microbial activity, has the highest values for pasture, while lowest values have been determined for vineyard.

The highest level of soil respiration was determined for P2 - pasture profile, reaching double values compared to similar horizons of the other profiles in the 0-40cm depth. We consider that situation is due to shallow water table and to annual Danube overflows, which favor vegetation development and create conditions for an intense microbial activity in that soil. That explain also the differences registered between P2 and P1, both soil profiles beeng under pasture.

The number of both fungi and bacteria showed a normal tendency to decrease with increasing depth, excepting P3 – vineyard, where the higher number has been reached wihtin the second (Ao2d) horizon.

The activity of bacterial microflora is substantialy higher within P2 - pasture and P5 - forest (the highest values were recorded in organic horizon of P5) than within arable and vineyard, while the number of fungal microflora has more closed values within the four land uses (excepting the first horizon of P2 and the second organic horizon of P5).

The lowest values of bacterial microflora and fungi on the soil profile were determined for P3 - vineyard compared with the rest of the analyzed soil profiles, as result of the low amount of annual organic residues within that agricultural use.

Evaluation of the degree of development of bacterial and fungal microflora in examined horizons of the soil profiles shows an average to high load of soil bacteria and fungi. The lowest values for bacterial and fungal microflora were recorded under vineyard, reflecting soil life response to anthropic interventions.

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HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY FOR CONVENIENT ANALYSES OF POLAR PESTICIDES

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Pesticides are used in plant protection products. Once applied appear the question of their dosing both in plants intended for consumption and the environment. Liquid chromatography may be successfully used for the analysis of nonvolatile and thermolabile pesticides. The most common type used is reversed phase (RP-HPLC). However, very polar and ionic pesticides shall not be retained in RP-HPLC. Therefore, a convenient solution is HILIC. Lipophilic Hydrophilic Chromatography is a type of liquid chromatography that used mobile phases for RP-HPLC on normal-phase stationary phases. This paper aims to highlight applications of HILIC in the analysis of a polar pesticide using liquid chromatography coupled with mass spectrometry.

Keywords: HILIC, liquid chromatography-mass spectrometry, pesticides residues.

INTRODUCTION

Modern analytical chemistry is dominated by separation techniques. For pesticides' analysis of have been posted two techniques for separating: gas chromatography and liquid chromatography. Gas chromatography, as the first separation technique with a long tradition, may be successfully applied to the analysis of volatile and thermostable pesticides (Niessen, 2001). It is worth noting that the first generation pesticides consist with those possibilities analysis by gas chromatography. Besides the main organochlorine pesticides (DDT, lindane) are persistent organic pollutants. A measure of the polarity of organic molecules is the octanol-water partition coefficient (K_{ow}) expressed as logaritm (Leo *et al.*, 1971). This parameter has been defined in particular for toxicological reasons to measure the extent of penetration of substances that pass the brain barrier. Then this parameter was correlated with year retention on reversed-phase liquid chromatography. Fosetyl is a very polar molecule, characterized by the log P = -2.7, that means that is highly soluble in water and practically insoluble in any organic solvent. It is used extensively as a fungicide for fruit and vegetables. The main advantage of fosetyl toxicity is low toxicity (Tomlin, 2003). As a general principle of retention in reversed phase liquid chromatography on C18 column, the retention time increases with increasing of log K_{ow} (or logP). This is true in general for logP > 1 But there are many highly polar organic substances (sugars, water soluble vitamins) or ionic (amino acids, nucleotides) that have logP <1 or negative. There is no retention on reverse-phase liquid chromatography for these substances. For this reason for the ionic substances it is preferable ion exchange chromatography. Fosetyl was successfully analysed by this method (Metrohm). However, ion-exchange chromatography is especially suitable when we use conductivity detection and most times it is necessary to suppress the mobile phase noise. To obtain retention of very polar substances and ionic in liquid chromatography we can use a very elegant mode with stationary phases for normal phase liquid chromatography with mobile phases for reversed phase. Such conventional silica column with mobile phase gradient from 97% acetonitrile, 3% water (100 mmol of ammonium formate pH 3 with formic acid) was successfully used to analyze free amino acids in aqueous solutions (Majors, 2014). The most often used for formulated plant protection products analysis is high performance liquid chromatography with ultraviolet-visible detection. Unfortunately, fosetyl has no UV

chromophore and is therefore difficult to analyze from formulated products due to interference of coformulants and other active substances that are present in products mix. As an alternative to UV-VIS detection mass spectrometry is used more extensively for polar and ionic analytes due to suitable interfaces such as electrospray (ESI). In this paper we aimed to develop a method for the analysis of fosetyl-based products by high performance liquid cromatografy coupled with mass spectrometry, and a method applicable to scale traces.

EXPERIMENTAL, RESULTS AND DISCUSSION

High performance liquid chromatography was carried out with a liquid chromatograph consisting of a ternary pump ProStar 240 SDM, an automatic injector Prostar 430 and a mass spectrometer 1200 L / MS / MS with electrospray interface, all from Varian. Ultrapure water was obtained from a DirectQ purifier (Millipore), acetonitrile and formic acid from Sigma, ammonium formate from Roth. Chromatography column was Polaris Amino (Varian) 150x4,6 mm (Lxi.d.) and 3 µm particles. The mobile phase consisted of water (100 mM ammonium formate pH = 3.7 with formic acid) 75% and 25% acetonitrile at a flow rate of 2 ml/min. Before entering the ESI interface was made a division of the flow of mobile phase 1/10, so to the reach only 0.2 ml / min of mobile phase. Drying gas was air at 21 psi and 400^0C, nebulizer gas was nitrogen at 42 psi, ESI needle was subjected to a voltage of -4500 V. The mass spectrometer was programmed in MRM (Multiple Reactions Monitoring) using Ar as collision gas. Fosetyl reference and sample Profiler 71.1 WG were from Bayer. Calibration of the mass spectrometer was performed with solutions between 5.3-28.5 µg/ml for macro analysis and 113-576 ng / ml for traces. Before all of that was performed parameters optimization by infusion of a solution containing ~ 1 µg/ml fosetyl. Breakdown curves from fig. 1 were used as basis of MRM merged program for chromatographic aquisition using three MRM (109 to 81 12 CE, 109 to 79 20 CE, 109 to 63 22CE).

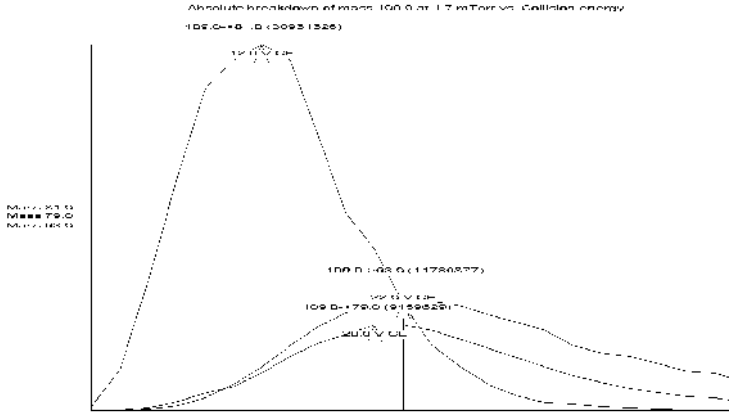

Figure 1. Breakdown curves for molecular ion [M-H]- of fosetyl m/z=109 to give main fragments m/z=81 (ethene loss), m/z=63 (ethylene oxide loss)

By replicated injections of calibrations solutions for *macro* analysis was obtained a linear calibration curve with a good parameters, comparable by one obtained in HPLC-UV detection presented in fig. 2.

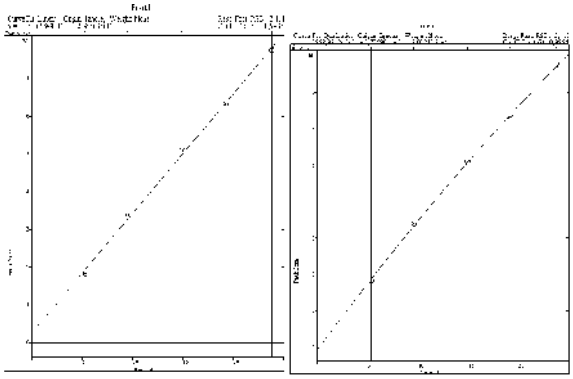

Figure. 2 Calibration curves with linear and quadratic fitting model for macro analysis

Relative standard deviation of response factors is good for a LC-MS external standardization method with a good correlation coefficient. Product *Profiler 71.1 WG* is a granular material consisting in a mixture of 66.67 % fosetyl, 4.44 % fluopicolid and some detergents for dispersing. By analyzing a *Profiler* sample, using instead MRM a full-scan MS program we can see that the potential interferences were removed by filtering information. Reconstructed ion chromatogram (RIC) for ion *m/z* = 109, corresponding to fosetyl, and for ions *m/z* = 381, 383, 385 corresponding to fluopicolid are presented in figure 3. Sample analysis is biased with 5 % because results were between 70-74%. A possible cause could be that calibration was *external.*

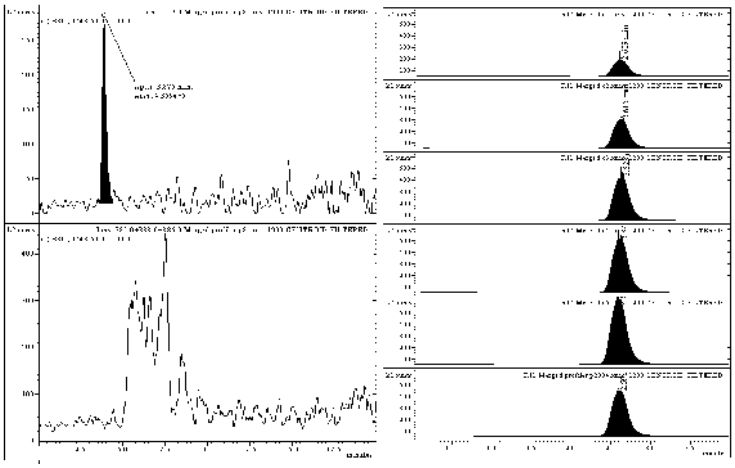

Figure 3. Extracted ion chromatogram for m/z=109 (fosetyl) and m/z=381,383,385 (fluopicolid) and stacked peaks from filtered MRM analyses

For traces calibration curve was not so easy to obtain because of highly noise baseline. However, the most diluted solution still gave a chromatographic peak with a signal/noise ratio S/N=52. Despite that detection limit was defined as the concentration that will generate S/N=3, it was very difficult to obtain such as peak. This time was obtained as most fitted model a quadratic curve, demonstrating the range of analyses was near the start of dynamic range. So we consider that as quantification limit 100 ng/ml because the chromatographic peak still remain with a good shape and exceed the noise (figure 4). Fragmentations pattern is almost the same for the first and for the last calibration level.

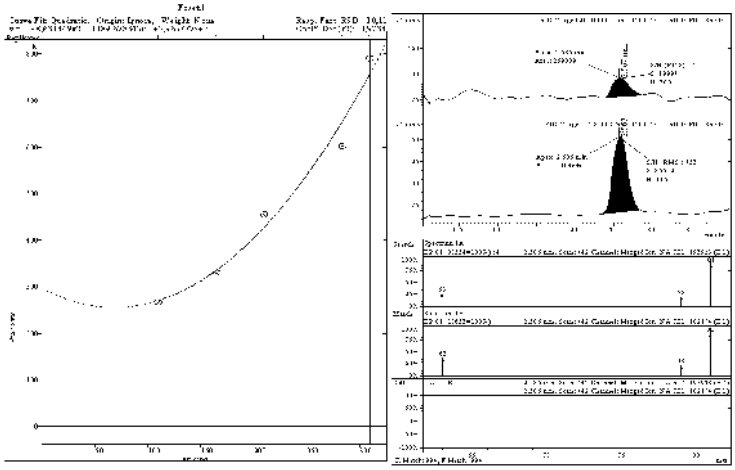

Figure 4. Trace analysis quadratic curve and fosetyl first and last calibration chromatographic peaks. Fragmentations patterns was maintained for all calibration levels

CONCLUSIONS

Using HILIC MS/MS for fosetyl anlysis from formulated product, were obtained comparable parameters with that from an HPLC-UV analysis. Main advantage was selectivity and the main disdvantage was the biased results. Probably using an appropriate internal standard and checking bias with another reference material the method will gain trueness. For residues from water or extracts from soils and plants was developed a trace analysis. Possible improvements could be using a narrow-bore column and concentration step before determination.

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NEW METHOD FOR BIODEGRADABILITY OF COLLAGEN AND KERATIN BASED MATERIAL ASSESSMENT

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Biodegradability of widely-used materials has become an important environmental property and a market tool for high quality articles intended for responsible consumers. Assessing biodegradability of natural furskins is a difficult endeavour due to the complex composition of natural fur skins. Originality of research consists in elaborating a method of assessing biodegradability of sheep furskins based on specific microorganisms for collagen and keratin degradation. In this regard, preparations with collagenase and keratolytic activity were developed and characterized by determining enzymatic activity before and after concentration and conditioning. Strains were identified using BIOLOG – Microbial Identification system. Experiments on biodegradation of natural furskins were performed in the WTW – OxiTop device and have enabled assessment of biodegradability by determining biochemical oxygen demand every 3 days for a 45-day period. Selected strains have shown ability to hydrolyze furskins at the end of their life cycle. The elaborated method enables biodegradability assessment of natural furskins in a shorter span of time compared to similar methods for plastics, which take minimum 6 months, and require much higher costs.

Keywords: biodegradability, furskin, collagen, keratin, enzymatic activity, specific enzymes.

INTRODUCTION

The research on biodegradability assessment of natural leather is of high interest as a scientific tool to eliminate the misunderstanding regarding the commercially "ecological" synthetic furskins and leathers. The recent discussions regarding the need for supporting through EU legislation the labeling of natural leather authenticity of leather goods, garment and upholstery (international Leather Maker, 2014) represent another tool for consumer education and protection. The development of a method for biodegradability assessment of natural leathers and furskins represents another instrument for leather goods labeling and consumer perception improving in connection to sustainable environmental protection as compared to synthetic materials with less durability and non biodegradability.

The definition (van der Zee, 2011) of polymers biodegradability and assessment was delimited from the other meanings as a process which best simulates the disposal pathway, keeps the accumulation under control, generates as end products CO_2, water, minerals, intermediate biomass which includes humic materials and the polymers biodegrade safety or the use of end products must be friendly.

Even though there are enough standards for polymer biodegradability testing many works need to be done to understand the mechanism of biodegradation. The lack of methods for the survey of intermediate materials generated by biodegradation is the most important blocked progress in biodegradability assessment.

The recent literature reports the biodegradability assessment of wet-white, vegetable or chromium tanned leathers (Qiang *et al.*, 2011). Composting for 150 days of leathers tanned with different organic phosphates, vegetable tannin and chromium salts showed a rate of degradation between 6-80% depending on crosslinking strength. The influence of leather manufacturing technology and consequently of the leather composition on biodegradability is stressed in different studies. The first tannery who claims to be the most ecological and certified for the first time a biodegradable product is Dutch Hulshof with Piuro product, a fully organic product (Hulshof).

The efforts to adapt the methods for plastics biodegradability assessment to leathers were already reported and consist in measurement of CO_2 released by biomass biodegradation (Bertazzo *et al.*, 2011; Calise, 2011; Lombardi, 2013). Even under these conditions the test is time-consuming and needs a step forward in microorganism selection and rapid assessment of biodegradability of leathers. The first step in biodegradability assessment is the evaluation of structural changes of materials under the composting conditions and understanding the mechanism of biodegradation initiation.

Furskins are complex materials composed of keratin and collagen, two proteins with different reactivity, and literature does not report any study regarding the biodegradability of furskins. Even the processing of furskins must comply with fashion trends and chemical materials are very complex (from syntans to finishing binders), the main components are collagen and keratin, biodegradable materials. In comparison to synthetic leathers and furskins, wrongly called “ecological”, natural furskins are more durable and less pollutant materials.

The paper presents some progresses in bacterial preparation for sheepskin biodegradability evaluation and a proposed method for biodegradability assessment.

MATERIAL AND METHODS

Selection of Bacterial Preparations Specific to Fur Substrates

To screen microorganisms specific to fur substrates two different sources were taken into account; the first was to isolate them from natural environments, and the second was the microorganism collection of the Microbial Biotechnology Centre (BIOTEHGEN).

In the experiment four bacteria strains were used, marked: BN7, Bl, 7.2 and Omf. Strains BN7, 7.2 and Omf are isolated naturally from the soil, and Bl is a strain from the collection (Bacillus licheniformis ATCC 14580).

To identify how these bacterial strains act on natural furs, they were grown in a minimal medium with pH=7.5 with the following composition: NaCl 1 gL^{-1}; $CaCl_2$ 0.05 gL^{-1}; KH_2PO_4 0.7 gL^{-1}; sucrose 3 gL^{-1}; $MgSO_4$ 0.9 gL^{-1}; K_2HPO_4 2.38 gL^{-1}, with fur samples as source of carbon and nitrogen. Bacteria strains were grown at 30-32°C under stirring (140 rpm).

Determining Collagenase and Keratinase Activity of Bacterial Preparations

Samples were analyzed in terms of enzymatic activity (EA) of collagenase and keratinase, as well as soluble protein content.

The method of determining collagenase activity is based on hydrolysis of collagen under the action of collagenase, as a result of which peptides are released. The degree of proteolysis is measured spectrophotometrically at 570 nm in the presence of ninhydrin.

Determination results were expressed in U/ml. One unit of collagenase is the amount of enzyme which releases peptides from collagen equivalent in color intensity determined spectrophotometrically using ninhydrin with 1.0 µmole of leucine in 5 hours at pH 7.4 and temperature of 37°C in the presence of calcium ions.

The method of determining keratinase activity is based on hydrolysis of the substrate (keratin azure) under the action of keratinase. The change in absorbance at 595 nm due to the intensifying coloration by substrate hydrolysis is determined spectrophotometrically. Determination results were expressed in U/ml. A keratinase unit is the amount of enzyme which causes an increase in absorbance of 0.01 at 595 nm after one hour reaction at 60°C while stirring at 150 rpm.

Identifying Bacteria Strains Using BIOLOG – Microbial Identification System

To identify bacterial strains isolated from natural environments, marked B7.2, BN7 and Omf using BIOLOG - Microbial Identification system the steps were the following: strains were inoculated on BUG agar medium and incubated for 24 hours at 33°C; the device was calibrated by densimeter calibration and verification of inoculum medium density; medium was inoculated with a bacterial colony of 84-89% density; GENIII plates were inoculated with 100ul of the inoculum; and incubated at 33°C for 20-22 hours and results were read using the Biolog spectrophotometer.

Concentration of Bacteria Preparations

In order to increase the stability of microbial enzyme preparation, the process of lyophilization was applied (freeze-drying under vacuum) to strains B7.2, BN7 and Bl. Lyophilization consisted in adding 10% trehalose as cryoprotectant agent, freezing the samples and then lyophilization (Labconco freeze dryer) at a pressure of 0.04 mbar, at temperatures between -40 and 45°C for 18 hours. Lyophilized powder reconstitution was performed using sterile distilled water.

Biodegradability Assessment of Natural Furs

Incubation is done in the dark or dim light in brown pots sterilized at constant temperature of 35°C ± 2°C under magnetic stirring in the WTW - OxiTop device on finished sheep furskin samples. At least two samples of fur are inserted into vessels in sterilized water with the bacterial preparation and two control samples without bacterial preparation. Any toxic contaminants or inhibitors for enzyme preparations are avoided and all working vessels and manipulation tools are carefully sterilized.

Biochemical oxygen demand (BOD) is automatically measured in the range 0-400 mg/L starting with time 0 and every 3 days for at least 45 days. The degree of biodegradability is calculated as follows:

$$B\% = (x\ mgO_2/L : 360\ mgO_2/L)x100 \quad (1)$$

where: x is the amount of O_2/L released after biodegradation of fur; 360 is the theoretical amount of O_2 required for complete decomposition of 0.6 g fur with 11% moisture in CO_2 (ThOD).

The test is considered valid if the control vials consume less than 20% of the oxygen demand consumed by the test sample.

RESULTS AND DISCUSSIONS

Collagenase and Keratinase Activity of Bacterial Preparations Specific to Natural Furskins

The test results on collagenase and keratinase activity of selected bacterial preparations are summarized in Table 1.

Table 1. Activity of the enzymatic complex synthesized by bacterial strains on natural furskins

Sample	Protein (mg/ml)	Collagenase EA (U/ml enzyme)	Keratinase EA (U/ml enzyme)
B7.2	1.59	14.56	2.76
BN7	1.45	18.07	2.54
Omf	1.58	13.13	1.08
Bl	1.42	23.7	4.44

In samples containing sheep furskin amounts of protein were determined between 1.42 - 1.59 mg/ml, indicating that the microorganisms synthesize their required amount of protein from carbon and nitrogen sources provided. The highest collagenase activity was recorded for Bl strain in samples containing sheep furskin (23.7 U/ml enzyme). Another strain with remarkable collagenase activity, of 18.07 U/ml enzyme, was BN7.

The concentration of the bacterial enzyme complexes has led to the results shown in Table 2.

Table 2. Activity of the enzymatic complex in concentrated enzymatic preparations

Initial sample	Protein (mg/ml)	Collagenase EA (U/ml enzyme)	Collagenase SEA (U/mg protein)	Keratinase EA (U/ml enzyme)	Keratinase SEA (U/mg protein)
BN 7	1.28	14.44	11.27	2.45	1.92
BI	0.89	16.33	18.21	5.09	5.67
B7.2	0.57	3.7	6.49	0.39	1.46
Lyophilized sample	Protein (mg/ml)	Collagenase EA (U/ml enzyme)	Collagenase SEA (U/mg protein)	Keratinase EA (U/ml enzyme)	Keratinase SEA (U/mg protein)
BN7	0.97	20.49	21.06	2.96	3.04
BI	0.76	17.81	23.53	4.69	6.19
B7.2	0.52	3.28	6.3	0.37	1.72

In Table 2, Bl strain stands out, with the highest initial specific collagenase activity (18.21 U/mg protein), which increased 1.3 times by lyophilization as a result of concentration processes it has been subjected to.

Good specific collagenase activity was also determined for initial BN7 strain (11.27 U/mg protein), which increased 1.87 times by lyophilization.

Identifying Bacterial Strains Isolated from Natural Environments

BIOLOG – Microbial Identification system is an advanced system for the identification and characterization of microorganisms, which allows rapid identification

of over 1,900 species of aerobic and anaerobic bacteria, pathogenic bacteria, filamentous fungi (500 species) and yeasts (250 species), as well as analysis of microbial communities. GENIII BIOLOG system is based on the analysis of metabolic pattern resulting from the breakdown of the major classes of biochemicals by microbial cells (71 carbon sources) and determination of important physiological properties such as pH, tolerance to salinity, acidity, and testing sensitivity to various chemicals.

Three bacterial strains isolated from natural environments, marked B7.2, BN7 and Omf were identified using BIOLOG – Microbial Identification system. To identify strains, the following are taken into account: index of similarity value (SIM), which is a comparison between the results of the biochemical characteristics of the tested bacterial strain and biochemical test results obtained with strain collection (ATCC) used to obtain BIOLOG databases and the distance (DIST), which is a comparison between the first two results obtained. For a conclusive identification it is necessary that SIM>0.5, and DIST value should be at least 2 (Table 3). Figure 1 shows how to identify strains isolated from natural sources on GENIII plates.

Table 3. Identification of bacterial strains using the BIOLOG system

Samples	Strain	Similarity	Distance
B7.2	Bacillus amyloliquefaciens	0.603	5.814
BN7	Bacillus amyloliquefaciens	0.589	6.006
Omf	Bacillus amyloliquefaciens	0.575	6.230

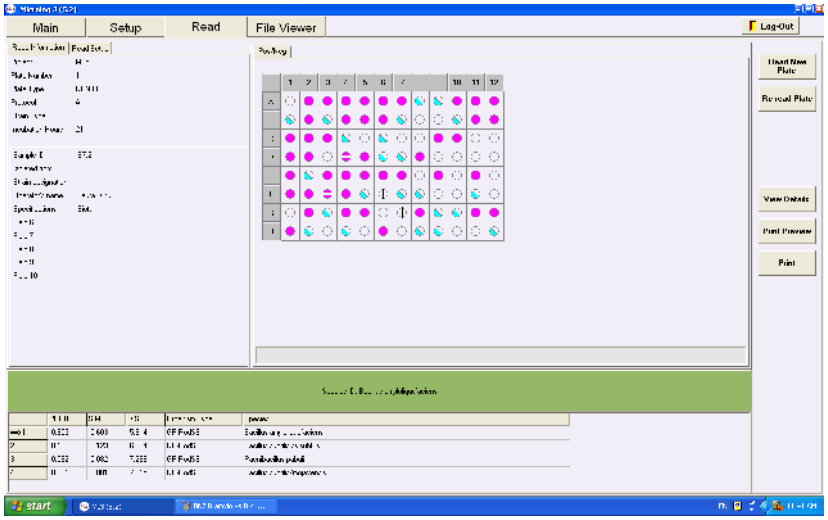

Figure 1. Identification of B7.2 strain on GENIII plate

Biodegradation of Natural Furskins

The results of the accelerated biodegradation of sheep furskin samples are presented in Figure 2. Figure 2 clearly shows a significantly higher oxygen consumption in the case of inoculum based on BI with the maximum value of 363 mg/L (100% biodegradable), while other types of inoculi generate consumption between 75-220 mg/L, the control shows a BOD value of 2.8-16.9 mg/l.

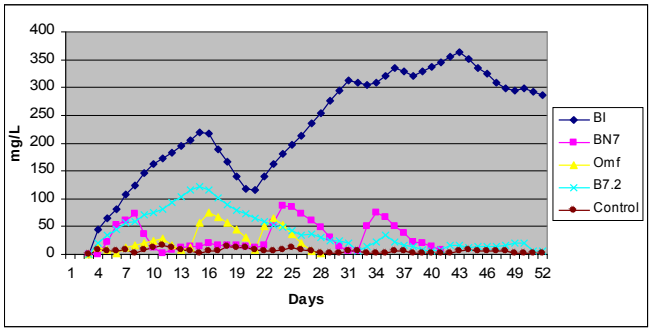

Figure 2. BOD of the sheepskins incubated with BI, BN7, Omf, B7.2 as compared to the control sample

CONCLUSIONS

Enzymatic preparations based on Bacillus licheniformis ATCC 14580, grown in specific environments for furskins and concentrated by lyophilization allow accelerated biodegradation of natural fur and are a useful material for the development of methods to assess biodegradability of natural fur. The research paves the way for the development of enzymatic preparations allowing faster evaluation of biodegradability of leather or fur in order to increase confidence in the environmental value of natural products.

Acknowledgement

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MODELING THE MOVEMENT OF NITRATES THROUGH THE SANDY SOIL CONSIDERING HOMOGENOUS SOIL PROFILE

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Nitrate is one of the most common chemical contaminant found in groundwater because it is a moderate solute in soils and could move quickly through the soil profile leading to plant nutrient loss and groundwater pollution. The intensive application of nitrogen fertilizers in agriculture can cause nitrate contamination of ground water above the 50 mg NO3-/L (WHO guideline value for drinking water).This paper reports an application of a model in order to investigate the migration process of nitrates through the sandy soil under different pore water velocities. The tests were carried out using two dimensional numerical models SEEP/W and CTRAN/W with homogeneous soil. SEEP/W computes the water flow velocity, volumetric water content, and water flux and CTRAN/W uses these parameters to compute the contaminant migration. These models are useful tools in predicting the effects of measures and can be used to optimize agricultural practice aiming to minimize the impact on the environment. For the sandy soil, the amount of nitrate adsorbed into the soil is higher than any other soil types such as loam or clay. Nitrate sorption in the sand is influenced by environmental Conditions such as temperature, humidity, type of natural soil and common irrigation practices. The results also show that water pressure and nitrate concentration was highly affected by soil type and water application boundary conditions. All of these variables are contributing to the migration process of nitrate in soil.

Keywords: nitrate concentration, Advection-dispersion Process, Two-dimensional Model, Homogeneous soils.

INTRODUCTION

The leakage of agriculture wastewater through vadose zone to the groundwater is considered a massive problem to the public health because of possible contaminants of drinking water in case of groundwater is the source of drinking. Agricultural wastewater is mainly consists of Nutrients (nitrogen and phosphorus) that is typically applied to farmland as commercial fertilizer (inorganic nitrogen fertilizers) and animal manure. Nitrates are mainly produced for use as fertilizers in agriculture because of their high solubility and biodegradability (Wang and Wang, 2008).

The infiltration of N- containing pollutants from surface water and the transport of nitrate contaminants through soil and groundwater occur via a series of complex chemical and hydraulic phenomena (Wang and Wang, 2008). Therefore, in many agricultural areas the values of nitrate in groundwater are higher (Bonton et al., 2012) than the 50 mg NO3-/L guideline value for drinking water of World Health Organization (WHO, 2011).

The groundwater pollution by nitrate is an international problem (Roberts and Marsh, 1987; Meybech *et al.*, 1989; Spalding and Exner, 1993; Zhang *et al.*, 1996; Lerner, 1999; Wakida and Lerner, 2002). Nitrate (NO3-) is a leaching pollutant from fertilizer application in soil and groundwater (Chotpantarat *et al.*, 2011). The pollution of groundwater by nitrate (NO3-) has been a frequent matter in aquifers in the world (UNEPE, 1991).

The transport process of nitrate contaminants through soil and groundwater occurs via a series of complex chemical and hydraulic phenomena (Wang and Wang, 2008). The high doses of nitrogenous/phosphorous fertilizer applied to the soil immediately

followed by massive irrigation water causes some nitrogen losses and risk of nitrates penetration to subsoil, Thus once ecosystems are contaminated by these elements, they become potential threat for many years (Shivasharanappa *et al.*, 2013; Novakova and Nagel, 2009). The choice of using two software, CTRAN/W is used in conjunction with SEEP/W, makes it possible to analyze problems varying from simple particle tracking in response to the movement of water, to complex processes involving diffusion, dispersion, adsorption, radioactive decay and density dependencies.

METHODS - NUMERICAL SOLUTION OF THE PROBLEM

There are many numerical solution methods such as Finite Differences (FDM), Finite Elements (FEM) and Boundary Elements (BEM). The FEM is an effective numerical technique because of its numerous applied fields such as groundwater flow, multiphase flow, and mass flow through pours medium. It is flexible in simulation.

General Description of SEEP/W and CTRAN/W Models

SEEP/W is a finite element software product for analyzing groundwater seepage and excess pore-water pressure dissipation problems within porous materials such as soil and rock. SEEP/W can model, in addition to traditional steady-state saturated flow, both saturated and unsaturated flow, that makes it possible to analyze seepage as a function of time and to consider such processes as the infiltration of precipitation.

CTRAN/W is a finite element software product that can be used to model the movement of contaminants through porous materials such as soil and rock. CTRAN/W utilizes the SEEP/W flow velocities to compute the movement of dissolved constituents in the pore-water. The following is the one-dimensional form of the advection-dispersion equation (Geo-Slope User's Guide). Where C is the concentration, θ is the volumetric water content, D is the hydrodynamic dispersion coefficient, U is the Darcy velocity, S is the adsorption, ρd is the bulk mass density of the porous medium, t is the time, and x is the distance in the x direction. The first term in the equation represents transport by dispersion, the second represents transport by advection, the third represents decayed mass loss in the fluid phase, and the fourth represents decayed mass loss in the solid phase. The term on the right side of the equation represents storage of mass in the fluid phase and in the solid phase due to a change in concentration.

The aim of this study is to model the movement of nitrates as a fertilizer applied in agricultural lands towards drain side and the risk which may be occurred when this contaminant reach the drain's water in sand soil. The flow is considered to be steady-state, two dimensional in a homogeneous isotropic porous media.

MODEL DESCRIPTION

The self-diffusion coefficient for representative anion (NO3-) at infinite dilution in water at 250C is D0=19*10-10 (m2/s). The model is two dimension, the hydraulic conductivities Kx/Ky =1, the porosity (n) = 50%, the longitudinal dispersivity / transverse dispersivity =2 and the time step sequence consists of ten steps. Time starts in zero days and ends in 100 days.

Table 1. Parameters of hydraulic functions for sand used in the simulation

Parameter	Value
Saturated water content (θ_s)	(0.65 m^3/m^3)
Residual water content (θ_r)	($1.01*10^{-4}$m/s)
Saturated hydraulic conductivity (K_s)	(0.30 m^3/m^3)

Table 2. The adsorption capacity for sand at 15°C, 25°C, and 35°C

C_0 (mg/L)	15°C *Qeq* (mg/g)	25°C *Qeq* (mg/g)	35°C *Qeq* (mg/g)
25	0.038	0.040	0.027
50	0.091	0.087	0.060
100	0.199	0.186	0.131
200	0.425	0.412	0.300
500	1.249	1.132	0.851

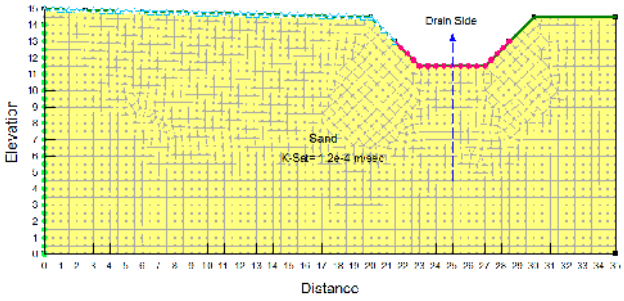


Figure 1. The domain mesh showing the boundary conditions for SEEP/W analysis

MODEL RESULTS AND DISCUSSION

In order to model the contaminant migration in unsaturated soil, SEEP/W was firstly run. After set the geometry (35 m in length x 15 m in depth soil) and the grid (mesh 0.5x 0.5 m) as shown in figure 1.

The SEEP/W contour function allows one to graphically view the results by displaying velocity vectors that represent the flow direction. A vector is drawn in each element. The vector represents the average velocity within the element. The seepage flow velocities computed from SEEP/W are then used by CTRAN/W for the contaminant transport analysis.

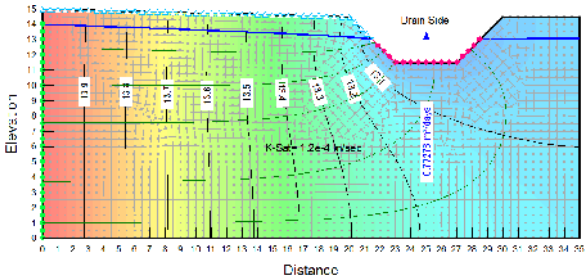
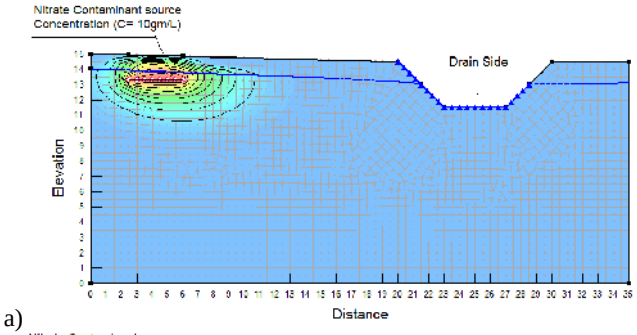
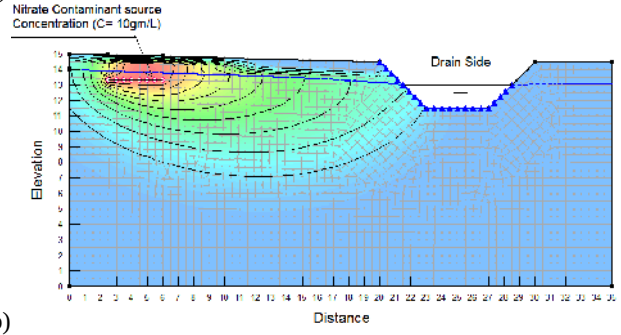


Figure 2. Total head distribution and flux section at the drain side

In the advection-dispersion analysis, adsorption of contaminant on the soil particles is linearly related to concentration. This is the concept of chemical partitioning between the fluid and solid phases. This means that the chemical partitioning coefficient, (which is the slope of the adsorption/concentration function), can be specified as a function of concentration. Figures 3(a-d) are graphical representations of advection-dispersion of nitrate; starting concentration (10 gm/l) = (10000 gm/m^3).



a)



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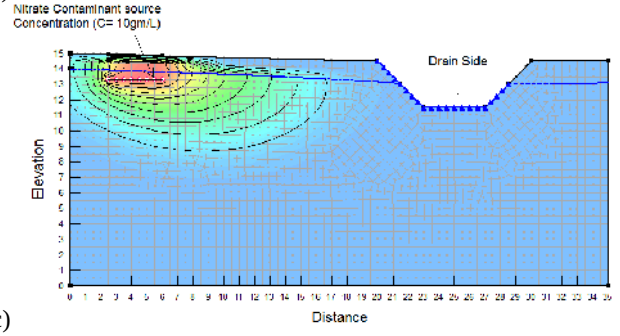

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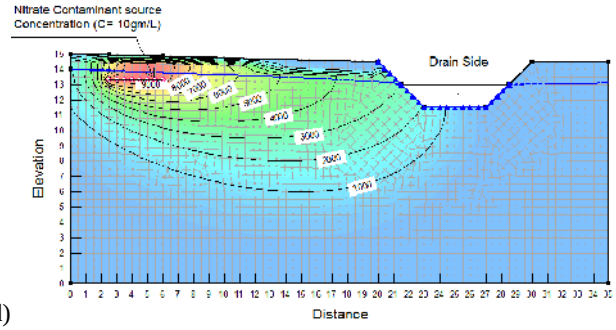

d)

Figure 3. Advection-dispersion analysis after: a) 10 days, b) 30 days, c) 60 days, d) 100 days

In case of using sheet pile, the water flux at the drain side equals (0.62258 m3/days) this value is less than the flux without using the sheet pile, this reflects that the water flow velocity has decreased.

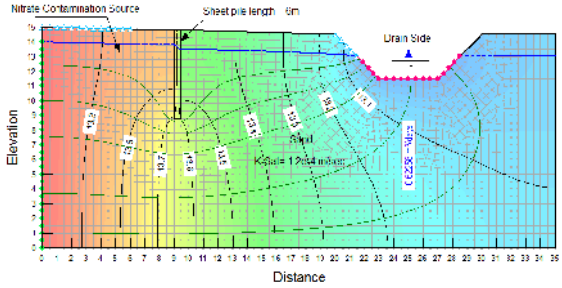

Figure 4. Total head distribution and flux at the drain side in case of using sheet pile

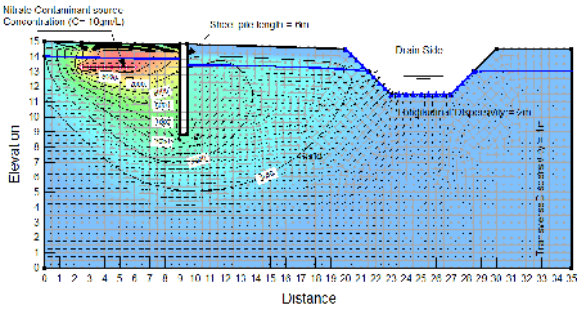

Figure 5. Advection-dispersion analysis after 100 days using sheet pile

CONCLUSION

The following conclusions are made, based on the studies in the literature and on the discussion presented in this study:

The movement velocity of contaminants in course soils (gravel and sand) is greater than its movement in fine soil (silt and clay). In case of increasing time, the existence of the contaminants is in a far distance in the soil media.

The distance of contaminant transport significantly depends on the hydraulic conductivity of the soil and the diffusion coefficient (D_0) of contaminant Anion or Cation.

The average particles tracking velocity in the soil significantly depends on the water velocity and their total distance traveled depends on the time.

The total flux at drain side is decreased in case of using the sheet pile rather than the useless of the sheet pile because the velocities magnitudes have decreased.

The head of water above the contaminant source affects significantly on the contaminant transport process rather than the difference in water level between the agricultural land and the drain side.

Acknowledgment

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POLLUTANTS MINIMISATION AND INNOVATIVE MONITORING TECHNIQUES TOWARD A SUSTAINABLE LEATHER INDUSTRY

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Sustainable development is a deep-seated value of the EU and encompasses issues of great importance to citizens, whether it be maintaining and increasing long-term prosperity, addressing climate change or working towards a safe, healthy and socially inclusive society. It is an overarching objective of the EU set out in the Treaty, governing all the Union's policies and activities. It aims at the continuous improvement of the quality of life and well-being on Earth for present and future generations. It promotes a dynamic economy with full employment and a high level of education, health protection, social and territorial cohesion and environmental protection, in a secure world, respecting cultural diversity as set out by Brundtland in 1987. Leather production fulfils a fundamental role in our society. It recovers the hides and skins resulting from the production of meat (for human consumption) and transforms them into a noble material that finds applications in a myriad of consumer goods. It thus prevents a difficult waste disposal problem and contributes with a useful and appealing material to our modern lifestyle, generating wealth and employment. Leather industry is, however, an environmentally intensive activity that can carry adverse effects to water, air and soil if the plant does not apply pollution prevention techniques. The new innovative technological & monitoring system for pollutants minimization, presented in the paper, will catalytically act for: improving the quality of the working environment, reducing/eliminating the pollutants, facilitating the implementation of the EMS and eco-labeling in the Romanian leather sector, contributing directly to its competitiveness and sustainable development.

Keywords: tanning & footwear industry, monitoring system, emissions, sustainable production

INTRODUCTION

Leather processing is one of the mankind's oldest occupations in the world. The tanning industry provides a high added value material to a number of value chains, notably in the fashion, furniture and automotive sectors.

The raw materials of the European tanning industry are hides and skins of which over 99% are derived from animals that have been raised primarily for milk, meat or wool production. This is revealing one more the important ecological role of tanneries: they recover a by-product (a food industry's waste which in the absence of the leather industry would have to be disposed of) transforming into a wonderful and spectacular material that all of us find in everyday goods which make our lives better and beautiful.

In Romania, the leather and footwear industry has a long tradition and many historical records.

In 2013, the leather sector (Tanneries and footwear companies) accounts for the following shares of Romania's macroeconomic indicators.

Table 1. Indicators of the Romanian leather & footwear industry

Indicators	Leather & Footwear industry
% of Romanian GDP	0,85
% of Romanian industrial production	1,34
% of Romanian exports	2,85
% of Romanian imports	2,14
% of Romania's industrial employment;	3,86
Number of companies	about 1900

Source: Ministry of Economy, Romania

While being a final product for tanneries, leather represents a "raw" material for other industries such as: footwear (about 62%), clothes (about 24%), leather goods (about 12%), upholstery and automotive leathers (about 2%) (Figure 1).

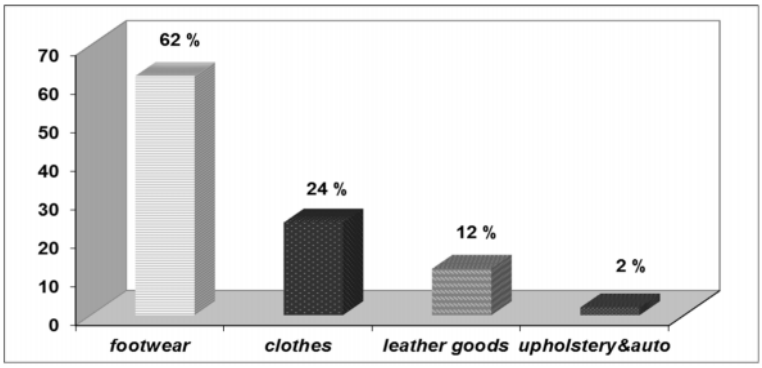

Figure 1. Leather destinations

In our days, the sustainability of production processes, both in tanning and footwear sectors, became a key factor in companies' competitiveness and general economic growth. So, for each company it's increasingly important to obtain certifications, according to the specific standards, for their performance in quality, environment and ocupational health & safety. In recent years a public awareness is also represented by the energy efficiency improving and CO_2 emissions reducing (COTANCE, 2012).

Romanian tanning and footwear companies have the same goals and concerns regarding their production sustainability.

EXPERIMENTAL

Having in view all these considerations, the general objective of the research is the development of an innovative minimization concept and of an integrated, computer aided-monitoring system for pollutants to aid a sustainable production in the leather industry. The major scientific and technical objectives were: (i) identification of the significant environmental aspects for each activity by monitoring emitted pollutants; (ii) identification of new ecological techniques for minimizing pollutants in leather manufacturing process and implementation of an innovative monitoring system; (iii)

optimization of pollutant control/analysis methods by designing and implementation of an innovative monitoring system.

In order to prevent, reduce and as far as possible eliminate pollution arising from industrial activities in compliance with the 'polluter pays' principle and the principle of pollution prevention, it is necessary to establish a general framework for the control of the main industrial activities, giving priority to intervention at source, ensuring prudent management of natural resources and taking into account, when necessary, the economic situation and specific local characteristics of the place in which the industrial activity is taking place.

Hide processing results in environmental pollutants which are toxic to both human health and the environment and need to be carefully and closely monitored.

The leather making process is the transfer of a 100 % renewable ressource to a highly valuable and toxicological safe substrate:

- ✓ In fact, leather manufacturing is part of a big recycling industry and solves a huge waste problem of the meat industry;
- ✓ Leather can be made without sustainable damage of the environment if best available technologies are followed. (2010/75/EU Directive IPPC, 2010)

The tannery operation consists of converting the raw hide or skin, a highly putrescible material, into leather, a stable material, which can be used in the manufacture of a wide range of products. A significant amount and variety of chemicals and specific products are used in the processes. The whole process involves a sequence of complex chemical reactions and mechanical processes. Amongst these, tanning is the fundamental stage, which gives leather its stability and essential character.

The tanning industry is a potentially pollution-intensive industry. The environmental effects that have to be taken into account comprise not merely the load and concentration of the classic pollutants, but also the use of certain chemicals: e.g., biocides, surfactants and organic solvents.

Environmental issues associated with tanning sector include the following: waste-water, air emissions, solid wastes and hazardous materials.

Due to the wide versatility of tanneries, both in terms of the types of hides and skins used and the range of products manufactured, the reported emission and consumption levels are generally indicative. The environmental impacts of tanneries originate from liquid, solid and gaseous waste streams and from the consumption of raw materials such as raw hides, energy, chemicals and water. The main releases to waste water originate from wet processing in the beamhouse, the tanyard, and the post-tanning operations. The main releases to air are due to the dry-finishing processes (Albu, 2005).

For footwear and leather goods companies the most important ecological issues are: air emissions, solid wastes and hazardous materials.

In order to establish the initial environmental impact of the most important environmental factors from a tannery (SC PIELOREX SA, Jilava, Ilfov), a footwear & leather goods company (SC MUSETTE SRL) have been monitored. The levels of emission analytically determined are presented in the following graphs and tables.

In tannery the hydrogen sulphide and ammonia levels were monitored for 18 hours into the beamhouse & tanyard workshop (Figure 2) and in wet finishing (post-tanning) area the ammonia emissions were registered for 24 hours (Figure 3).

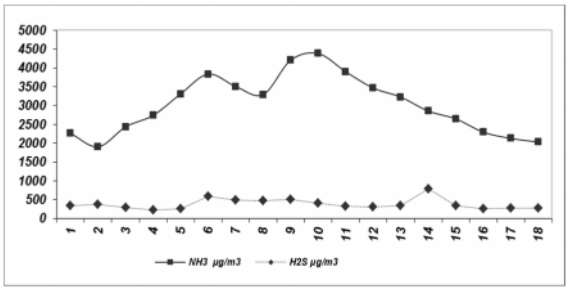

Figure 2. Emissions in beamhouse & tanyard workshop

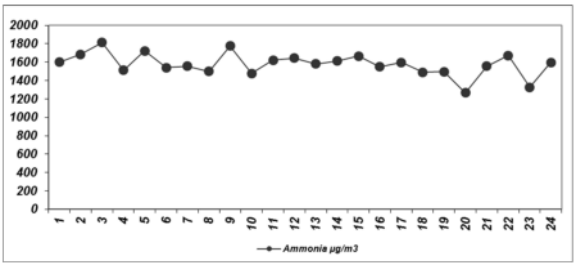

Figure 3. Emissions in wet finishing workshop

For finishing operations buffing dust and volatile organic compounds (VOCs) were measured (Table 2).

Table 2. Emissions in finishing workshop

Operation	Measurement point	Dust	VOCs as total organic Carbon
Buffing	Buffing equipment	2083 µg/mc; 845 µg/mc	-
	Exhaust cart from buffing	42.6mg/Nmc	-
Surface finishing	Entrance on finishing spraying line		7.1 mgC/Nmc
	Exit from finishing spraying line		6.9 mgC/Nmc
	Environment area of finishing spraying line		2.5 mgC/Nmc

The tannery waste waters and the characteristics for each operation and for final treated effluent were registered too.

For footwear and leather goods company the emissions measured – dust and VOCs - are presented in Table 3.

Table 3. Emissions in footwear & leather goods company

Measurement point	Dust	VOCs as total organic Carbon
Stitching area	-	450 mgC/Nmc
Lasting & Finishing	0.32 mg/mc	420 mgC/Nmc
Spraying cabin for adhesives	-	250 mgC/Nmc
Bonding area	-	450 mgC/Nmc

RESULTS AND DISCUSSION

The quantity of air emissions depend on the substance used in each industrial process (EPA, 1993). The purpose of the developed process is to minimize the quantity of emissions driven into the atmosphere in order to respect both environmental restrictions and occupational health and safety.

Having in view the emissions identified and measured in tanning and footwear & leather goods companies an intelligent system have been designed, patented and the prototype implemented.

Other monitoring systems (Filip, *et al.*, 2007; Moldovan, *et al.*, 2009; Clotan-Turcu, 2003) were studied and a series of disadvantages were identified: (i) the coupling of sensors to a single microcontroller, if it fails or needs to be replaced, then the whole system stops working; (ii) high difficulty in introducing a new sensor in the system this being caused by the fact that a single microcontroller can be interfaced with a limited number of sensors.

The problem solved by the new intelligent system is monitoring levels of toxic emissions resulting from industrial processing and alarming the workers when the pollutants exceed limits.

The proposed monitoring system (Figure 4) is made using sensitive specific sensors for the desired monitored pollutants. Each sensor (S) is connected to a data acquisition board (PA), which has included a microcontroller (uC). The microcontroller collects data from the sensors and, by using the industrial Ethernet communication network (CE), transmits it to the process computer (CP) which runs a software application (AS) in order to saving to the database (BD) and visualizing the acquired data. The microcontroller is connected to one acoustic alarm system (A). The alarm system is activated automatically when the toxic emission exceeds the maximum allowed values.

The new intelligent monitoring system has the following advantages:

- The system can operate with an unlimited number of sensors;
- Specific sensors can be added for other pollutants;
- Any time you can remove/add sensors as they operate independently of each other;
- The software application which runs on the process computer is modularized so that changes which should be made when a sensor is removed/added to system are minimal;
- Sensors and related acquisition boards are directly connected to AC power, ensuring continuous operation and continuous monitoring;
- Connection between modules (acquisition boards, computer process switches) is made by using UTP cable (not wireless). So, the effect of disruptive industrial environment on communications is minimized and the communication security is improved (no user from outside the company can intercept/decrypt data packets).

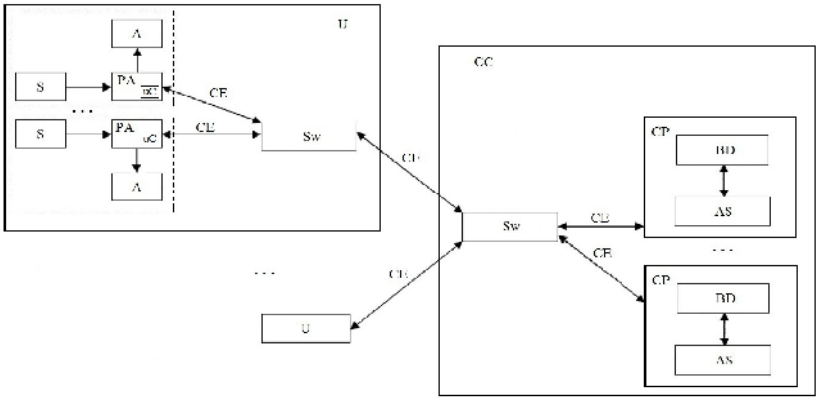

Figure 4. The basic structure of the intelligent monitoring system

Research work will continue in order to implement such intelligent monitoring systems in all companies participating in the project and to develop the systems by adding new sensors according to the specific activities and emissions.

In the same time good practice and eco-friendly technological solutions will be identified and implemented in the production processes (mainly in tanning sector), in order to minimize the ecological impact.

CONCLUSIONS

The monitoring system is intended to be a real-time one, in order to signal out any exceeding of the imposed limits.

The new innovative complex (technological & monitoring) system resulted will catalytically act for improving the quality of the working environment, reducing/ eliminating the pollutants, facilitating the implementation of the environmental management systems (EMS) and eco-labeling in the Romanian leather sector, contributing directly to its competitiveness and sustainable development.

Acknowledgements

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EXTRUDED AND SINTERED CLAY CERAMICS CONTAINING STEEL-MAKING DUST

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In the present work, the feasibility of recycling a steel-making by-product into extruded clay-based ceramics is examined, with the emphasis put on their mechanical performance. Actually, the utilization of massive amounts of solid residues recovered in steel production plants worldwide, such as steel-making dust (electric arc furnace dust, solid waste from gas treatment), is of increasing importance. This fine powdery residue, however, contains several oxides (mainly iron and zinc oxide phases), and therefore it could be considered as secondary material for substituting traditional clayey materials in ceramics manufacturing. For the fabrication of extruded specimens, a laboratory pilot-plant simulation of the industrial processes was employed. Clays appropriate for standard brick manufacturing were selected as the base materials and characterized. Then, various clay/steel dust mixtures were prepared and mixed with water to form a plastic mass for extrusion of specimens. The extrusion procedure and drying behavior of specimens were optimized in order to obtain integral specimens possessing sufficient green density and strength for the subsequent sintering at 850, 950 and 1050^{o}C, in a controlled furnace. The effect of the % by-product content, and also of the firing temperature, on shrinkage, bulk density, water absorption capability and mechanical strength of the fired specimens was investigated. According to the results, the addition of steel-making dust up to 15 wt. % in clay-based bricks is tolerable without significant variations in the mechanical performance, while the open porosity slightly increases, which could be of importance in terms of heat insulating behavior.

Keywords: Ceramics, extrusion, sintering, steel-making dust.

INTRODUCTION

The management and valorization of massive quantities of solid residues recovered in steel-making plants worldwide, such as electric arc furnace dust, electric arc furnace slag and ladle furnace slag, represents a significant issue.

In steel industry, the production of 1 ton of steel results to generation of 2-4 tons of various types of waste by-products (Das *et al.*, 2007), while 1 ton of stainless steel waste is produced per 3 tons of stainless steel making (Huaiwei and Xin, 2011). Therefore, taking into account the huge quantities of steel wastes, their utilization is environmentally and financially beneficial, the proper disposal and handling remaining both dangerous and expensive task. Blast furnace slag and steel slag are competitive raw materials in the mineral industry, and blast furnace slag utilization in the cement industry currently increases resulting in the reduction of the production cost. Furthermore, electric arc furnace slag is widely used in the road and pavement construction and is also recently studied for the development of vitreous ceramic tiles (Das *et al.*, 2007; Khan *et al.*, 2002; Sarkar *et al.*, 2010).

Particularly, the recycling of steel dust (electric arc furnace dust - EAFD) is very important, because it is one of the major steel by-products, included in the European Waste Catalogue (Commission decision 2000) with code no. 10 02 07* (solid wastes from gas treatment containing dangerous substances), and produced worldwide in large quantities (Krztoń 2010; Martins *et al.*, 2008; Tang *et al.*, 2008; Sofilić *et al.*, 2004). EAFD is generated from the volatilization of heavy metals when steel scrap is melted in the electric arc furnace. Volatilized metals are oxidized and subsequently solidified and detained in the form of fine powder in specially designed filters, which are placed in the EAF gas stream cleaning system (Salihoglu and Pinarli, 2008, Guézennec *et al.*, 2005, Kashiwaya *et al.*, 2004, Gritzan and Neuschütz, 2001). The use of EAF technology in the

steelmaking industry has been increasing considerably over the last decades resulting in the production of significant quantities of solid residues. The world generated steel dust per year has been estimated to be around 3.7 million tons (Néstor and Borja, 2003).

Since EAF dust mainly contains zinc and other metals, it is recycled in USA for recovering zinc and lead from the industrial waste stream, utilizing the Waelz Kiln technology. Main product is Waelz oxide, which is transformed into zinc oxide, zinc sulfate or zinc metal by zinc smelters. Waelz iron product, an iron concentrate, is also produced (Steel Dust Recycling 2014). Moreover, this by-product is examined as an additive in asphalt cement mixtures for road construction (Alsheyab and Khedaywi, 2013). Nevertheless, EAFD contains several valuable oxides, and thereby it could be considered as secondary raw material for substituting traditional clayey materials in bricks manufacturing. On the other hand, huge quantities of clays are annually needed for the production of considerable amounts of fired ceramic bricks worldwide, and therefore, much research focuses on the utilization of alternative raw materials from various origins in clay mixtures at different combinations and proportions for the fabrication of conventional sintered bricks (Karayannis *et al.*, 2013). The recycling of steel-industry byproducts as raw materials in bricks production would contribute to the conservation of natural resources, from the environmental point of view. Moreover, the low cost of these solid wastes and even possible energy savings during clay/waste mixtures firing in bricks manufacturing should also be considered, particularly taking into account recent targets of the EU's energy policy (Vogl, 2013; Antoniadis *et al.*, 2014). So far, limited studies are reported on the utilization of steel-making dust in the fabrication of construction materials including ceramics, vitreous and glass-ceramic products (Lis and Nowacki, 2012; Machado *et al.*, 2011).

In the present work, the feasibility of recycling steel-industry waste byproducts into extruded clay-based bricks is studied, this being an undertaking of technological, environmental and economic interest. Specifically, EAFD was incorporated in clayey raw material mixture, and the effect of the % dust content as well as of the firing temperature on the physico-mechanical properties of the extruded and sintered brick specimens is examined.

EXPERIMENTAL

Raw Materials

Three clay samples from different deposits in Greece, considered representative of the main types of clayey raw materials utilized by the ceramic industry (A, B and C), were selected and characterized (XRF-analysis). The CaO content of the clay samples used ranges from 3.51 to 11.82 wt.%, but they contain no sulphur. The main mineralogical phases identified were albite, enstantite and illite.

Main constituents of the EAFD (red/brownish fine powder), which was used as a secondary raw material in the current research, are iron and zinc, this being in accordance with several other studies on the characterization of steel-industry dust. Certainly, each particular dust is site-specific. However, the zinc in the dust typically exists as zinc oxide (ZnO) and as a mixed zinc-manganese ferrite spinel or ZMFO ($(Zn_xMn_yFe_{1-x-y})Fe_2O_4$) (Pickles, 2010).

Specimen Preparation and Testing

80x43.5x18 mm specimens of were prepared employing a pilot-plant simulation of the industrial brick manufacturing processes (Spiliotis *et al.*, 2013): the clay samples were pulverized and mixed in proportions appropriate for standard brick fabrication.

Various clay/EAFD mixtures with 0-15 wt.% dust content were prepared and mixed with water to form a plastic mass for extrusion. The plasticity of the mass was evaluated using a thermo-balance. After gradually drying, a) in air for 24 h and b) subsequently in an oven at 105°C for 48 h, the specimens were fired in a chamber furnace for sintering and consolidation. The first heating step of 500°C, was reached after controlled heating at 1.7°C/min for 5 h, followed by further heating at 4.5°C/min up to a peak temperature. The specimens remained at the max. temperature only for 15 min to attain energy savings, and then they were cooled to room temperature in the furnace.

After optimizing the % EAFD content in the mixture by firing at 1050°C, similar heating procedure was followed by lowering the maximum firing temperature to 950°C and also down to 850°C, to assess the possibility for attaining energy savings.

Shrinkage, bulk density, open porosity, water absorption (%), mechanical strength and thermal conductivity were determined on sintered specimens and studied in relation to the admixture percentage as well as to the firing temperature. In order to determine the water absorptivity, the sintered specimens were weighed before and after immersion in water for 2h. Mechanical behavior was assessed by 3-point bend testing. Tests were performed on 20 specimens of each composition and firing temperature, and the average values were reported in the results. Then the modulus of rupture (MOR) was calculated.

RESULTS AND DISCUSSION

Brick Specimen Preparation

Plasticity differences were observed when steel dust was added in various amounts in the clays, but they did not caused significant problems in the specimen preparation, which had the strength required to ensure safe handling in the subsequent fabrication steps. Hence, no additive was demanded to facilitate plastic extrusion of mixtures containing EAFD. Therefore the extrusion behavior of the green (unfired) specimens can be considered satisfactory. Drying behavior of the green specimens was quite satisfactory, and only limited shrinkage was observed during this first step of thermal treatment (drying in air). In all cases, the shrinkage appeared relatively restricted and remained within tolerable limits for standard brick manufacture. Upon firing up to 1050°C, specimen coloring turns gradually from lighter to dark brown when the EAFD percentage in the raw material mixture is increased, due to its noticeable % iron content.

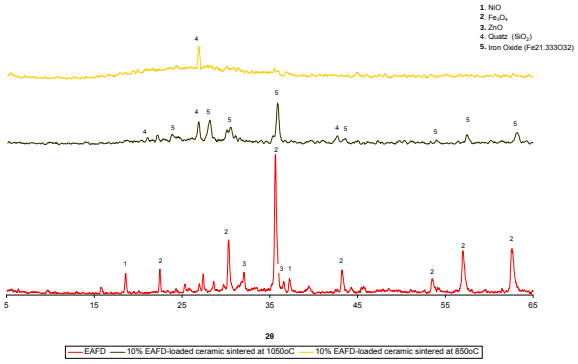

Figure 1. Typical XRD spectra of EAFD and 10 wt.% EAFD-loaded clay ceramics sintered at 850°C or 1050°C

Open Porosity - Water Absorption (%)

The influence of the % EAFD addition into the clays for specimens fired at 1050°C, as well as of the firing temperature for 10 wt.% EAFD-loaded specimens, on the open porosity and the % water absorption of the sintered ceramic specimens is depicted in Figs. 2a and 2b respectively. Fig. 2a shows that the open porosity does not vary significantly with the % EAFD content. The lower open porosity is determined when 10% EAFD is added into the clay mixture (1050°C). The trend in the results for the % water absorption presented in Fig. 2b is generally similar to that for the open porosity in Fig. 2a. Specifically, the water absorption of the bricks sintered at 1050°C slightly decreases as the EAFD amount in the mixture increases up to 10 %, while further waste addition (15%) leads to a slight water absorption increase. Regarding firing temperature effect on 10% EAFD specimens, it can be seen that the water absorption remains almost constant when the sintering temperature is increased from 850 to 950°C, but it clearly decreases when firing at 1050°C, following the decrease in porosity respectively.

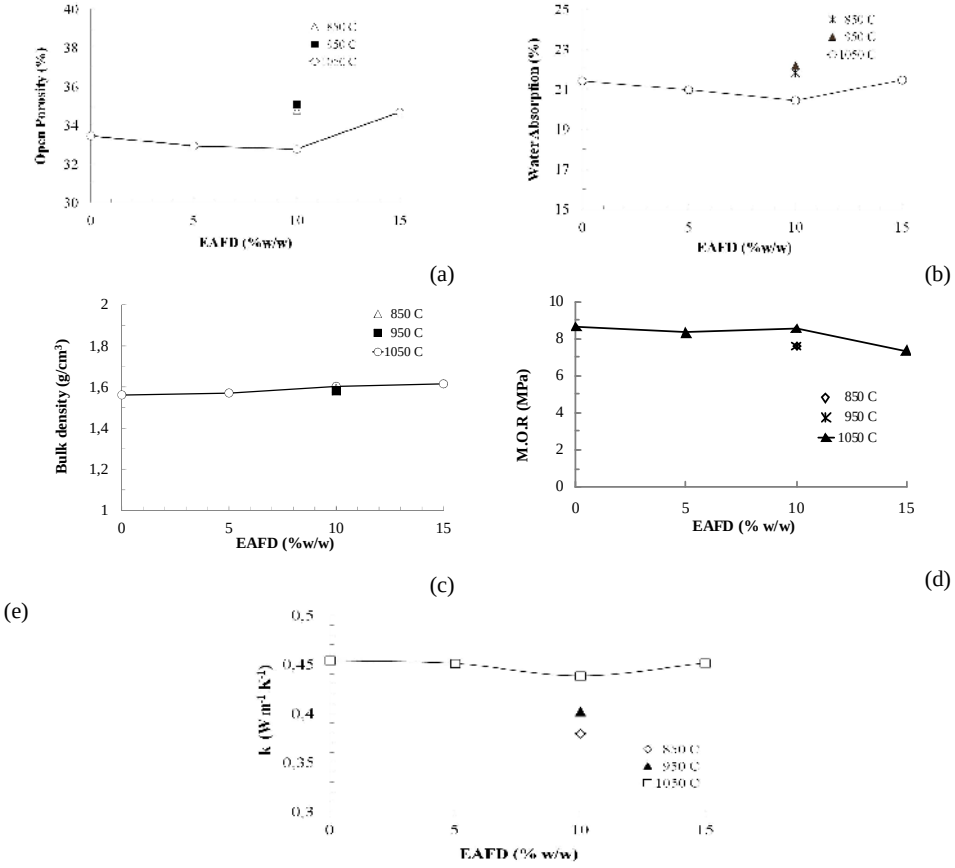

Figure 2. Effect of the % EAFD embodiment and also of the firing temperature on the open porosity (a), water absorptivity (b), bulk density (c), modulus of rupture (MOR) upon bend testing (d), and thermal conductivity (e) of sintered specimens

Bulk Density

The effect of the % EAFD addition into the clay mixture for specimens fired at 1050°C, as well as of the firing temperature for specimens containing 10 % EAFD, on the bulk density after sintering is presented in Fig. 2c. According to the results, bulk density of the sintered specimens is only slightly affected by the % EAFD content and the firing temperature. These findings are in accordance with the experimental results for the weight loss upon sintering, which does not vary substantially and lies approximately in the range of 9.5-10.5%.

Mechanical Strength (Modulus of Rupture)

The effect of the % EAFD addition into the clay mixture for specimens fired at 1050°C, as well as of the firing temperature for specimens containing 10 % EAFD, on the modulus of rupture (MOR) calculated upon three-point bending of the bricks is shown in Fig. 2d. The experimental data indicate that steel dust addition up to 10 wt.% into the clay mixture does not deteriorate the bending strength (expressed in terms of MOR) of the sintered bricks, while further waste addition (15 wt.%) leads to a noticeable decrease of approx. 15% in MOR. With regard to firing temperature effect, no difference in MOR is observed for a temperature increase from 850 to 950°C, but the MOR increases by approx. 12% when firing the specimens at 1050°C, which should be associated with the aforementioned decrease in open porosity at this firing temperature.

Thermal Conductivity

Use of ceramics as thermal insulators represents one of the main applications for this category of materials. The usefulness of a ceramic for these applications is largely fixed by the rate of heat transfer through it under a particular T gradient. The basic equation (1) to define thermal conductivity coefficient is (Kingery *et al.*, 1976):

$$dQ/d\theta = -(kAdT)/dx \quad (1)$$

where: dQ = the amount of heat flowing normal to the area A in time dθ; -dT/dx = the temperature gradient; k = thermal conductivity coeff., the proportionality factor, a material constant.

Fig. 2e shows how k of sintered bricks is affected by a) the % EAFD in the clay mixture (at 1050°C) and b) the firing temperature (for 10% EAFD-loaded specimens). It is apparent that k does not vary considerably with the EAFD addition up to 15% (1050°C) and remains relatively constant (around 0.45 $Wm^{-1}K^{-1}$). On the other hand, k increases when the sintering temperature of 10 wt.% EAFD specimens is increased from 850 to 950°C, and especially up to 1050°C. This variation in ceramic thermal conductivity should mainly be attributed to the corresponding decrease in open porosity.

CONCLUSIONS

- Extruded and sintered clay ceramics incorporating steel-making dust are successfully produced using brick manufacturing pilot-plant simulation procedures.
- EAFD (steel dust) embodiment up to 15 wt.% in clayey mixtures does not prevent an effective extrusion of ceramic bricks without significant variations in both their mechanical performance and thermal conductivity after sintering.
- EAFD does not act as a pore-forming agent in the ceramics so-produced, as only slight increase in the open porosity is obtained, even with 15 % dust addition into the clays. Further % admixture use would endanger the extruded product quality.

- Sintering of 10% EAFD-loaded clays at 850^{o}C or 950^{o}C results in ceramics with similar mechanical and thermal behavior. At 1050^{o}C however, the MOR and thermal conductivity increase as a result of porosity reductions and higher crystallinity.

Acknowledgment

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NOVEL FLOCCULANTS BASED ON ACRYLAMIDE AND ACRYLIC ACID OBTAINED BY ELECTRON BEAM IRRADIATION

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Water pollution results from all human activities: domestic, industrial and agricultural. The literature reports a multitude of processes for the decontamination of contaminated water and wastewater such as coagulation, precipitation, extraction, evaporation, adsorption on activated carbon, ion-exchange etc. Coagulation/flocculation is a frequently applied process in the primary purification of industrial wastewater. There are two major classes of materials used in coagulation/flocculation processes: (1) inorganic and organic coagulants including mineral additives, hydrolyzing metal salts, pre-hydrolysed metals and polyelectrolytes (coagulant aids); (2) organic flocculants including cationic and anionic polyelectrolytes, non-ionic polymers, amphoteric and hydrophobically modified polymers, and naturally occurring flocculants (starch derivatives, guar gums, tannins, alginates, etc.). In Romania, the obtaining and using of polyelectrolytes for residual and surface water treatment is not so developed because the advantages of their use are not well known, these being the following: (a) reduce the quantity of classic electrolytes needed by 25% up to 50%; (b) concentrations of 10 to 100 times smaller of classic materials are used, the final volumes of reagents which are used in water treatment are considerably decreased, saving space, labour, energetic consume, means of transport; (c) they do not produce metallic residuals in the mud left after water purge; (d) reduce by almost 60% the volume of the resulted mud by using them in comparison with the volume resulted from classic material treatment, which it reflects in the space economy for depositing the mud resulted from the purifying stations; (e) reduce by approximately 5-10 times the contact, stationary and decantation time which determines a shorter process of water purifying; etc.

Keywords: flocculants, copolymerization, acrylamide, acrylic acid, electron beam

INTRODUCTION

Wastewater and industrial effluent treatment require removal of suspended solids for purification and possible re-usage. The removal can be accomplished by gravitation (very slow), by coagulation (dependent on electric charge situation) and by flocculation (not dependent on electric charges and the fastest) (Brostow *et al.*, 2009). Coagulation and flocculation are phenomena which give names for two distinct stages of physical and chemical treatment processes applied in water and waste water treatment. Coagulation is the phenomenon in which the system consisting of colloidal particles from water is destabilized. It is the result of the adhesion of chemical reagents (ferric chloride, ferrous sulfate, aluminum sulfate) to the suspended particles from the water and results in the formation of larger aggregates. Flocculation is the phenomenon in which destabilized colloidal particles join together in larger agglomerations. It is caused by the addition of small quantities of chemicals known as flocculants and the effectiveness is manifested especially in the situations where colloidal particles are already destabilized (Singh *et al.*, 2000). During the last three decades, polyacrylamide and acrylic acid converted by different methods in polyelectrolytes are used increasingly as flocculants worldwide. These polymers are relatively nontoxic, but for health concerns, even for wastewater treatment has been subjected to closer evaluation

during the last decade (Fetting *et al.*, 1991). For this reason, a strict condition of use is that the residual acrylamide monomer content in the final product to be less than 0.05% (Martin *et al.*, 2006). From their apparition until today, flocculants were obtained by different chemical methods, but the method of producing by irradiation and particularly by electron beam irradiation has now become more attractive. The major advantages of radiation induced polymerization processes are: (1) very easy to manipulate the molecular weight, from low to very high, by simply changing the feed composition as well as the composition of the product by incorporating different monomers; (2) precise control of charge density as the monomer feed composition is controlled at the initial stages only; (3) precise control of molecular weight distribution; (4) no flammable and toxic solvents used; (5) no production of waste matter or evolution of noxious gases; (6) no production of hazardous effluents; (7) very low monomer contents; (8) very clean process (Martin *et al.*, 2006). Physical and chemical properties of flocculants based on polyacrylamide and acrylic acid obtained by electron beam irradiation are strictly related to the efficiency of waste water treatment expressed by the level of treated water quality indicators. There are many situations in which organic flocculants should be used together with classic coagulation aids (inorganic flocculants) such as $Al_2(SO_4)_3$, $FeSO_4$ or $Ca(OH)_2$ because in the case of very charged waste waters, treatments based only on organic flocculants or inorganic flocculants (classical treatment) are less efficient than in the case of their combined use. Moreover, the so-called flocs are larger and more strongly bound than the aggregates obtained by coagulation (Brostow *et al.*, 2007). It is well known that each waste water type, depending on its origin, has its particularities and more than that the same type of waste water shows significant variation during the same day. For this reason treatments based on polyelectrolyte should be seen as treatment schemes for each type of waste water. These treatment schemes should also be easily adaptable to the significant variations of the same type of waste water characteristics. There is no polyelectrolyte with universal destination regardless of the method of production. This is one of the reasons why the field of flocculants obtaining by radiation technologies is still open (Craciun *et al.*, 2013). Grafting of synthetic polymers on natural polymers has become increasingly attractive for scientists and technologists on the one hand because it provides a potential biodegradability and on the other hand because it reduces the amount of synthetic monomers used in the reaction. Among grafted guar gum, xanthan gum, carboxymethyl cellulose, and starch, grafted starch performs the best (Singh *et al.*, 2000).

The goal of the paper is to present some flocculation results obtained on kaolin suspension (0.2 wt %), at room temperature (20-25°C) using a novel flocculant based on grafted starch with acrylamide and acrylic acid, obtained by electron beam irradiation.

EXPERIMENTAL

Materials

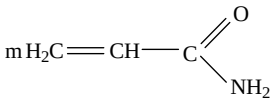
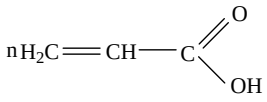
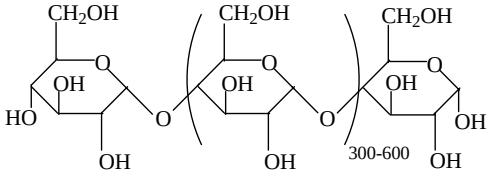
For flocculants obtaining the following materials were used: acrylamide (molar mass 71.08 g mol^{-1}; density 1.13 g/cm^3; solubility in water 2.04 kg/L at 25°C); acrylic acid (molar mass 72.06 g mol^{-1}; density 1.051 g/mL; solubility in water: miscible); sodium formate (molar mass 68.01 g mol^{-1}; density 1.92 g/cm^3; solubility in water 97 g/100 mL at 20°C) - serves as chain transfer agent in the copolymerization process; potassium persulfate (molar mass 270.322 g mol^{-1}; density 2.477 g/cm^3; solubility in water 1.75

g/100 mL at 0°C), - serves as initiator in the copolymerization process; starch (molecular weight 342.3 g/mol, density 1.5 g/cm^3).

Preparation and Irradiation of the Samples

Two classes of flocculants having the same quantities of acrylamide (AMD), acrylic acid (AA), chain transfer agent (CTA) and initiator (I) but with and without starch (S) were synthesized. The chemical structures of the synthetic and natural polymers used in grafting reaction by electron beam are presented in Table 1, as follows:

Table 1. The chemical structures of the synthetic and natural polymers used in grafting reaction

Chemical structures of the synthetic polymers used for grafting	
	
Acrylamide (AMD) structure	Acrylic acid (AA) structure
Chemical structures of the natural polymer which is grafted	
	
Starch (S) structure	

Flocculant synthesis details are presented in Table 2.

Table 2. Flocculants synthesis details

Sample code	AMD (mol/L)	AA (mol/L)	I (mol/L)	CTA (mol/L)	S (mol/L)	Irradiation dose, (kGy)
F1						0.5
F2	5	0.05	2.75×10^{-6}	1.1×10^{-4}	-	1
F3						1.5
F4						0.5
F5	5	0.05	2.75×10^{-6}	1.1×10^{-4}	0.07	1
F6						1.5

Experiments were carried out with an experimental installation consisting mainly of the following units: an electron linear accelerator (ALIN-10) of 6.23 MeV energy and 75 mA peak current of the electron beam and an irradiation chamber containing the samples of monomer solution. The ALIN 10 is a travelling-wave type, operating at a wavelength of 10 cm and having 164 W maximum output power. The accelerating structure is a disk-loaded tube operating in the π/2 mode. The optimum values of the EB peak current I_{EB} and EB energy E_{EB} to produce maximum output power P_{EB} for a fixed pulse duration τ_{EB} and repetition frequency f_{EB} are as follows: E_{EB} = 6.23 MeV, I_{EB} =75 mA, P_{EB} = 164 W (f_{EB} = 100 Hz, τ_{EB} = 3.5 μs). The EB effects are related to the absorbed dose (D) expressed in Gray or J kg^{-1} and absorbed dose rate (D*) expressed in

Gy s^{-1} or J kg^{-1} s^{-1}. Electron beam dose rate was fixed at 2kGy/min in order to accumulate doses between 0.5-1.5 kGy and samples were irradiated in atmospheric conditions and at room temperature of 25°C (Craciun *et al.*, 2011).

Methods for the Physical and Chemical Characteristics Determination

Flocculants thus obtained should present a good solubility in water and high flocculation capacity, and for this they must have specific physical and chemical characteristics such as: conversion coefficient (C_c), residual monomer concentration (M_r), intrinsic viscosity (η_{intr}) and linearity coefficient expressed by Huggin's constant (k_H). The conversion coefficient (C_C) and the residual monomer concentration (M_r) are determined on the basis of the bromation reaction of the double-bond (Dimonie *et al.*, 1986). The intrinsic viscosity (η_{intr}) and the Huggins' constant (k_H) are determined by the viscosimetry method, using a Hoppler BH-2 (Dimonie *et al.*, 1986). Sodium nitrate was used as a solvent 1N ($NaNO_3$) and the working temperature was 30°C.

RESULTS AND DISCUSSION

Conversion coefficient (C_c), and residual monomer concentration, M_r are the first important parameters in polyelectrolyte characterization. The first one is required to be higher than 90% and the second less than 0.05 % in accordance with rules established by the IPCS - International Programme in Chemical Safety in the document named "Environmental Health Criteria-49-Acrylamide". A high value of conversion coefficient demonstrates a good monomer transformation efficiency in polymerization process and ensures a substantial reduction in residual monomer concentration. This is a very important aspect in polimerization process because of the well known toxicity of acrylamide in the monomer state. Regarding intrinsic viscosity, η_{intr}, and linearity constant, k_H, they must be such as to ensure linearity and water solubility of polymeric flocculant. All those characteristics are influenced by the following factors: chemical composition of the solutions to be irradiated, absorbed dose level (D = energy quantity per unit mass in Gy or J kg^{-1}) and absorbed dose rate level (D* = energy quantity per unit mass and unit time in Gy/s or J kg^{-1} s^{-1}). In our experiments we obtained two classes of flocculants: the first one based only on AMD and AA and the second one based on grafted starch with AMD and AA. For getting both of them, CTA and I were used. Differences between physical and chemical characteristics of them come not only from the chemical composition but from irradiation treatment also. In Table 3 are presented the above mentioned physical and chemical characteristics of flocculants from both classes.

Table 3. Physical and chemical characteristics of flocculants

Sample code	Conversion coefficient C_c (%)	Residual monomer M_r (%)	Intrinsic viscosity η_{intr} (dL/g)	linearity constant k_H	Irradiation dose (kGy)
F1 (class 1)	97.51	0.025	2.8	0.6	0.5
F2 (class 1)	97.83	0.021	2.6	0.8	1
F3 (class 1)	99.28	0.028	3.6	0.5	1.5
F4 (class 2)	99.36	0.016	3.8	0.5	0.5
F5 (class 2)	99.68	0.011	4.5	0.2	1
F6 (class 2)	99.67	0.012	4.6	0.2	1.5

As can be seen, the conversion coefficient C_c and intrinsic viscosity η_{intr} increases slightly with the electron beam absorbed dose. The highest C_c values obtained in the same time with the best values of η_{intr} were obtained for the samples from class 2. For all samples the M_r is much under the limit value of 0.05 %. All samples obtained by grafting AMD and AA on starch (F4-F6/class 2) present superior physical and chemical characteristics than the samples from class 1. This last result is in accordance with our expectations.

Flocculation tests were carried out on kaolin suspension (0.25 wt%), at room temperature (25°C) using the standard Jar test apparatus (Velp FC 6S, Italia). For these experiments, solutions of four different concentrations of flocculants from each flocculant listed in Table 2 were prepared: 5, 10, 15 and 20 mg/L. Due to their good linearity (k_H <1) all flocculants were well dissolved in water. Under a slow stirring condition, the flocculant solution was added by means of a pipette in each beaker of 500 mL, in order to determine the polymer concentration influence (5-20 mg/L). Immediately after the addition of polymer solution, the suspensions were stirred at a constant speed of 60 rpm for 15 min, and then allowed to sediment for 15 min. Clear supernatant was drawn from the top layer and its transmittance was measured at 203-206 nm using a Cary Bio-100 UV-VIS spectrophotometer. The results are presented in Table 4.

Table 4. The results of flocculation tests

Flocculation characteristics	Flocculant concentration (mg/L) 5	10	15	20
F1				
Sediment (mm)	40	50	60	60
Transmittance (%)	95.13	92.46	92.3	90.41
F2				
Sediment (mm)	40	40	50	50
Transmittance (%)	95.24	93.12	92.17	91.85
F3				
Sediment (mm)	40	50	40	60
Transmittance (%)	95.13	95.74	95.46	92.64
F4				
Sediment (mm)	40	40	60	90
Transmittance (%)	97.33	97.12	96.43	95.12
F5				
Sediment (mm)	40	40	50	40
Transmittance (%)	98.25	97.46	97.38	96.41
F6				
Sediment (mm)	40	40	40	40
Transmittance (%)	98.63	98.84	97.43	97.22

The pH of the kaolin suspension was measured and it was found as been 6.99. The pH of the clear supernatant was also measured and it was found between 7.02 and 7.30. We can say, however, that the treatment with flocculant improved the pH of kaolin suspension. During the flocculation experiments, pH corrections were not necessary.

As can be seen there are not significant differences between the values of sediment, except for the flocculant concentration of 20 mg/L. All samples obtained by irradiation grafting of AMD and AA on starch exhibit very good values of light transmittance. But

it is easy to observe that for all samples, the flocculant concentration increasing decrease the light transmittance.

CONCLUSIONS

The grafting of synthetic polymers (acrylamide and acrylic acid) on a natural polymer (starch) by electron beam irradiation of 6.23 MeV was carried out. The obtained flocculants exhibit very good conversion coefficients (>99%) and as a consequence small residual monomer concentrations (<0.05%). Also, this class of flocculants has a better intrinsic viscosity than the class of flocculants obtained by simple polymerization of acrylamide and acrylic acid. Both class of flocculants were found to have good linearity (k_H <1). Flocculation tests were carried out on kaolin suspension (0.25 wt %), at room temperature (25°C). For these experiments solutions of four different concentrations of flocculants from each obtained flocculant were prepared: 5, 10, 15 and 20 mg/L. Even there were not significant differences between the values of sediment (except for the flocculant concentration of 20 mg/L) notable differences were obtained in light transmittance. Also, is seams that the flocculant concentration increasing decrease the light transmittance of clear superantant. Further studies regarding the biodegradability potential of this class of flocculants will be performed.

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RED MUD AS MULTIFUNCTIONAL MATERIAL FOR POLLUTANTS CAPTURING FROM WASTEWATER

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Red mud is a waste of alumina manufacturing from bauxite using the Bayer process, containing a mixture of minerals with ion exchange properties. Previous experiments have demonstrated the ability of red mud, raw or processed, to capture and retain chromium ions from wastewater and sludges. The present study highlights the ability of chemically modified red mud to capture other organic and inorganic substances, such as those found in effluents of hide processing. Laboratory analysis of the residual solutions, before and after treatment with the mineral complex of red mud, by potentiometric, gravimetric, photocolorimetric, and spectrophotometric methods, has shown that: the phosphate and sulphate content can be reduced by 80-99%, depending on their pH, silicon content can be reduced by 93%, the content of metal-complex dyes can be reduced to 100 % for blue dyes range, the chemical oxygen demand can be reduced by approximately 85%. The mineral complex of red mud is a multifunctional material for wastewater treatment, by simple, effective, and reproducible processes, which can be embedded in the composition of building materials and design of roads.

Keywords: mineral matrix, multifunctional, wastewater.

INTRODUCTION

Tannery wastewater is difficult to treat due to its high content of low biodegradability chemicals (Banuraman *et al.*, 2013).

For tannery wastewater treatment, various treatment options (Sivaprakasam *et al.*, 2008; Imran *et al.*, 2012; Kanagasabi *et al.*, 2013) were studied. Despite these efforts, most treatment plants for such tannery wastewater operate based on the principle of chemical coagulation of pollutants in water cumulated from natural leather processing operations. Following the global chemical treatment in wastewater treatment plants, most of inorganic and organic content of wastewaters from natural leather processing is transferred into residual sludge, whose traceability is not always very clear and which must be subsequently managed in accordance with environmental protection regulations.

Depending on the particularities of each tannery, various procedures can be adopted in order to reduce wastewater treatment costs, which are generally significant. The pre-treatment of individual wastewaters may be a solution for both reducing the amount of residual mud resulted in wastewater treatment and for an expansion of mud recovery possibilities.

A special category of wastewaters from tanneries is that of wastewater resulting from preliminary operations designed to prepare the dermis for tanning: washing, soaking, liming, deliming, pickling. In addition to a high load of organic substances, these wastewaters also contain many inorganic compounds.

Due to their high organic matter content, these wastewaters have a particularity, given their very high chemical oxygen demand. In previous studies (Niculescu, 2013) it was shown that, for this type of industrial effluents, chemically modified red mud has the ability to reduce chemical oxygen demand by approximately 85%.

In this study we considered the possibility of treating industrial effluents for controlled capture of various inorganic substances using a mineral complex made by chemical modification of red mud (Niculescu *et al.*, 2009), a waste of manufacturing alumina from bauxite using the Bayer process.

Red mud, raw or processed, has a great ability to capture and retain a wide range of pollutants, such as dyes, anions, heavy metals (Fu *et al.*, 2011; Huang *et al.*, 2008; Vaclavikova *et al.*, 2006). Chemical modification of red mud to develop its ability to capture a specific compound (such as chromium, for example) and physical conditioning (Niculescu *et al.*, 2009; Niculescu *et al.*, 2010) do not cancel the affinity of this material for other chemical species. Recent results (Wendling *et al.*, 2012) have set apart the mineral complex from red mud waste as a tool for managing the aquatic ecosystem due to its sorption capacity for dissolved organic carbon, phosphorus, and for all nitrogen species found in water.

In this work we studied the possibility of reducing phosphates which, although they are not specific to hide processing, can be found in larger or smaller amounts in wastewaters from all operations preceding tanning, as well as the sulphates found in significant amounts in waters resulting from pickling operation, discharged at a rate of approximately 50% before leather tanning using basic chromium salts. Simultaneously, the mobility of iron and silicon in the mineral complex composition was tested, due to their instability at pH variations in experimental conditions.

EXPERIMENTAL

Experimental Techniques

Experiments were conducted to transfer phosphates and sulphates from wastewaters into the chemically modified and conditioned red mud.

The mineral complex (chemically modified red mud) was dispersed in wastewater samples, in a solid/liquid ratio of 1/10. The adsorption process was carried out at room temperature (approximately 20°C), under stirring for 4 h. After stirring time lapse, each dispersion was vacuum filtered. After stirring program completion, dispersions were vacuum filtered, and filtrates were analysed to determine colour changes and sulphate, silicon and phosphate content.

Methods of Analysis

Residual solutions from preliminary operations of natural leather tanning, before and after treatment with the mineral complex, were analysed using: potentiometric methods to determine pH, according to STAS 8619/3-1990, gravimetric methods to determine sulphate content, according to STAS 8601-1970 and photocolorimetric methods to determine phosphate and silicon content, using a HANNA Multi-Parameter Ion Specific Meter C209.

RESULTS AND DISCUSSIONS

Waste waters from operations preceding tanning (washing, soaking, liming, deliming, pickling) were analysed both before and after treatment with the mineral complex based on chemically modified red mud.

Wastewaters from the following operations before treatment: washing (pH 6.80, phosphate content 85 mg/l); soaking (pH 6.80, phosphate content 130 mg/l, silicon content 10.55 mg/l); liming (pH 12.11, phosphate content 255 mg/l); deliming (pH 8.70, phosphate content 10.3 mg/l, silicon content 1.06 mg/l); pickling (pH 2.55, phosphate content 30.2mg/l, sulphate content 34000 mg/l, silicon content 1.24 mg/l).

Table 1 presents codes assigned to studied wastewaters.

Table 1. Origin of studied wastewaters

Sample code	Sample W	Sample S	Sample L	Sample D	Sample P
Technological operation	Washing	Soaking	Liming	Deliming	Pickling

Analytical results, illustrated in figure 1, demonstrate that values of phosphate ion content decrease significantly after treatment of wastewaters with chemically modified red mud. The capture and retention rate of phosphate ions by chemically modified red mud may be influenced to a certain extent by the amount and nature of the other compounds found in wastewaters (particularly organic substances), which compete for the adsorption centres of red mud particles, but the significant influence is that of pH value at which the adsorption process starts.

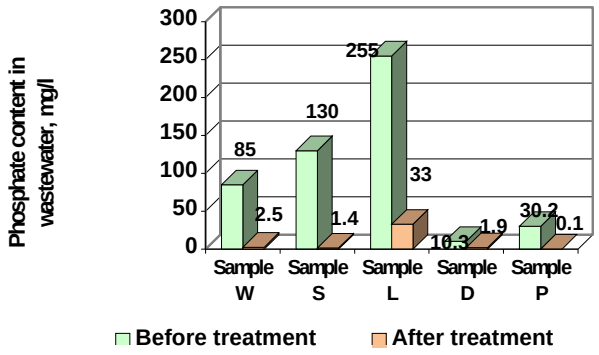

Figure 1. Reduction of phosphate content

Table 2 presents initial pH values of wastewaters, an important parameter in relation to the point of zero charge of the mineral complex.

Table 2. Initial pH value of wastewater

Sample	Sample W	Sample S	Sample L	Sample D	Sample P
Initial pH	6.80	6.80	12.11	8.70	2.55

The proportion in which phosphate ions can be removed from wastewaters using chemically modified red mud is given in figure 2.

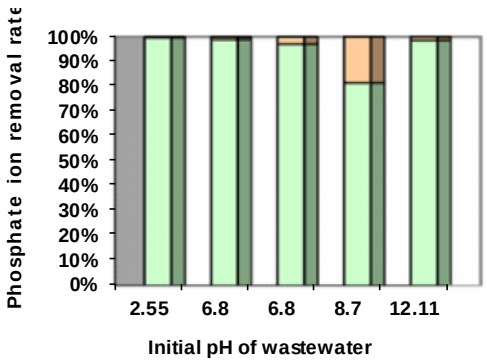

Figure 2. Influence of pH on the phosphate ion removal rate

It should be noted that the higher the difference between the initial pH of the solutions and the pH of the aqueous extract of chemically modified red mud used as adsorbent, the more effective the removal of phosphate ions.

Results of the experiment to capture phosphate ions from wastewaters of leather processing are in accordance with recent results of experiments to remove phosphorus from water, using either raw red mud, or improved by thermal processing or combined with photocatalysis, in which case phosphorus removal may exceed 94% (Qin *et al.*, 2012; Tie *et al.*, 2013; Zhang *et al.*, 2013).

In the case of wastewater from the pickling operation, an 85% reduction in sulphate content is also noticed, figure 3, as well as a significant increase in pH value, from 2.55 to 7.54, until it falls into the range regulated for discharge into sewers and natural receptors (6.5-8.5), in accordance with national (NTPA-001/2002 and NTPA-002/2002 with subsequent amendments) and European standards.

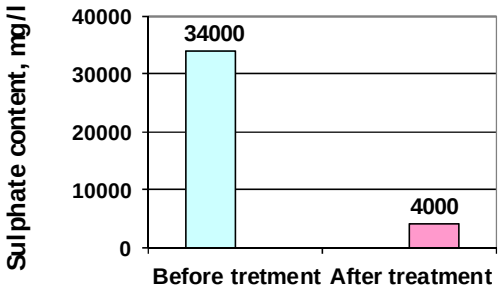

Figure 3. Reduction of sulphate content

This is an important aspect, because in most of the technologies applied in chromium tanning, half the residual float from pickling process is discharged, and sulphate content of over 30,000 mg/l and pH < 3 is a significant burden for wastewater treatment plants.

Because the pH values of wastewaters from tannery have a very wide range, and chemically modified red mud has a complex mineral matrix, random tests were carried out by photocolorimetry on iron and silicon content. The results of these tests confirmed that the iron is not solubilized in the mineral matrix, being undetectable after wastewater treatment, while in the case of silicon, both content decreases in wastewater after treatment with chemically modified red mud, and content increases in wastewater after treatment with chemically modified red mud were recorded, figure 4.

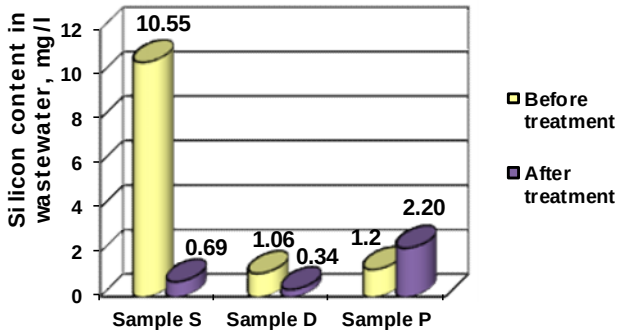

Figure 4. Evolution of silicon content in wastewaters

This behaviour is the consequence if significant differences in pH of the tested wastewaters. Experimental results prove the ability of the material to retain silicon from wastewaters with alkaline or slightly acid pH, and transfer the silicon from the mineral matrix into the wastewaters at highly acid pH.

This study was focused on the composition of the liquid phase, to establish the effectiveness of removing some anions, but for practical applications, it is important to establish also the composition of solid phases, as well as their properties, given that previous papers (Niculescu *et al.*, 2011) have demonstrated the importance of the presence of phosphate ions in the inertisation process of chromium captured by red mud from wastewaters of leather tanning. In this regard, it is noteworthy that an individual treatment of wastewaters from leather processing, carried out in cascade, using the same charge of mineral complex based on red mud, could set the premises both for recirculating wastewaters and for enriching the mineral complex with various compounds to enhance its chemical stability, until reaching the point where it could be directed to applications not yet studied.

CONCLUSIONS

The material made of chemically modified red mud, designed to capture chromium and render it inert, is able to retain phosphates from wastewaters. The mineral complex based on red mud can reduce the phosphate ion content by over 80% from tannery wastewaters. By treating wastewaters from preliminary operations of leather tanning, using the mineral, the higher the difference between the initial pH of the solutions and the pH of the aqueous extract of chemically modified red mud used as adsorbent, the more effective the removal of phosphate ions.

In the acid pH range, the mineral complex is able to reduce sulphate content by over 85%.

In wastewater with slightly acid or alkaline pH, the mineral complex can reduce silicon content by 30% to 80%.

The mineral complex made by chemical modification of the alkaline red mud waste is a multifunctional material.

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ASSESSMENT OF LEATHER AND LEATHER SUBSTITUTE WASTE BIODEGRADABILITY UNDER AEROBIC CONDITIONS IN LIQUID ENVIRONMENT

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The leather and footwear industry is one of the industries that generate large amounts of leather and leather substitute waste. Waste biodegradation is assessed through tests performed under aerobic conditions in the soil, in an aqueous medium under composting and anaerobic conditions in an organic waste anaerobic digester or under similar conditions in the laboratory. The aim of this paper is to comparatively study biodegradation under aerobic conditions in liquid medium of three types of materials used in the leather and footwear industry, namely: chrome-tanned leather, vegetable-tanned leather and synthetic leather. Biodegradability study was conducted in accordance with EN 13432/02 in a facility for waste biodegradation in liquid medium under aerobic conditions (EN ISO 14852-05). To characterize the biodegradation process, the following were monitored for 100 days: conductivity, total organic carbon content (TOC), total organic nitrogen content (TON) and the degree of waste biodegradation.

Keywords: biodegradation, chrome-tanned leather, vegetable-tanned leather, synthetic leather

INTRODUCTION

The leather and footwear industry is one of the industries that generate large amounts of solid waste, which until recent years have been ignored and huge amounts have been discharged directly into the environment (Zerdan *et al.*, 2004).

Throughout the life cycle, from processing finished leather for shoes, the resulting waste and chemicals contained by some waste can be hazardous to health and to the environment (Stefan *et al.*, 2012b).

In accordance with European legislation, waste from the leather and footwear industry must be exploited by the same methods: reuse, recycling, energy recovery, recovery by chemical and biochemical degradation, recovery of useful organic compounds (Aftab *et al.*, 2006; De Gisi *et al.*, 2009; Dogruel *et al.*, 2004; Mandal *et al.*, 2010; Stefan *et al.*, 2012a; Zaharia *et al.*, 2013).

One way for low-cost recovery of solid waste is biodegradation (Ruggieri *et al.*, 2008).

The aim of this paper is to comparatively study biodegradation under aerobic conditions in liquid medium of three types of materials used in the leather and footwear industry, namely: chrome-tanned leather, vegetable-tanned leather and synthetic leather. Waste pieces are placed in the composting system in a nutrient solution which provides the required C, N, P, Ca and Mg and the inoculum of microorganisms able to biodegrade them. In order to characterize the biodegradation process, the following were monitored: the conductivity and the degree of waste biodegradation by determining the resulting amount of CO_2.

MATERIALS AND METHODS

For the study of biodegradability three types of materials were used, common to the leather and footwear industry, namely: chrome-tanned leather (PCr), vegetable-tanned leather (PTan) and synthetic leather (PS). The biodegradability study was performed

according to EN ISO 13432-02: 2002 - Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging. The facility in which the study was conducted in accordance with EN ISO 14852:1999 - Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium - Method by analysis of evolved carbon dioxide, and is shown in Figure 1.

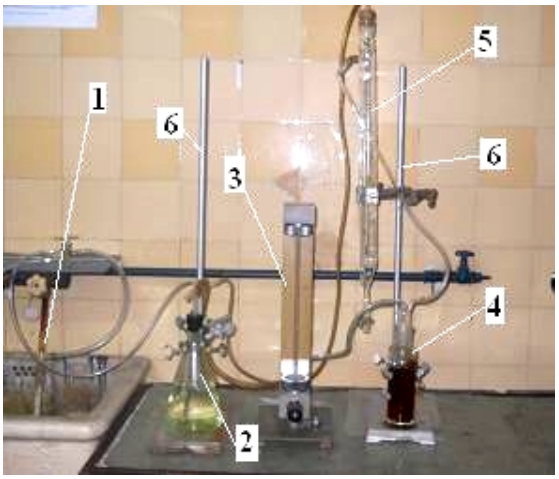

Figure 1. Facility for waste biodegradation in aqueous medium, under aerobic conditions: 1 - vacuum system, 2 - vessel for CO2 absorption from air, 3 - rotameter, 4 - reaction vessel, 5 - CO2 absorption column, 6 – frame.

With the vacuum pump air is circulated through the apparatus, the flow rate measured using the rotameter varies around the value of 2 L/h. The absorption vessel contains 300 mL of KOH in concentration of 10 mol/L and is designed to retain CO_2 from the air. The air free of carbon dioxide enters the reaction vessel which contains about 0.4 g of a waste sample and 250 mL of nutrient solution (prepared from: KH_2PO_4, K_2HPO_4, $Na_2HPO_4*2H_2O$, NH_4Cl, $MgSO_4*7H_2O$, $CaCl_2*2H_2O$, $FeCl_3*6H2O$ dissolved in distilled water according to EN ISO 14852-05) and 10 cm^3 of inoculum. The inoculum was prepared from 10 g of compost collected from the biodegradation landfill of leather waste (INCDTP-ICPI) that were put into contact with 100 mL of distilled water. The suspension was stirred for 30 minutes and then filtered; 10 mL of the solution was used as inoculum in the reaction vessel.

In the reaction vessel, degradation processes occur, resulting in carbon dioxide, which is absorbed in the absorption column containing 100 ml of NaOH solution with the concentration of 0.05 mol/L. Carbon dioxide concentration was determined daily by titration with HCl 0.05 mol/L in the presence of phenolphthalein in the first stage and methyl orange in the second step.

In the liquid phase the following were also determined: conductivity, using a conductometer, Jenway 470; total organic carbon content, TOC, using a LiquiCOT analyzer; and total organic nitrogen content, TON, determined by the Kjeldahl method using a Velp Scientifica UDK 130 D apparatus.

The degree of biodegradation was determined using equation 1:

$$R, \% = \frac{Ct}{Ci} \times 100 \quad (1)$$

where: Ct - the amount of carbon dioxide resulting from the biodegradation facility when the analysis was performed, mg/g; Ci - total organic carbon content corresponding to 1 g of sample, mg/g.

RESULTS AND DISCUSSIONS

Using miocroorganism populations found in the composting landfill of leather waste which were inoculated in a nutrient solution, their degree of biodegradation was monitored. Figures 2, 3, 4 and 5 present variations of conductivity; total organic carbon content, in the liquid phase, TOC; total organic nitrogen content, TON; and the degree of biodegradation. The system was monitored for a period of 100 days.

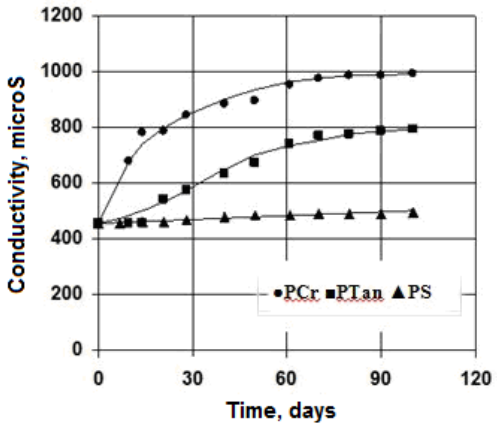

Figure 2. Variation over time of conductivity in the liquid phase

Conductivity in the system increases for all three samples, phenomenon due to salt solubilization and degradation processes that occur.

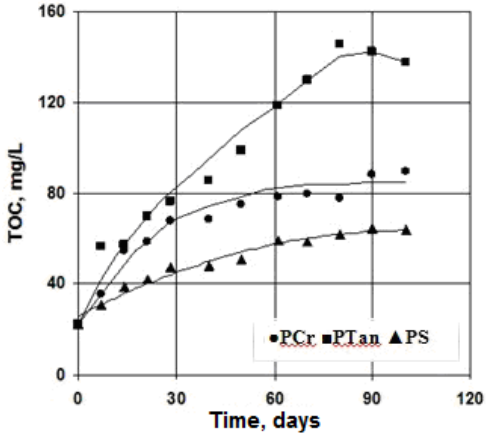

Figure 3. Variation over time of TOC in the liquid phase

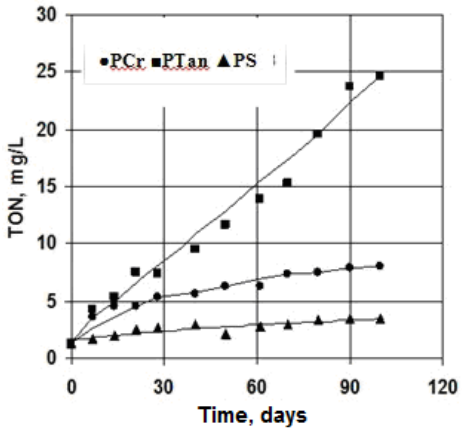

Figure 4. Variation over time of TON in the liquid phase

The increase of total organic carbon and total organic nitrogen concentration in the studied systems prove that degradation processes occur in the system. It is noticed that they are more intense for vegetable-tanned leather waste and much weaker for chrome-tanned leather and synthetic leather.

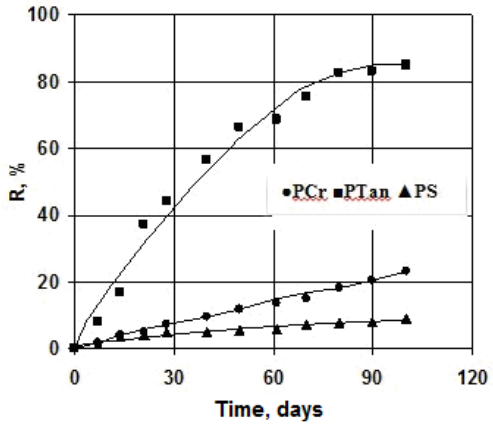

Figure 5. Variation over time of waste biodegradability

Upon analyzing Figure 5, it can be noticed that the degree of biodegradation of vegetable-tanned leather reaches 84% in the 100 days of the study.

The degree of biodegradation of chrome-tanned leather waste and synthetic leather waste reaches 23% and 9%, respectively. Vegetable tanning is a great step towards biodegradation of leather waste; therefore it is recommended to replace the chrome-tanning process with vegetable-tanning, which enables leather waste biodegradation.

CONCLUSIONS

None of the materials subjected to analysis fully complies with the conformity criteria, but vegetable-tanned leather has the closest degree of biodegradation to the required one.

As a result of this study, it was found that vegetable-tanned leather has a biodegradation capacity of 84.6%, chrome-tanned leather of 23%, and synthetic leather of only 9%, which proves that vegetable-tanned leather is biodegradable and comes very close to biodegradability conditions required by EN ISO 13432:2002.

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RECOVERY OF TANNERY WASTES FOR FUNCTIONAL MICROENCAPSULATED PRODUCTS

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Leather is one of the most used materials in the footwear and leather goods industries, and is also employed in the manufacture of a variety of products in the clothing/garment industry as well as in furniture upholstery. Even though the tanning industry is considered to play an important environmental role as users of a by-product of the meat industry, the different stages involved in the transformation of hides and skins into leather generate a significant amount of wastes, both liquid and solid. In this sense, the advancement of European policy and legislation protecting the environment has prompted the transformation of tannery solid waste materials into valuable co-products that can be recycled or employed in other industries. The paper focuses on the recovery of collagen derivatives from untanned solid wastes, more specifically by isolating gelatine in order to use it as a natural microencapsulating agent in the production of active materials with functional properties. Gelatine was the first shell-forming material used in microencapsulation and, nowadays, it is still a promising material for the creation of natural and biodegradable microcapsules. In the footwear industry, microencapsulation can transform a traditional shoe into an "active shoe" that ensures the continuous care of our feet by the incorporation of microencapsulated products with therapeutic and/or antimicrobial properties. This work describes the project and the results obtained to date.

Keywords: gelatine, tannery wastes, microencapsulation.

INTRODUCTION

The tanning processes carried out during the different processing stages involved in the transformation of hides into leather generate significant amounts of wastes, both liquid (wastewater) and solid (tanned and untanned waste and sludge). Several approaches have been suggested for the minimisation, treatment and valorisation of effluents and solid wastes generated by the leather industry. In this sense, the advancement of European policies and legislation protecting the environment has prompted the transformation of tannery solid waste materials into valuable co-products that can be recycled or employed in other industries, for instance for the preparation of organic fertilisers, the production of biomaterials, gelatines or collagens, and the production of biofuel (Schrieber and Gareis, 2007).

Gelatine is a soluble protein compound obtained by the partial hydrolysis of collagen. The most abundant sources of gelatine are pig skins, bovine hides and pork and cattle bones. The gel-forming properties of gelatine are the basis of classical applications in food, photographic, cosmetic and pharmaceutical industries. Recently, new applications have arisen, such as its use as a shell-forming polymer for microencapsulation applications (Schrieber and Gareis, 2007, Perez-Liminana *et al.*, 2014; Sánchez-Navarro *et al.*, 2013).

Microencapsulation is a coating technology by which active substances are encapsulated in a polymeric shell, leading to core-shell particles called microcapsules (Figure 1). Microencapsulation is an effective method to protect reactive, sensitive or volatile chemicals from reaction with moisture, light and oxygen. Furthermore, this technology allows a controlled release of the active substance and enhances stability

against external factors. Indeed, microcapsules, when firmly anchored to a material can add new smart functionalities without affecting the look and feel of the material. Therefore, this technology holds great promise for the future of the footwear industry since it can transform a traditional shoe into an "active shoe" that ensures the continuous care of our feet by the incorporation of microencapsulated products with therapeutic and/or antimicrobial properties improving the comfort and welfare of the user (Morace, 2010).

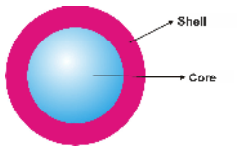


Figure 1. Microcapsule structure. Shell: polymeric cover; Core: encapsulated active chemical

One of the most widely used shell-forming materials is gelatine. Nevertheless, gelatine shows a wide range of properties such as gel strength, film-forming capability, and emulsion properties, among others, which determine its final application. Those properties are governed by extraction conditions (temperature, pH, time, etc). Therefore, the optimisation of the transformation process of collagen into gelatine is necessary in order to obtain specific properties suitable to their use in added value applications, for instance as a shell forming biopolymer for microencapsulation (Schrieber and Gareis, 2007).

The extraction process of gelatine from untanned wastes consists of several steps. As a first step, collagen must be acid or alkaline pre-treated since its hieratical fibrous structure makes it insoluble in water. Such pre-treatment breaks non-covalent bonds so in order to produce fibre swelling and collagen solubilisation, thus enabling its extraction. The alkaline pre-treatment is usually preferred when bovine hides are used as a raw material. During the treatment – a long process that takes several months – the non-protein substances such as mucopolysaccharides and sulphur-containing compounds, as well as non-collagenous proteins, especially albumin and globulin, which are always contained in the raw material, are reliably dissolved out. Subsequently, the treated material is washed free of alkali and neutralised by the addition of acid. Most of the neutral salts produced during this process are then removed by numerous washes (Schrieber and Gareis, 2007).

Because of the high water requirement and the high content of protein in wastewater, the gelatine industry views the improvement of the process along with savings in energy and water as being of high priority. The energy balance is similar in the case of the alkaline procedure using hides and in the acid procedure using pigskins. However, the water requirement increases to 400 L kg^{-1} gelatine for the alkaline procedure versus 150 L kg^{-1} gelatine for the acid procedure. The reason for this is the fact that water has to be replaced about 20 times during the conditioning and washing processes. So, this implies the treatment and management of large amounts of wastewater generated. Nowadays, the enzymatic pre-treatment as an alternative to the alkaline pre-treatment for gelatine manufacture is raising interest in order to save costs by reducing wastewater and time (Schrieber and Gareis, 2007; Zhang *et al.*, 2006).

Proteolytic enzymes (proteases) are commonly used in the leather industry for dehairing, bating and soaking processes, as well as in the detergent industry for

breaking down proteinaceous matter caused by body secretions, food stuff, and blood. As in the pre-tanning process, the use of enzymes during the conditioning of hides prior to gelatine extraction opens up a new alternative to the alkaline pre-treatment to reduce time and wastewater (Kanagaraj, 2009).

The selection of the pre-treatment procedure (acid, alkaline or enzymatic) and also the extraction conditions (temperature, pH, time) will influence the final properties of the gelatine and, therefore, its final application. For technical applications such as microencapsulation, gel strength (Bloom strength, related to gelatine's average molecular weight), film-forming or emulsifying properties of gelatine affect to some extent the quality of the microcapsules and, therefore, the microencapsulation process (Schrieber and Gareis, 2007).

Currently, INESCOP is working on the LIFE microTAN project (LIFE12 ENV/ES/000568) which focuses on the recovery of collagen derivatives from untanned solid wastes, more specifically by isolating gelatine in order to use it as a natural microencapsulating agent in the production of active materials with functional properties. This work proposes the enzymatic pre-treatment as an alternative to the current alkaline pre-treatment process in order to save costs and reduce time and, finally, the use of gelatine for microencapsulation applications. This paper describes some results obtained to date.

MATERIALS AND EXPERIMENTAL TECHNIQUES

Bovine Hides

Bovine hides were supplied by a local tannery as a by-product (INCUSA, Valencia, Spain). Limed bovine pelt wastes were used, which were in the previous condition to the tanning process. The bovine hides were frozen for their conservation up to their use. Previously, the samples were cut into pieces of approx 0.5x0.5 cm.

Gelatine Extraction Process

Prior to gelatine extraction, the hide-waste pieces were conditioned by applying an alkaline pre-treatment using a sodium hydroxide solution (1%) or by applying an enzymatic pre-treatment. After conditioning, the gelatine was extracted under different extraction conditions (pH, time, temperature). The extraction was carried out in a three-neck, 3-L Pyrex glass flask to which a condenser (vertical position), a thermometer and a mechanical stirrer were fitted. The flask was placed in a water bath at a determined temperature. The gelatine extracts were filtered under vacuum. Next, it was concentrated by evaporation in a water bath and kept at a constant temperature using a rotavapor device. Finally, the concentrated gelatine solution was placed in a PTFE mould and left to dry overnight in a furnace at T=45ºC to obtain the gelatine films for further characterisation.

In addition, type B commercial gelatine G9382 supplied by Sigma-Aldrich was used as a reference of suitable properties for microencapsulation applications.

The properties of the gelatine films were evaluated by Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Infrared Spectroscopy (FTIR) and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The microencapsulation capability of the extracted gelatines was also evaluated.

Assessment of Microencapsulation Capability

The assessment of the microencapsulation capability was carried out by the complex coacervation method. The complex coacervation process is based on the phase separation that takes place spontaneously when in an aqueous phase, two or more colloids of opposite charges (a polycation and a polyanion) are mixed in the presence of an active substance dispersed (oil phase). In this work almond oil was chosen as the oil phase. The extracted gelatines (polycation) and sodium carboxymethylcelulose (CMC) (polyanion) were used as biodegradable shell-forming polymers.

The morphology of the microcapsules were analysed by Scanning Electronic Microscopy (SEM) and optical microscopy. The thermal properties of the microcapsules were evaluated by Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC), and their chemical composition was determined by Infrared Spectroscopy (FTIR).

RESULTS AND DISCUSSION

The degree of conversion of collagen into gelatine depends on the processing conditions. The collagen conversion was reduced when no pre-treatment was carried out and a high amount of solid wastes was not transformed. The hide pre-treatment (enzymatic or alkaline) prior to extraction doubled the yield of the extracted gelatine due to the collagen swelling, which enabled its solubilisation and made the extraction easier. Furthermore, the yield increased as temperature raised and the pH decreased. The enzymatic treatment of the bovine hide wastes produced higher or similar gelatine yields than the alkaline pre-treatment.

The results obtained from SDS-PAGE assays showed that the different extraction conditions greatly affected the molecular weight distribution (Mw) of gelatine. An increase in temperature or a decrease in pH decreased the Mw of gelatine. The enzymatic treatment produced gelatine with lower molecular weights than the alkaline treatment.

By way of example, the chemical composition of the extracted gelatines as a function of the type of treatment was determined by FTIR spectroscopy (Figure 2). The infrared spectra of the extracted gelatines were similar to those of commercial gelatine. The gelatine spectra showed vibration bands at 3400-3100 cm^{-1} for N–H stretching (amide A and B), 3100-2800 cm^{-1} for alkenyl C–H stretching, 1635 cm^{-1} (Amide I) for C=O stretching, a band at 1550 cm^{-1} (Amide II) for out of phase combination of the N–H in plane bend and the CN stretching vibration, 1480-1300 cm^{-1} for CH2 bending, 1249 cm^{-1} (Amide III) for in phase combination of the NH bending and CN stretching vibration (Nagarajan *et al.*, 2012; Barth and Zscherp, 2002). Additionally, the gelatine prepared using the enzymatic pre-treatment showed typical bands of fatty acids due to the presence of the C=O stretching band characteristic of ester groups and also an important increase in C-H stretching bands. In contrast, the alkaline pre-treatment used was more effective than the enzymatic one for the removal of fat from hides.

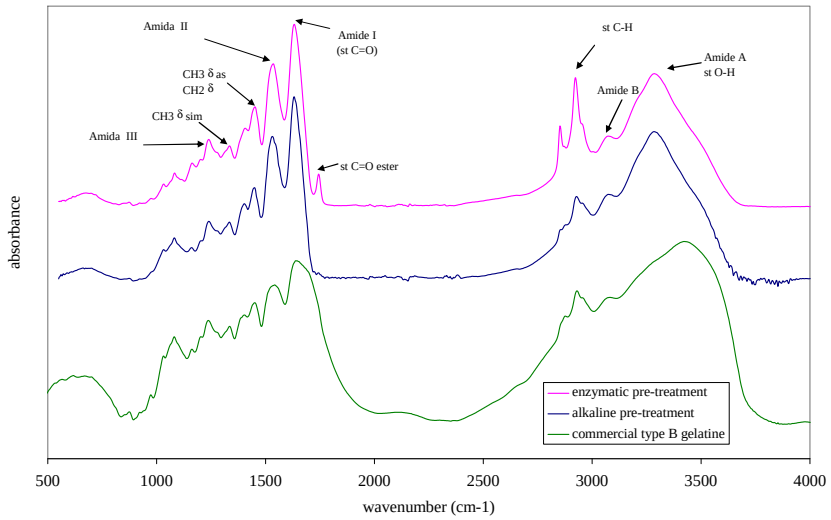
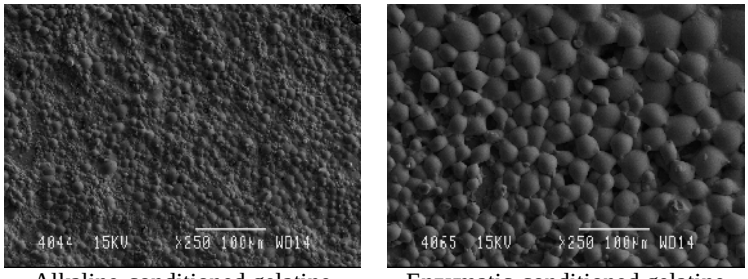


Figure 2. Infrared spectra of enzymatic or alkaline pre-treated gelatines compared with commercial gelatine

Microencapsulation

The microencapsulation capability of the extracted gelatines was evaluated by the complex coacervation process. Microcapsules containing an oil phase as a core material were successfully prepared using both alkaline and enzymatic pre-treated gelatines. Optical microscopy showed the typical elongated-shaped shell around the oil of the microcapsules obtained by complex coacervation. SEM images of the different microcapsules obtained are shown in Figure 3. The particle size of the microcapsules obtained from the enzymatic-treated gelatine was higher than that of the microcapsules obtained from the alkaline-treated gelatine. The particle size was affected by gelatine properties, acting as an emulsifier and as a shell-forming polymer. The emulsion properties of gelatine depend on its chemical composition (based on the presence of hydrophilic/hydrophobic amino acids) as well as the gelling power, both parameters being governed by manufacturing conditions.



Alkaline-conditioned gelatine Enzymatic-conditioned gelatine

Figure 3. SEM images of microcapsules containing almond oil using the extracted gelatines

CONCLUSIONS

Gelatine from bovine untanned wastes can be successfully extracted as valuable co-products using alkaline or enzymatic pre-treatments prior to extraction. It shows suitable properties as a shell-forming polymer allowing the microencapsulation of oils by complex coacervation.

The degree of conversion of collagen into gelatine depends on the processing conditions (pre-treatment, T, t, pH). The pre-treatment of bovine hides is a fundamental step during gelatine manufacture since it allows the swelling of collagen and enables its solubilisation, thus increasing the yield. Moreover, an increase in temperature or a decrease in pH also increases the conversion of collagen into gelatine yield.

The enzymatic pre-treatment is an efficient procedure to open the collagen fibres and remove non-collagenous proteins and other substances. However, some fats from original bovine hides are present in the extracted gelatine, which can be successfully removed when the alkaline pre-treatment is carried out. This indicates that the enzymatic procedure needs to be optimised in order to improve the removal of impurities.

Finally, the particle size of the microcapsules is influenced by gelatine properties (molecular weight, conformation, and chemical composition), which depend on extraction conditions.

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FORMULATION OF NANOCOMPOSITES FOR FOOTWEAR WITH ENHANCED COMFORT AND SAFETY PROPERTIES

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Over the last years, huge research has been made on the developing of advanced, innovative, high-performance, nanotechnology-based polymeric materials. As a result, the development of nanofilled plastics, the so-called polymer nanocomposites, has allowed the introduction of new combinations of properties, which consequently enables new applications for plastics. For this purpose, INESCOP is working in the European project NANOFOOT (FP7-SME-2013-2-606570) which focuses on the implementation of nanotechnology in footwear materials and components in order to impart antimicrobial properties, electrical and thermal conductivity, water resistance, breathability, etc., with the main objective of obtaining high added value and marketable materials and footwear. The development of thermally conductive nanocomposites is expected to contribute to an improvement of comfort, since such materials would improve the dissipation of overheating which is produced within the footwear during use. With regards to antistatic nanocomposites, their implementation in the footwear industry would improve both comfort and security as they will reduce the electrostatic charges accumulation. Last but not least, the development of nanocomposites with antibacterial and antifungal properties will improve both comfort and foot health. This paper focuses on the development of foamed EVA-base nanocomposites for insoles, with expected electrical and thermal conductivity properties. Several aspects related to the nanocomposite processability have been evaluated.

Keywords: insole, nanofiller, antistatic.

INTRODUCTION

Over the last years, huge research has been made on the developing of advanced, innovative, high-performance, nanotechnology-based polymeric materials. As a result, the development of nanofilled plastics, the so-called polymer nanocomposites, has allowed the introduction of new combinations of properties, which consequently enables new applications for plastics (Ray, 2013).

The interest in applying nanoscaled fillers into polymeric matrices is the expected achievement of unique properties attributable to the nanoscopic dimensions and intrinsic extreme aspect ratios of nanofillers. Thus, the possibility of incorporating nanofillers to polymers opens a new range of innovative solutions for the footwear industry and related fields. Specifically, there is a trend towards the implementation of nanotechnology in footwear components in order to impart antimicrobial properties, electrical and thermal conductivity, water resistance, breathability, etc., with the main objective of obtaining high added value and marketable materials and footwear.

Thermally conductive polymer nanocomposites have been developed as a light-weight, corrosion-resistant substitute for metallic parts in several applications, such as electronic appliances, electric motor and generators, heat exchangers etc. In the case of footwear, the development of thermally conductive nanocomposites is expected to contribute to an improvement of comfort, since such materials would improve the dissipation of overheating which is produced within the footwear during use.

Different nanoparticles have been described in the literature to improve thermal conductivity of polymers. Silica, boron and aluminium nitrides and aluminium and magnesium oxide nanofillers are considered as convenient fillers in the formulation of

composites for electronic appliances, due to their capability to impart thermal dissipative properties and their electrically insulating character (Kochetov, 2012). Boron nitride has been reported to improve thermal conductivity of poly(dimethyl siloxane) (Yu and Cennini, 2014) and polypropylene (Muratov *et al.*, 2014) for LED applications. Boron nitride nanotubes (BNNT) have been reported to impart up to 21.1-fold improvements of thermal conductivity to polystyrene, poly(ethylene vinyl alcohol), poly(vinyl butyral) and poly(methyl methacrylate) based nanocomposites (Zhi *et al.*, 2009). Silica nanofibres can lead to 2-fold improvement of thermal conductivity of epoxy resins (Ren *et al.*, 2014).

With regards to carbon-based nanofillers, graphene nanosheets have proved to improve thermal conductivity of nanocomposites based on polyimide (PI) and on poly(ethylene-vinyl acetate) (Song *et al.*, 2012). Carbon nanofibre and carbon nanotubes (either single wall-SWCNT or multi wall-MWCNT) have also been reported to improve thermal conductivity of polymer composites.

What is more, some authors have reported a synergic effect when formulating nanocomposites with a mixture of nanofillers. Polyphenylene sulphide (PPS) filled with a mixture of boron nitride and MWCNTs can reach a thermal conductivity of 1.74 W/mK (Pak *et al.*, 2012), and epoxy resins filled with a mixture of single-layer graphene and boron nitride can reach thermal conductivity values of ~21.6W/mK (Liem and Choy, 2013).

The implementation of antistatic nanocomposites in the casual and mainly safety footwear industry would improve both comfort and security as they will reduce the electrostatic charges accumulation.

Carbon-based nanofillers, including nanotubes, carbon black and nanofibres, have been reported by several authors to improve electrical conductivity to different polymeric matrices, such as polyurethane (Orgilés-Calpena, 2013), EVA (Sohi, 2011), polyethylene (Jin *et al.*, 2013), polyethersulphone (Jin *et al.*, 2013), among others. Metal-based nanofillers and organometallics (Wypych, 2014) are also able to impart antistatic properties to polymers. Other antistatic additives that can be explored in combination with nanofillers, include inorganic and organic chemicals, as well as inherently conducting polymers (Casado *et al.*, 2014).

Nevertheless, processing of polymer nanocomposites is still a challenge, particularly using conventional melt-blending technologies, mainly due to their low loading concentrations and difficulties in achieving adequate nanofiller dispersion. In general, nanoparticles tend to aggregate during processing with polymers, limiting their possible nanoscale reinforcement effect and resulting in defect sites that limit the overall mechanical and transport performance of the resulting composite. It is crucial to adequately control the addition of nanoparticles into the polymer matrix and later processing in order to minimize re-aggregation or re-agglomeration of the nanoparticles in the resulting nanocomposite.

Currently, INESCOP is working in the European project NANOFOOT (FP7-SME-2013-2-606570) which focuses on the implementation of nanotechnology in footwear materials and components in order to impart antimicrobial properties, electrical and thermal conductivity, water resistance, breathability, etc.

In this work, foamed EVA formulations were produced. Thermal and electrical conductive nanoparticles of different nature were considered, including carbon based nanoparticles, metallic-based nanoparticles, and other organic antistatic additives.

MATERIALS AND PROCEDURES

Materials

Ethylene-vinyl acetate copolymer (EVA) was used as polymeric matrix. Standard EVA formulation and components were provided by the company EVATHINK, S.L. (Aspe, Alicante, Spain), according to technical and processing requirements for EVA as insole material, provided by Todo Para Sus Pies, S.L. (Elda, Alicante, Spain).

Antistatic and electrical/thermal conductive fillers used in this work are listed in Table 1. Fillers were dosed as recommended by suppliers.

Table 1. Selected fillers used in this work

Reference	Description
NF1	Alkaline salt +quaternary N-compound
NF2	Masterbatch of MWCNT in EVA
NF3	Ionomer resin
NF4	Organo-Ti(IV) mixture + silica

Nanocomposites Processing

EVA nanocomposite blend formulations were made in a laboratory-scale internal batch mixer (Banbury-type). The blend-batch was dropped onto a two-roll mill mixer where crosslinking and foaming agents were incorporated and the nanocomposite was sheeted out. Nanocomposite sheets were compression-moulded using a hydraulic press. During the moulding process, EVA curing and foaming took place.

Nanofillers and the rest of additives were handled and processed following the safety recommendations given by the National Institute for Safety and Health at Work and the required personal protective equipment was wore by the personnel.

Composites Characterisation

The curing characteristics of EVA composites were analyzed in a moving die rheometer (MDR). The stiffness change of the nanocomposite, which is compressed between two heated dies is oscillated and measured by the rotor. As a result, a cure curve representing the evolution of the elastic modulus (S', also known as storage modules or in phase modulus, measured in lbs·in) as a function of time is produced by the MDR. In this work, the evolution of S' as a function of the time was measured at 170ºC, with an oscillation frequency of 1.6 Hz.

The homogeneity of the dispersion of Ti based additive in blends was assessed by Inductively coupled plasma mass spectrometry (ICP-MS). Three samples were taken at different positions in the uncured nanocomposite sheets and Ti concentration was determined.

RESULTS AND DISCUSSION

Blank EVA and 7 formulations including antistatic nanoadditives have been prepared (see Table 2).

Table 2. EVA base nanocomposites formulations

Batch Reference	Antistatic additive	Dosage
Blank	-	
PNC1-1	NF1	recommended
PNC1-2		>
PNC2-1	NF2	recommended
PNC3-1	NF3	<
PNC3-2		recommended
PNC4-1	NF4	recommended
PNC4-2		>

PNC1-1 and PNC1-2 nanocomposites showed an oily aspect and migration of some component seemed to occur in the uncured sheet. Even though this aspect disappeared after curing in the curemeter, a further processing of the sheets in a calander prior to press-moulding produced the exfoliation of PNC1-2, showing the typical aspect of incompatible polymer blends.

With regards to nanocomposite PNC2-1, the dispersion of black nanofillers within the EVA matrix leads to the formation of uniformly black samples. The carbon base nanofillers dispersion and distribution throughout the EVA matrices can thus be considered as macroscopically homogeneous. Nevertheless, EVA nanocomposites should be evaluated by transmission electron microscopy (TEM) in order to asses the suitable dispersion of the nanofillers into the polymer matrix at nanoscale.

Some difficulties have arisen when compounding PNC3 formulations. They were too sticky and difficult to be handled during processing. A revision of the formulations was made. Nevertheless, composites based in NF3 still showed difficulties in handling.

The increment of nanoadditive concentration in PNC4 series had a plastifying effect and in the case of PNC4-2 a reduction of the shear during blending in the Banbury-type mixer is produced. This effect was considered to potentially lead to a poor dispersion of the nanofiller and other components into the polymer matrix. This assumption was confirmed by ICP-MS analysis. Three samples were taken at different positions in the uncured nanocomposite sheet. Concentration of Ti was evaluated and standard deviation of measured values was considered as an indicator of dispersion effectiveness.

It has been observed that a homogeneous dispersion of the nanofiller has been achieved in PNC4-1 (standard deviation $\sigma = 0.45$). Nevertheless, PNC4-2 shows a heterogeneous distribution of the additive (standard deviation $\sigma = 253$). Such result confirms the assumption that a poor mixing of components could have taken place during PNC4-2 formulation due to a plastifying effect of this nanoadditive. Therefore, the whole EVA nanocomposite formulation and mixing process must be revised in a next step.

Therefore, and from a point of view of processability, PNC1-1, PNC2-1 and PNC4-1 are potential candidates for the development conductive EVA-based nanocomposites.

Finally, Figure 1 shows, for each reference, the curing curve from the moving die rheometer. Curing curves of the different EVA nanocomposites are compared with the one obtained for blank EVA. The effect of the nanoadditive concentration has also been assessed.

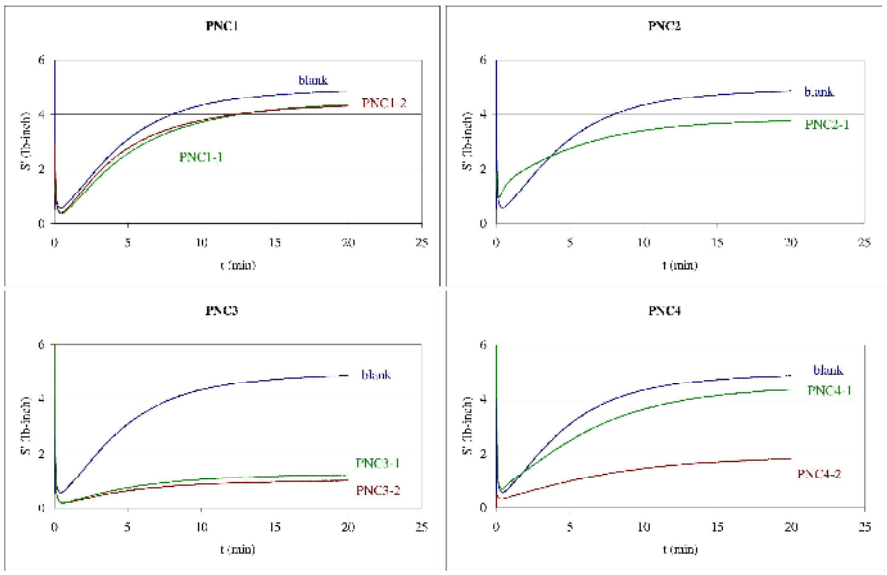

Figure 1. Nanocomposites of EVA. Effect of nanofillers in the curing curve

Curing curves show that, in general, the value of maximum elastic modulus (S'max) decreases after the addition of nanofillers. This decrease is more evident when increasing the percentage of nanoadditive. Therefore, the incorporation of nanofillers can affect both curing and foaming processes during moulding, thus affecting the final physical-mechanical properties of the nanocomposites. An important loss of properties is expected in those nanocomposites showing a more dramatic drop in S'max value, namely PNC3 series and PNC4-2.

CONCLUSIONS

In general, the addition of nanofillers affects processability of EVA-based formulations, being this effect more evident when increasing the percentage of additive.

Furthermore, the curing curve is affected in some extent by the addition of nanofillers. Therefore, an effect on the physical-mechanical properties of the EVA foam is expected.

According to this work, PNC1-1, PNC2-1 and PNC4-1 seems to be the most promising nanocomposites considered, since they show adequate processability, and an apparently good dispersion of the nanofiller. Furthermore, they show a low decrease in the maximum elastic modulus in the curing curve, and minor effects in the mechanical properties of the composite are expected.

Nevertheless, care must be taken in the dosage of NF1 and NF4, since composites formulated with higher percentages can show incompatibility with EVA matrix (PNC1-2) or an ineffective dispersion of the filler plus expected low properties of foamed material (PNC4-2).

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GROWTH OF LEATHER SECTOR IN ASIAN COUNTRIES AND RECENT ENVIRONMENTAL DEVELOPMENTS IN WORLD LEATHER SECTOR

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The leather production activities, specifically raw to semi-finishing processes have been shifted from United States, West European countries to Asian, South American and other developing countries. Annual World Leather process is estimated at 16 million tons of hides and skins. More than 50% of World leather production is done in Asian countries such as China, India, Vietnam, Bangladesh, Pakistan, etc. Wastewater discharge from Asian tanneries is more than 350 million m3/annum. Solid waste generation is about 4 million tons/year. Safe disposal of chrome containing sludge which is about 6 million tons/year from the entire World leather sector is one of the major unresolved issues. Environmental regulations and standards are similar in developing and developed countries. Certain parameters are more stringent in developing countries when compared to the developed countries. Major investments are being made for the environmental protection systems and resettlement of tanneries from urban areas to the industrial parks with Common Effluent Treatment Plants (CETPs). New regulations such as restriction on use of chemicals, control on salinity and water recovery under zero discharge concepts, management of chrome containing sludge etc. envisage continued applied Research & Development activity. Asian International Union of Environment (AIUE) Commission has got about 30 technical members from all major Leather producing countries in Asia, Russian Federation, IULTCS, UNIDO and European Union (EU). The recent technical developments to meet the environmental challenges with specific reference to Asian countries, Europe and Latin America are dealt in this technical paper.

Keywords: AIUE, Environment, Asian Leather

INTRODUCTION

The Asian International Union of Environment (AIUE) Commission is being launched in association with 10th Asian International Conference on Leather Science and Technology (AICLST). There are 30 technical members from various countries and invitees from IULTCS, United Nations Industrial Organization (UNIDO), European Union (EU) and other relevant international organizations.

AIUE has developed the following documents and they are being updated periodically. The list of AIUE Commission documents are given in Table 1.

Table 1. Documents of AIUE Commission

Doc.No.	Title
AIUE 1	Viable cleaner technologies for leather production
AIUE 2	Management of chrome containing waste
AIUE 3	Document on total dissolved solids in tannery effluent
AIUE 4	Options for tannery solid by- product management
AIUE 5	Typical performance for tannery wastewater treatment
AIUE 6	Typical pollution values related to tannery processes
AIUE 7	Odour control options in tanneries & effluent treatment Plants
AIUE 8	Special sewer system for conveyance of tannery effluent
AIUE 9	Recommendations for occupational safety and health aspects
AIUE 10	Concept and Guidelines for Environmental Footprint for Leather Sector
AIUE 11	Environmental update in World Leather Sector

WASTE DISCHARGES FROM ASIAN TANNERIES

Annual World leather process is estimated at 16 million tons of hides and skins. More than 50% of World leather production is done in Asian countries such as China, India, Vietnam, Bangladesh, Pakistan, etc. Wastewater discharge from World tannery sector is about 600 million m^3/annum. From Asian tanneries more than 350 million m^3 of waste water is discharged per annum. Solid waste from Asian tanneries and sludge generation from effluent treatment plants in Asian region are comparatively higher. This is mainly due to the use of poor quality chemicals in liming and other operations and also in effluent treatment plants for physiochemical treatment.

Safe disposal of large volume of chrome containing sludge which is about 6 million tons/year from the World Leather Sector is one of the major unresolved issues. Due to this, many tanneries started using quality chemicals and adopt waste minimization practice. Many individual and Common Effluent Treatment Plants (CETPs) in Italy and other countries have avoided chemical treatment and adopted total biological oxidation system with high detention time of 4-6 days to minimize sludge generation.

ENVIRONMENTAL REGULATIONS & MANAGEMENT

Almost all the leather processing countries including Asian and African countries have introduced pollution control standards similar to the standards adopted in United States, European Union and other developed countries. In view of the serious environmental issues, cleaner production and implementation of Common Effluent Treatment Plants (CETPs) in tannery clusters, relocation and resettlement of tanneries from urban towns to designated industrial areas are the recent development in countries such as Spain, Turkey, India, China etc with major investments. Countries such as Bangladesh, Egypt etc. have planned to relocate the cluster of tanneries from the cities to new industrial zones with CETPs. In many countries including in India new tanneries or expansion of the existing tanneries are permitted only in authorized industrial parks with Common Effluent Treatment Plants (CETPs).

The sustainability of the small-scale units has become a serious issue in leather sector due to enforcement of environmental regulation in many countries 400 small-scale tannery units have been closed in China during recent years. Currently environment is the major area of research carried out by the leather research institutes and Universities. More than 50% of the research publications in the World Leather Sector deal with cleaner production & waste management.

With a view to control salinity and environmental protection in countries such as Brazil the hides and skins from the slaughter house needs to be processed immediately without preservation using common salt. During the International recession period there was no demand for the wet blue/finished leather, and the disposal of unsalted hides and skins had become a major environmental issue in Brazil. The organized slaughter houses in Brazil and other countries are building their own tanneries to process fresh hides and skins without applying salt for preservation. Management of high chlorides and salinity in the tannery effluent has become a serious environmental threat in many countries including Spain, India & China etc. They have started adopting membrane system for water recovery and costly treatment of the saline rejects from the membrane system. Multiple stage evaporators have been adopted for evaporation of the saline stream from membrane system with huge cost. Proper environmental solution is to be

developed for the disposal of the mixed contaminated salt recovered from the evaporator particularly in land locked areas.

The recent developments in cleaner production and waste management in selected leather producing countries are given in Table 2.

Table 2. Research & Development in Environmental Protection

S.No.	Country	Research & Development
1.	ARGENTINA	Cleaner Production and establishing new standard procedures through Commission of Ecology Control are current R & D activities. Restriction / Refusal for the disposal of chrome containing sludge to the common landfill "Green Peace" Movement targets for effluent treatment and management are some of the recent challenges in Argentina.
2.	BRAZIL	Applied R & D activities such as photo-electro oxidation and electro dialysis for water recovery and reuse are being carried out in Federal University of Rio Grande do Sul and SENAI Leather Center. Controlled incineration of chrome tanned wastes and development of constructed wetlands for effluent treatment in some tanneries at pilot scale are some of the recent field applications. Meeting toxicity standards, restriction in the disposal of chrome containing sludge even in common secure land fill site are some of the recent challenges.
3.	CHINA	Currently there are about 800 tanneries. Till now, about 13 CETPs are in operation, some more are under planning. Planned to reduce the volume of water usage and pollution load at source through cleaner production programme. The tanneries are permitted to expand the capacity without increase in the water usage. One of the major tanneries has implemented the MBR and RO system for water recovery and reuse. As such there is no specific restriction on the Total Dissolved Solids (TDS) or salinity norms for the disposal of treated effluent. However meeting the BOD, COD norms for the saline streams from RO is one of the issues being addressed by new technological development. As a sustainability measure new licenses are given to tanneries with a processing capacity of more than 3000 tons /year of raw hides and skins.
4.	COLOMBIA	In view of the serious environmental issues, cleaner production, implementation and maintenance of Effluent Treatment Plants have become necessary in all the tanneries in Colombia. During the recent years, there had been many changes in the regulations related to environmental impacts for the general industry in Colombia. Those changes are related to waste water discharges and now the latest addition is odour control.
5.	FRANCE	Tallow extracted from fleshing converted into alternative energy source, Reed bed system is installed for effluent treatment.
6.	INDIA	A biggest CETP in Asia with a capacity of 48,000 m^3/day (48 MLD) for 450 tanneries is being planned with a budget of about 60 million USD in Kanpur city. Zero Liquid Discharge concepts by adopting membrane system for recovery of water from tannery effluent have been implemented in the South Indian tanneries at a cost of about 100 million USD. Disposal of the saline stream from membrane units in land locked areas is one of the unresolved technical challenges. Decentralized secure landfill system linked with CETPs for leather sector had been implemented in many tannery clusters. (First of its kind in the World). R&D activities on bio processing are under progress.

S.No.	Country	Research & Development
7.	NEW ZEALAND	Enzymes and unhairing process is becoming more popular. Elimination of salting of skins by introducing chilling process in selected areas, Sulphide oxidation, pH & settleable solids control and discharge of effluent into public sewer system.
8.	POLAND	Processing of organic materials and converting into fuel called as bio-coal, Co-fermentation of chromium-free tannery wastes with municipal sewage sludge and conversion into fertilizer are the recent developments.
9.	ROMANIA	Applied R & D programme on Cleaner Production and technology dissemination are being carried out with the co-operation of INCDTP / ICPI, Institutions, Universities & organization such as COTANCE, European Union, etc.
10.	SPAIN	The tannery clusters with CETP are located in Igualada near Barcelona and Lorca near Murca a coastal town in the southern part of Spain. The CETP in Igualada with a capacity of 9 MLD has been established with a capital cost of 13 million Euros. Membrane Bio Reactor with Reverse Osmosis (RO) for water recovery has been established in the CETP near Lorca. The water recovery system from a tannery CETP is first of its kind in the world and was commissioned during 2004-2005. The system has faced with some technical and economical issues in saline water evaporation system in the landlocked area. R & D activities on cleaner production and waste minimization are being carried out by the institutions in Spain: INESCOP, AIICA and EEI (Universitat Politecnica de Catalunya)
11.	TAIWAN	Currently there are about 50 tanneries in operation in Taiwan. The tanneries are having individual treatment plants with capacities ranging from 300 m^3–2000 m^3/day. They adopt conventional physio-chemical and biological treatment systems.
12.	TUNISIA	Integrated cleaner production programme has been carried out for 12 vegetable tanneries in Tunisia; Research & Development on solid sludge is under progress in co-operation with CTC.
13.	TURKEY	There are about 540 tanneries existing in 14 zones viz. Tuzla(51), İzmir(24), Çorlu(78), Gerede(120), Bursa(20), Uşak(26), Gönen(18), Manisa(30), Biga(13), Denizli(20), Isparta(23), Bor(75), G.Antep(10) and Antakya(33). Eight Common Effluent Treatment Plants (CETPs) have been established and are in operation. The biggest CETP with a capacity of 36000 m^3/day has been established in Tuzla Industrial estate near Istanbul. The other CETPs are having capacities ranging from 1800 m^3/day to 36000 m^3/day. The tanneries had been resettled in industrial zones. R&D activities on cleaner production and environmental protection are being continued in universities such as Ege University, Izmir etc. Sludge disposal is a major problem similar to other countries.
14.	UNITED KINGDOM	Bio-diesel from tallow, Bio-ethanol from protenised wastes; short-term preservation of raw hides; technical assistance on cleaner production; adoption of membrane system etc. to other countries.
15.	URUGUAY	There are about 23 working tanneries in Uruguay. Two big and some of the medium tanneries have effluent treatment plants and they have also specific secure landfill places to dispose the solid wastes. Many cleaner production projects are being carried out with the involvement of several organizations. Currently, the main environmental problem to be addressed in

S.No.	Country	Research & Development
		Uruguay is the disposal of solid waste generated by the tanneries located on the Southern part of the country.

SUSTAINABILITY IN MEETING ENVIRONMENTAL CHALLENGES

The leather production activities especially raw to semi-finishing process are being shifted from the developed nations such as United States, West European countries, to Asian, North African and Latin American countries. The major leather producing countries such as China, Italy, India etc. are facing problems due to enforcement of stringent environmental regulations. The sustainability of the small-scale units is becoming a serious issue to meet the environmental requirements. Major investment is being made for environmental protection and resettlement of tanneries from the urban areas to the industrial parks with common effluent treatment plants. New regulations and restrictions such as REACH on the use of certain chemicals, salinity and water recovery under zero discharge concept, disposal/ management of chrome containing sludge etc. envisage continued Research & Development activity. Innovative tanning processes which will greatly reduce the water and chemical usage and minimize solid waste generation are needed together with overall environmental planning and management.

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BLOOD LEAD CONCENTRATIONS OF HORSES AND DONKEYS IN THE VICINITY OF HEAVILY POLLUTED RIVER BY INTENSIVE INDUSTRY IN SOUTHEASTERN TURKEY

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The aim of the present study was to determine the concentration of Pb were assessed in the blood of horses and donkeys which were living in the vicinity of Nizip River where is the discharging area of intensive industry of Gaziantep City. A total of 66 (< 15 years) male horses and donkeys (41 horses and 25 donkeys) were sampled during 2005-2006. The concentration of the blood Pb concentrations were determined by the atomic absorption spectrometry (AAS) method. Mean concentrations of Pb in horses blood serum ranged from 0.06 to 1.88 ppm, and in donkeys from 0.20 to 2,23 ppm. The study allows concluding that the levels of Pb in both horses and donkey are significantly high depending on the feeding or grazing by the products that are grown in the agricultural area where irrigated with pollutioned river water or using it as a drinking sources.

Keywords: blood serum, donkey, horse, lead, pollution

INTRODUCTION

Environmental pollution due to the heavy metals is an increasingly important problem (Arslan *et al.*, 2011; Yarsan *et al.*, 2014; Yipel and Yarsan, 2014). The accumulation of metals in soil and water is of great concern because has adverse effects on environmental health, crop growth, and potential health risk to the local inhabitants by ingested or transferred through food (Dounay *et al.*, 2013). Toxic metal levels such as lead (Pb) in biological samples like blood in donkeys and horses are used to assess diseases (Asano *et al.*, 2002). Because of contact with organs and tissues where chemicals are stored, blood is the ideal matrix for most chemicals (Esteban and Castano, 2009). The most commonly used biomarker of Pb exposure is the Pb concentration in whole blood because of %60-90 of absorbed Pb found in erythrocytes (Nordberg *et al.*, 2007). It has been known by current studies that Pb exposure connected to cardiovascular (Weisskopf *et al.*, 2009), gastrointestinal, renal, nervous, musculoskeletal and haematopoietic diseases (Puschner and Aleman, 2010), lower body mass index, obesity (Scinicariello *et al.*, 2013), infertility (Rahman *et al.*, 2013) and crosses the placental barrier (Puschner and Aleman, 2010). Additionally Pb has a special concern about its neurotoxical effects (Zubero *et al.*, 2010) on animals like muscle twitching, convulsive seizures, depression, eyelid snapping, teeth grinding and blindness (Arslan *et al.*, 2011; Puschner and Aleman, 2010). Besides it leads to anaemia and renal damage (Zubero *et al.*, 2010). The toxicity is due a to the disruption of the prooxidant and antioxidant balance of the cells by Pb (Arslan *et al.*, 2011). In horses 0.2-0.5 ppm of blood Pb levels associated with chronic intoxication while blood <0.2 ppm is acceptable (Palacios *et al.*, 2002; Puschner and Aleman, 2010). Donkeys and horses are exposed to environmental effects of metals and at risk of chronic Pb poisoning feeding or grazing on contaminated pastures by polluted river or using it as a

drinking sources due to their long life span (Donkeys 40 years, horses 50 years) (Janiszewska and Cieśla, 2002; Puschner and Aleman, 2010). The main entranceway of Pb to the food chain of animals are Pb accumulated plants. Thus the pastures near industrial sites or polluted rivers must be evaluated for potentially toxic concentrations of Pb and other pollutants (Puschner and Aleman, 2010).

The Gaziantep City is placed in the in the southeast of Turkey which is a vital commercial, economic and industrial center with a population of over 1.5 million people. Although there are 67 large-scale industrial firms (27 textile manufacturing, 17 chemical, 6 cement construction, 3 energy, 3 machine manufacturing and 11 in other industrial fields) the city has only one urban wastewater treatment plant. Also a number of industrial activities (leather processing etc.) which are still not registered. The main source of the wastewater is industrial zone that discharge into the Nizip River (Avci, 2012). In a current study that aims to determine the heavy metal levels of wastewater of factories involved in various sectors within the province of Gaziantep City, has been emphasized that even after waste treatment a significantly high level of Pb (especially in motor oil industries, battery manufacturing industry wastes) has been determined which is classificated as ''can not be discharged'' (Yılmaz and Dinç, 2013). The Gaziantep City is placed in the southeast of Turkey and has the most developed industries in the region with over 1.5 million population. The river is 80 km length and a large land area (over 65,000 acres) where the samples collected surround of it is 45 km far away the Gaziantep City that uses this river water for agricultural applications which is contaminated with chemicals especially heavy metals. The Nizip Town and their urban environment has been exposed to the chemical pollution heavily by rivers and air due to intensive industrial activities in the Gaziantep City over the past two decades. Especially the Nizip River is the main sources of the inorganic pollution due to being main discharge area of urban and industrial wastewaters of Gaziantep City. The heavy metals enter the food chain of horses and donkeys primarily by using the river water in the growing process of plants that they grazing or feeding by (Avci, 2012). In a current research that aims to determine the metal levels in some plants which irrigated by river water such as maize (Zea mays L.) that is mostly consumed agricultural plant by local inhabitants and animals which has been sampled from Salkım village that is located on the area 1 (Fig.1). The researchers determined the increased Pb levels ($P< 0.05$) in all plant, soil and water samples to compare with control area by repeated investigations (Kafadar and Saygıdeger, 2010). On the other hand there is no data currently about use of Pb-containing pesticides or insecticide in the study area.

The present paper is concerned with the possible effects of polluted river by Pb intensively on human being and others by determine the blood serum levels of the donkeys and horses from southeastern Anatolia of Turkey.

MATERIALS AND METHODS

Study Area and Sampling

The blood samples were collected during the period between 2005-2006 from the areas where people were using water of Nizip River in agriculture for a long time depending on their living at a smaller distance (≤ 1 km) to the river (see in figure 1).

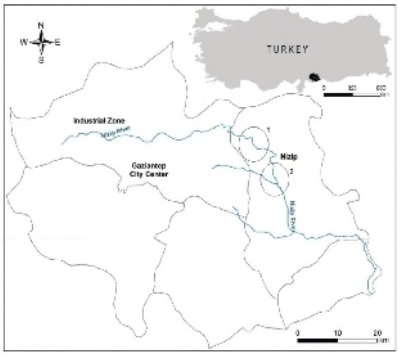

Figure 1. Sampling areas

The blood of 25 donkeys and 41 horses were used as the sample in this study because it has been considered as ideal matrix for biomonitoring of Pb (Esteban and Castano, 2009). All the animals that selected for the sampling were male and at the age between 1-15 years. The blood samples were taken 10 ml in to anticoagulant tubes from the vena jugularis after cleaned of the region by ethanol and deionized water by a sterile plastic syringe and needle and after immediately 20% trichloroacetic acid (TCA) was added was centrifuged (Arslan, 2011). The samples were frozen and stored at -20 until analysis.

Instrumental Analysis

Blood Pb levels were determined by atomic absorption spectrometry (ASS) (Unicam-929).

Statistical Analysis

The results were statistically analysed by using independent samples of t-test or Mann Whitney U-test. A p value less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Sample Profile

A total of 66 animals (Donkeys *n=25* and Horses *n=41*) that are members of farmer communities in the eastern traditional farming villages of Gaziantep City were studied. The ages of the included animals, who were divided in to two groups as under 5 years and over 5 years old, ranged from 1 to 15 years, with the mean age equalling 5.3 years in total *(n=66)*, 2.6 years in group 1 *(n=45)*, 9.2 years in group 2 *(n=21)*. 37.9 percent of the sample belongs to donkeys, and all the animals were feeding or grazing mainly by the agricultural plants which are grown in irrigated lands by Nizip River.

The majority part of the animals were healthy in terms of clinical findings (gastrointestinal, urinary, neurological, and dermatological vs.) outside of common intestinal parasite infestation.

Horses, Donkeys and Lead

Nizip River more than 8 mounts of a year. 38 percent of horses and 53 of donkeys use the river water for drinking if it is necessary.

Blood Pb Distributions of Horses and Donkeys

Among the study animals *(n=66)*, the median blood Pb level was 0.96 ppm in the range from the minimum 0.06 ppm, to the maximum 2.23 ppm 0.06-2.23 (Table1). Mean Pb concentration in the horse blood serum (*n=27*) was found as 0.82 ppm in the range from the minimum 0.06 ppm, to the maximum 1.43 ppm and in donkeys (*n=27*) 0.88 ppm in the range from the minimum 0.20 ppm, to the maximum 1.64 ppm for group 1 (<5 years old). While mean Pb concentrations in the horse blood serum (*n=14*) were found as 1.14 ppm in the range from the minimum 0.28 ppm, to the maximum 1.88 ppm and in donkeys (*n=17*) 1.37 ppm in the range from the minimum 0.54 ppm, to the maximum 2.23 ppm for group 2 (>5 years old). Blood Pb levels in the total horse samples ranged from 0.06 to 1.88 ppm, with the mean level 0.93 (SD=0.42) (*n=41*) and donkeys ranged from 0.20 to 2.23 ppm, with the mean level 1,01 (sd=0.46) (*n=25*). More than eighty percent of the horses and eighty four percent of the donkeys had blood Pb levels greater than or equal to 0.5 ppm that is recognized as a chronic Pb intoxication in references (Palacios *et al.*, 2002).

Table 1 gives the blood Pb distributions by groups of age. As can be seen, blood Pb levels were higher in 5 years old and younger group relative to 5 years old and older group (p=0.02). Horses also had the highest proportion (%54.5) of samples with blood Pb levels >5 ppm, compared to donkeys.

Table 1. Levels of Pb (ppm) in horses and donkeys

		< 5 years	> 5 years	Total	p[a]
Horse	N	27	14	41	0,02
	mean	0,82 ppm	1,14 ppm	0,93 ppm	
	Standard deviation	0,38	0,44	0,42	
	Min.–Max.	0,06-1,43	0,28-1,88	0,06-1,88	
Donkey	N	18	7	25	0,01
	mean	0,88 ppm	1,37 ppm	1,01 ppm	
	Standard deviation	0,37	0,51	0,47	
	Min.–Max.	0,20-1,64	0,54-2,23	0,20-2,23	
Total	N	45	21	66	0,02
	mean	0.84	1,22	0,96	
	Standard deviation	0,35	0,32	0,44	
	Min.–Max.	0,06-1,64	0,28-2,23	0,06-2,23	

[a]Mann–Whitney test

It has been demonstrated by findings of our investigation that higher blood Pb levels (>0.2 ppm) of healthy horses and donkeys were associated with Pb intake by ingestion over tolerable concentrations. As is well known feeding is one of the environmental factor that controls the concentrations of various elements in the animal body. Combined with results of current researches (Kafadar and Saygıdeğer, 2010; Yılmaz and Dinç, 2013) it has been understood that the source of the Pb is diet. The major part of their diets is forming by plants which accumulated Pb in high concentrations because of irrigation by Nizip River. The river has been heavily pollutioned by extensive

industrial activities of Gaziantep City which has an inadequate waste treatment process. Also one of the main way of Pb intake is inhalation but distance of city centre of Gaziantep (45 km) and industry area (50 km) and there is no road (closer than 15 km) with intensive traffic. Thus it can be considered as not necessary the Pb intake by inhalation. Statistical analysis showed that diet Pb levels has significant correlation with blood Pb level. Also, there was significant difference in Pb levels between group 1 (<5 years) and group 2 (>5 years).

The Joint FAO/WHO Expert Committee on Food Additives established a provisional tolerable weekly intake of 0.05 mg/kg b.w. for adult humans. However, increased blood Pb levels did occur when the dietary intakes of Pb were 8-9 µg/kg b.w./day (JECFA, 2013).

Horses from Gaziantep City exhibited relatively low blood Pb levels compared with horses from other cities, and higher Pb levels than donkeys. This may be related to leaded gasoline environmental pollution and children's hand-to-mouth activities.

CONCLUSIONS

Even after treatment of the Gaziantep City industrial wastewaters Pb level is high according to current studies. However, discharged wastewaters and an agricultural crops used for foodstuffs did not comply with the legislation of authorities. Due to discharging wastewater of intensive industrial and urban activities, the Nizip River and agricultural area that located around the river is strongly contaminated by Pb and create to risk on ecology. With this study the reflection of the ecological risks on some animals like donkeys and horses has been demonstrated. The elder participant's metal concentrations higher than young's associated with exposure time.

This observed results could have significant implications for assessing of health risks to local inhabitants and consumers of agricultural and livestock products of the region as well as investigations of ecological risk and monitoring studies.

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V.
CULTURAL HERITAGE

EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON VEGETABLE TANNED LEATHER STUDIED BY THERMAL ANALYSIS

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The present paper reports the results obtained by Differential Scanning Calorimetry (DSC) and Micro Hot Table method (MHT) for new vegetable tanned leathers exposed to 80°C and 80% RH for 1 to 32 days. DSC measurements were carried out both in water excess (heating rate 10 C•min^{-1}, temperature range 25 to 110°C), and under nitrogen flow (heating rate of 10 K•min^{-1}, temperature range 25 to 280°C). MHT method was used to measure the shrinkage temperature of collagen fibres. The results on hydrothermal stability obtained using these two techniques were compared. In general, collagen denaturation and shrinkage temperature decreased with time exposure, whereas the melting temperature of collagen crystalline fraction, obtained by DSC analysis in dry nitrogen flow, remained practically constant.

Keywords: vegetable tanned leather, DSC, MHT.

INTRODUCTION

The chemical degradation of vegetable tanned leather is mainly caused by acid hydrolysis and oxidation induced by environmental deteriorative factors such as air pollutants, heat and light. The type of tannin highly influences both the pattern and rate of deterioration of leather.

In the last two decades, great attention has been dedicated to the heritage materials, objects, and artefacts made of leather and parchment through several research projects (PERGAMO, PELRESTAURO, PN STEP, ENVIRONMENT, IDAP and MEMORI). One of their main aims has concerned with identification of physical-chemical changes that occurred in historic and naturally aged leathers and better understanding the relation between degradation observed at different levels, from macroscopic to molecular level. The effects of temperature and humidity on hydrothermal stability of collagen can assist in providing adequate microclimate conditions for the collagen-based collections. Degradation in parchment was more extensively studied by comparing with leather using various physical-chemical techniques such as optical microscopy and collagen fibre shrinkage measurement by Micro Hot Table (MHT) method (Larsen *et al.*, 1993), thermogravimetry (TG/DTG) and differential scanning calorimetry (DSC) (Badea *et al.*, 2011; Budrugeac and Miu, 2008; Budrugeac *et al.*, 2010; 2011; Badea *et al.*, 2008), infrared spectroscopy (FTIR) (Badea *et al.*, 2008; Odlyha *et al.*, 2009), Raman spectroscopy (Bicchieri *et al.*, 2011), X-ray diffraction, X-

ray scattering and Micro-X-ray fluorescence (Mozir *et al.*, 2012), nuclear magnetic resonance (NMR) (Badea *et al.*, 2008; Odlyha *et al.*, 2009; Bicchieri *et al.*, 2011; Mozir *et al.*, 2012; Mašić *et al.*, 2012), scanning electron microscopy (SEM) (Badea *et al.*, 2008) and atomic force microscopy (AFM) (Odlyha *et al.*, 2009).

In this paper we present the results obtained for artificially aged vegetable-tanned leathers using Differential Scanning Calorimetry (DSC) and Micro Hot Table method (MHT).

MATERIALS

New vegetable tanned leather from sheep and calf hides, each tanned with mimosa, quebracho and chestnut extracts (Table 1) were prepared at the Leather and Footwear Research Institute Bucharest according to traditional recipes.

Table 1. List of the new manufactured leathers

New leathers		
Animal species	Tannin type	Symbol
sheep	Mimosa	SM
sheep	quebracho	SQ
sheep	Chestnut	SC
calf	Mimosa	CM
calf	quebracho	CQ
calf	Chestnut	CC

ARTIFICIAL AGEING TREATMENT

The artificial ageing treatment consisted in heating the samples at 80°C in a thermo-controlled oven for 1, 2, 4, 8, 16 and 32 days. A controlled 80% RH was maintained by keeping samples in a desiccator over a saturated KCl solution. Samples were treated in the Institute for Science and Technology in Art, Academy of Fine Arts,Vienna, Austria.

METHODS

Differential Scanning Calorimetry

DSC measurements were made with a DSC 204 F1 Phoenix (Netzsch, Germany) instrument. Samples of about 1-5 mg were measured in:

i. *excess water conditions,* using hermetically sealed aluminum pans in which samples were stocked with 30 µl distilled water for 24 h. Samples were heated from 25 to 110 °C at 10 C·min^{-1} heating rate.

ii. *in dry conditions, using* open aluminum pans and nitrogen flow (20 mL·min^{-1}, gas purity: 99.999%). Samples were measured from 25 to 280 °C at 10·C min^{-1} heating rate.

Micro Hot Table Method (MHT)

MHT measurements were performed with an easy-to-use equipment composed of a stereo microscope Leica S4E with a camera and a hot table Caloris equipped with a

FP90 temperature processor and a home-made software F.L.T.K. 1.1.X for temperature regulation and data collection. Magnification used was x40.

Micro-samples of 10-15 fibres from the flesh side were thoroughly wetted and separated in demineralised water, placed on a microscope slide with a concavity and left 10 min for homogeneous hydration. Hydrated fibres were separated as much as possible under a light microscope using a pair of fine needles and then covered with a cover glass, placed on the hot table and heated at $2°C \cdot min^{-1}$. The shrinkage process was digitally recorded and shrinkage temperature determined.

RESULTS

Collagen Denaturation in Hydrated State

The thermal denaturation of collagen in water can be characterized by both the MHT method and DSC analysis in excess water conditions. The shrinkage temperature T_s of collagen fibres is determined by MHT method, whereas the extrapolated onset temperature T_{onset} of the DSC peak associated with collagen thermal denaturation is measured by DSC.

The DSC peaks of new vegetable tanned leathers measured in excess water conditions displayed sharp and symmetrical shapes, with an onset temperature ranging from 80°C (chestnut tanned sheep leather) and 86°C (quebracho tanned calf leather). These values are in good agreement with those in the literature (Budrugeac *et al.*, 2011). The symmetrical shape of the peaks suggests a uniform distribution of the collagen populations with different thermal stabilities (Larsen *et al.*, 1993) and, hence, a homogeneous tanning process. With ageing time, the DSC peaks shifted gradually to lower temperatures and became shorter and broader by comparison to the new, untreated sample, indicating an increasing heterogeneity due to the formation of collagen populations with distinct thermal stabilities (Figure 1).

DSC analysis of artificially aged leather samples showed that thermal behavior depends on both animal species (i.e. calf leather is more thermostable than sheep leather) and tannin type (i.e. mimosa and quebracho tanned leather are more thermostable than chestnut tanned leather).

The onset temperature T_{onset} measured by DSC and shrinkage temperature T_s measured by MHT are generally very close as they characterise the same proces, e.g. thermal denaturation of collagen at mesoscopic and macroscopic levels, respectively. Figure 2 shows the comparison between T_{onset} and T_s for mimosa tanned sheep leather exposed to artificial ageing. The small diference between these values can be related to the different heating rates used for the two types of measurement and to the measurement quality. In fact, T_{onset} is a bulk material property, while T_s reflects the hydrothermal stability of a few collagen fibres from surface (Budrugeac and Miu, 2008; Budrugeac *et al.*, 2010; 2011).

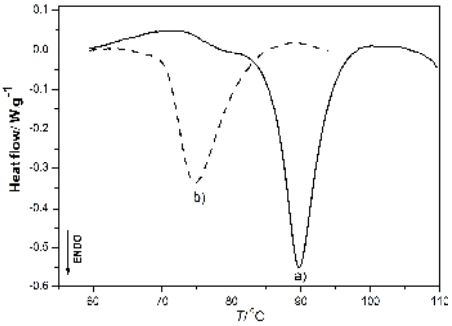

Figure 1. DSC curves obtained in sealed crucible for quebracho tanned sheep leather a) new, untreated and b) exposed to 80°C and 80% RH for 32 days

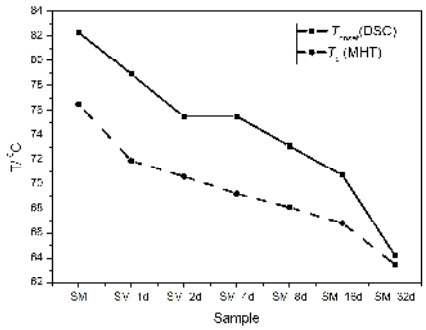

Figure 2. Comparison between the values of T_{onset}, measured by DSC in excess water conditions, and those of T_s, measured by MHT method, for mimosa tanned sheep leather during ageing

Collagen Denaturation in Dry State

DSC curves associated to the thermal transitions which tipically occur in parchment and leather samples measured in open crucibles and gas flow display a broad endothermic peak followed by one or more smaller endotherms (Figure 3). The larger DSC peak in the temperature range (50–110)°C corresponds to thermal dehydration of the sample. The first endotherm at about 129°C (T_{d1}) is related to denaturation of dehydrated collagen matrix, whereas the second peak at T > 220°C (T_{d2}) represents the thermal denaturation (or softening) of the crystalline collagen embedded in the amorphous matrix (Budrugeac *et al.*, 2011).

According to the literature (Budrugeac *et al.*, 2011), the tanning process stabilises the crystalline region by inducing cross-linking. The denaturation temperature of collagen crystalline fraction T_{d2} for the new vegetable tanned leathers showed to generally decrease on natural ageing and deterioration suggesting a progressive decrease of cross-linking degree (de-tanning process) (Budrugeac *et al.*, 2011). In our experiment, however, this value did not significantly change during the artificial ageing treatment, but a rather constant value of (245±5)°C was obtained for all investigated leathers.

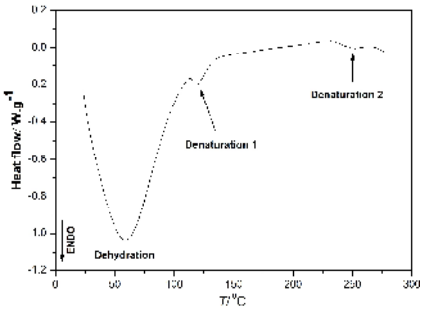

Figure 3. DSC curves obtained in open crucibles and nitrogen flow for new, untreated mimosa tanned sheep leather

T_{d1} values e.g. (122.0±2.9)°C were reported for new vegetable tanned leathers, while slightly high values, e.g. (125.7±2.9)°C were found for historical leathers (Budrugeac and Miu, 2008). In our experiment, this peak was observed for mimosa (113°C) and chestnut (104°C and 116°C) tanned leather only. Accelerated ageing did not induced significant variations of these values. The two DSC signals at 104°C and 116°C (Figure 4) may be ascribed to the presence of two collagen population with slightly distinct thermal stability.

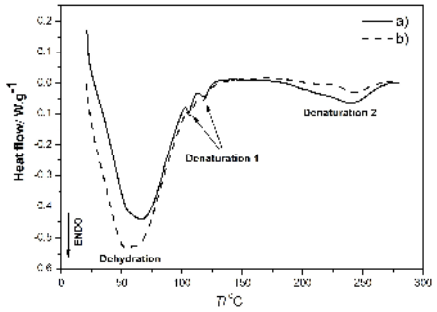

Figure 4. DSC curves obtained in open crucibles and nitrogen flow for chestnut tanned sheep leather a) new, untreated leather and b) leather exposed to 80°C and 80% RH for 32 days

CONCLUSIONS

The use of DSC and MHT method provides useful parameters as temperature of collagen denaturation in both hydrated and dry states, softening temperature of rigid, crystalline collagen and shrinkage temperature. The variations of these parameters enable us to evaluate the effect of accelerated ageing at 80°C and 80% RH for the vegetable tanned leather investigated.

In summary we observed:

(i) Temperature of denaturation measured in excess water, as well as shrinkage temperature decrease for all vegetable tanned leathers with time exposure.

(ii) Hydrothermal stability depends on both the animal species and tannin agent.

(iii) Softening temperature of crystalline collagen fraction does not significantly change during artificial ageing.

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EFFECT OF ACID RAIN ON VEGETABLE TANNED LEATHER

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Abstract: In order to study the influence of gaseous pollutants on leather, the artificial acid rain was used to soak the vegetable tanned leather, and then the leather was aged for 25 days in 50°C and 100% relative humidity to accelerate the aging speed. The mechanical properties, micro hot table (MHT), FT-IR, optical microscope, DSC and TG were used to analyze the change of leather during the aging process every 5 days. The results showed that the mechanical properties and shrinkage temperature of aged leather were decreasing, and the collagen fibers were damaged and leaded the amide I and amide II band moving to low wave number. Furthermore, the thermal denaturing temperature and the temperature of decomposition at max rate of aged leather were both dropped too. The longer aging time was, the more obvious impacts existed. In conclusion, the artificial acid rain has a significant aging effect on vegetable tanned leather.

Keywords: artificial acid rain; vegetable tanned leather; accelerated aging

INTRODUCTION

Vegetable tanning is one of the oldest tanning methods, therefore many collagen-based cultural relics are vegetable tanned leather. In addition, vegetable tanned leather is also used in modern society because of unique properties (Popescu *et al.*, 2008). However, vegetable tanned leather is often exposed in atmospheric air so that air pollution will lead to the aging and damage of vegetable tanned leather (Barbara *et al.*, 2012; Miu *et al.*, 2009; Deselnicu, 2010). Vegetable tanned leather is affected slowly by gaseous pollutants, which makes the study of leather aging difficult. Studying of the influence of the air pollution on vegetable tanned leather could provide theoretical support and advice for preservation of leather cultural relics and degradation of vegetable tanned leather.

In order to accelerate aging and simulate the effect of gaseous pollutants, the artificial acid rain was used to soak the vegetable tanned leather, and then the leather was aged for 25 days in 50°C and 100% relative humidity. With the persistent temperature and humidity, the leather aging was accelerated, and the samples were taken out every 5 days to characterize the change in the aging process.

EXPERIMENTAL

Materials

Quebracho extract tanned calf leather was obtained from Leather and Footwear Research Institute, Bucharest, Romania. Other chemical reagents used in this study were research grade.

Sampling

In order to reduce experimental error and increase the comparability of samples, six large leathers (10cm×7cm) were sampled along the back bone line adjacently and used for mechanical property tests. Another six small leathers (3cm×2cm) were sampled under the corresponding large leather and used for morphology, structure and thermal analysis. Among the twelve samples, five large and five small pieces were used for aging test, and the left two pieces were used as control.

Preparation of Artificial Acid Rain

The artificial acid rain (Lesu *et al.*, 2005) was prepared as follows: 0.04ml concentrated sulphuric acid, 0.06ml concentrated hydrochloric acid and 0.02ml ammonium hydroxide were dissolved in 80ml distilled water, then 0.0296g $Ca(OH)_2$ was added and diluted to 2L volumetric flask. The pH of artificial acid rain was 3.25.

Leather Aging

Ten pieces of leather samples were soaked in the artificial acid rain (liquor's weight was 20 times based on leathers) for 48 hours. Then the samples were sealed and aged in 50°C and 100% relative humidity (RH) for 25 days. A large and a corresponding small leather pieces were taken out every 5 days and dried in nature for 48 hours, then the samples were placed in a 65% RH desiccators for a week to equilibrium.

Analytical Method

Mechanical Properties

After conditioning, tensile strength, tear strength and elongation at break of leather were tested by tensile machine (AI-7000S, China) following a standard method.

Optical Microscope Analysis

The samples were sliced into 12 μm pieces by freezing microtome (Leica Company in German). After hematoxylin and eosin staining, the cross sections were observed by SZX12 optical microscope (Olympus Optical Co., Ltd) at 40 times magnification.

Shrinkage Temperature

Shrinkage temperature (Ts) was determined by the micro hot table (A WT2000, China). A few fibers were separated and wetted with distilled on microscope slide, and more than two fibers should be observed in the sight. Then the fibers were covered with a cover glass and heated at 2°C/min on the hot table and Ts was recorded when more than two fibers were shrinked simultaneously and continuously. The final results were the average of three tests (Larsen, R., 2002).

FT-IR Test

Samples were ground with KBr and made into pellets, then a Nicolet10 FT-IR (American Thermo Scientific Corporation) was used to scan in the wavelength range of 400-4000cm^{-1} for 32 times.

DSC Test

Samples were placed in 20°C and 65% RH for 24 hours. The samples were put into sealed stainless steel crucibles and heated at 10°C/min in N_2 atmosphere (flow N_2:100mL/min) with a Netzsch DSC PC200 calorimeter (Germany).

TG Test

Samples were placed in 20°C and 65% RH for 24 hours. The samples were put into Al_2O_3 crucibles and heated at 10°C/min in N_2 atmosphere (flow N_2:100mL/min); the range of temperature was from 40 to 800°C with a NETZSCH TG 209 F1 thermogravimetric analyzer (Germany).

RESULTS AND DISCUSSIONS

Mechanical Properties

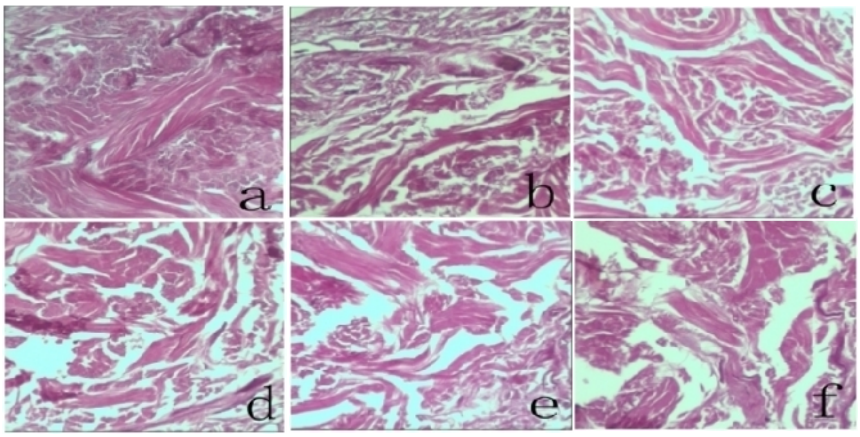
Table 1. Mechanical properties and Ts of leathers

Sample	Tensile strength N/mm^2	Tear strength N/mm	Elongation at break %	Ts °C
VL-0	7.6	31.4	26.674	83.7
VL-5	7.5	30.9	26.582	85.1
VL-10	7.2	28.7	25.899	80.7
VL-15	7.0	27.3	25.340	73.2
VL-20	5.9	24.8	25.095	71.0
VL-25	5.7	24.2	23.934	70.4

VL-0: control, VL-5: aging 5 days, VL-10: aging 10 days, VL-15: aging 15 days, VL-20: aging 20 days, VL-25: aging 25 days

As shown in Table 1, the tensile strength, tear strength and elongation at break of vegetable tanned leather were reduced with the increase of aging time, and the Ts was dropped too. Because of the damage of the artificial acid rain, the fibers and cross-linking between collagen were broken down, which decreased the stability and mechanical properties of leather.

Optical Microscope Photograph



a: VL-0 b:VL-5 c:VL-10 d:VL-15 e:VL-20 f:VL-25

Figure 1. Optical micrographs of aged leather (at a magnification of 40×)

As shown in figure 1, the collagen fibers of control were tight, but the gaps of collagen fibers were enlarged with the increase of aging time. These phenomena indicated that the collagen fibers were damaged by acid rain, which proved the conclusions of mechanical properties and Ts.

FT-IR Analysis

Table 2. The shift of Amide I and Amide II band of aged leather

Sample	Amide I cm^{-1}	ΔAmide I cm^{-1}	Amide II cm^{-1}	ΔAmide II cm^{-1}
VL-0	1648.66	0	1544.86	0
VL-5	1645.38	3.28	1532.60	12.26
VL-10	1639.81	8.85	1528.66	16.20
VL-15	1636.28	12.38	1525.49	19.37
VL-20	1631.85	16.81	1458.12	86.74
VL-25	1632.82	15.84	1457.90	86.96

The wave number of 1700 ~ 1500 cm^{-1} was a characteristic absorption peak of amide I and amide II band in leather, so they were selected to analyze the changing of leather during the aging process (Cyril *et al.*, 2006). As shown in table 2, with the increase of aging time, the amide I and amide II band were moved to lower wave number, and the amide II band was dropped significantly. During the process of aging test, main changes have been taken place on the group of C=O and NH, indicating that the collagen structure was changed because of acid rain.

Thermal Stability

Table 3. DSC and TG properties of aged leather

Sample	T_d/°C	ΔT_d	$\Delta_d H$/J/kg	T_{max}/°C	ΔT_{max}
VL-0	92.3	0	283.1	322.4	0
VL-5	88.3	4.0	260.7	313.8	8.6
VL-10	87.5	4.8	248.5	311.7	10.3
VL-15	86.5	5.8	235.3	310.7	11.3
VL-20	86.4	5.9	233.2	308.5	13.9
VL-25	83.7	8.6	230.8	300.4	22.0

For DSC, T_d was the thermal denaturing temperature of leather; $\Delta_d H$ was peak area of the DSC curves and represented enthalpy during heating process (Budrugeac *et al.*, 2010). In TG/DTG curve, T_{max} was the temperature of decomposition at maximum rate (Marcilla *et al.*, 2011). These values were listed in table 3. The results showed that T_d, $\Delta_d H$ and T_{max} were dropped with the increase of aging time. During the aging, multi-point hydrogen bonds between vegetable tanning agent and collagen peptide chains were destroyed and leaded to the cross-linking degree between collagen and tanning agent reduced, therefore thermal stability of leather was decreasing. After the destruction of the collagen fibers and cross-linking effect of tanning agent, it was easy to break down the structure of leather; therefore T_d, $\Delta_d H$ and T_{max} were also dropped.

CONCLUSION

The vegetable tanned leather was aged by artificial acid rain, with the increase of aging time, the mechanical properties were decreasing; the collagen fibers were broken down and the gap of fibers were enlarged; the Amide I and Amide band were moved to lower wave number; the thermal-stability was dropped obviously. In conclusion, the acid rain has a significant aging effect on vegetable tanned leather which leaded structure changed and performance reduced. Furthermore, with the increase of aging time, the impact on leather is more evident.

Acknowledgement

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CHARACTERIZATION OF THE EFFECT OF HEAT ON VEGETABLE TANNED LEATHER AND RESTORATION TRIALS THROUGH ENZYMATIC PROCESSES

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Cultural heritage artefacts made on leather may suffer from adverse condition during conservation that results in an irreversible change of their chemical and physical properties. Our research aims to develop a new restoration approach for leather having lost its flexibility after exposure to heat. The characterization of heat-damaged leather was performed by various technics such as Dynamic Mechanical Analysis (DMA) and contact angle measurement. Heat causes darkening, mass loss, shrinkage, stiffness increase and renders leather non wettable. Part of these changes can be due to an aggregation of leather proteins as a result of heat exposure. An innovative method relying on the use of biological molecules was developed in order to respect the nature of the object and preserve its past and future. Enzymes such as hydrolases able to break the protein aggregates have been used. One of the challenges was to provide water necessary for the enzyme activity without wetting the leather surface to avoid further damage of the leather. Several procedures were tested and compared to decrease water availability/activity, and first promising results were obtained with an enzymatic emulsion allowing a flexibility gain of about 20% of heated leathers. Moreover the efficiency of the enzyme in this treatment has been demonstrated. Attempts to restore will be pursued in this direction.

Keywords: leather, heat, enzymes

INTRODUCTION – BACKGROUND AND HYPOTHESIS

The research project named "BIORESTOCUIRS" focusses on cultural artifacts having leather, such as book bindings, that have been exposed to drastic conditions. Exposure to high heat during a fire is especially devastating and has for consequence to turn the items non-handable due to its rigidity and fragility. The first goal of this project is to characterize the changes induced to leather by heat in order to understand at various scales its consequences. The second objective is to elaborate an innovative treatment based on an enzymatic process to restore leather initial properties, especially its flexibility.

MATERIALS AND METHODS

Materials

Different new calf leathers tanned with vegetable sumac (hydrolysable) or mimosa (condensed) tannins were used for the experiments. Artificially altered samples were prepared by exposing the leather to dry heat at 160°C for 4 days.

Characterization Methods

To quantify the loss of flexibility after exposure to heat and the efficiency of the restoration treatment, dynamic mechanical analysis (DMA) was performed using a DMA Q800 (TA instrument) in tensile and frequency sweep mode between 0.5 and 60Hz at room temperature, under a controlled strain of 0.05 % and 0.01N static force; specimens are placed in the direction head-tail.

To determine the consequences of heat exposure on water absorbency, leather samples (unheated and heated) were immersed in pure water. Before and during the measurement (each hour), samples were weighted until an equilibrium state is reached.

To determine the sample surface hydrophobicity and wettability, a goniometer is used. A droplet of water (15µL) is deposited on the leather sample surface (grain side) and the droplet behaviour on the support is recorded. Contact angle value (θE) is determined by the software "Drop Shape Analysis". Material is defined as hydrophobic when θE is superior to 90° and wettable if the droplet can penetrate the material within 3 minutes.

RESULTS AND DISCUSSION

Characterization of Modifications Induced to Leather by Exposure to Heat

Following heat exposure, shrinkage and darkening of the sample were both accessed at a macroscopic scale.

First, dynamic mechanical analysis (DMA) was performed to quantify the loss of flexibility after heat exposure as shown in figure 1.

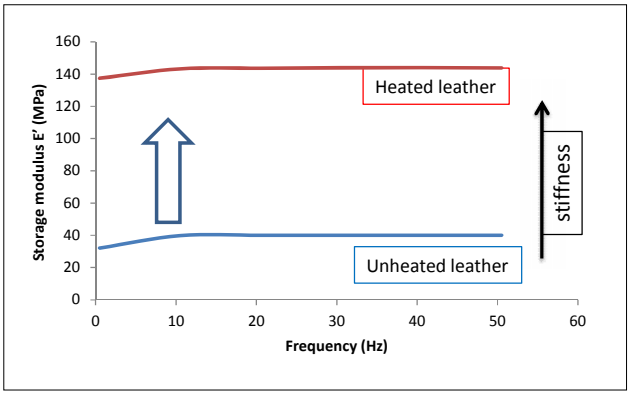

Figure 1. Storage modulus of an unheated leather and a heated leather as a function of frequency

The results show a large increase of the storage modulus (up to about 260%) after exposure to heat correlated with an increase in leather stiffness. Considering that one of the main objective of the restoration treatment is to restore leather flexibility, this method will be essential in evaluating the efficiency of the treatment.

Because the restoration treatment has to be applied on the surface through an aqueous solution, the wettable and absorbency properties of the leather with water has

to be determined. The contact angle, wettability and water absorbency measurement are shown in figure 2.

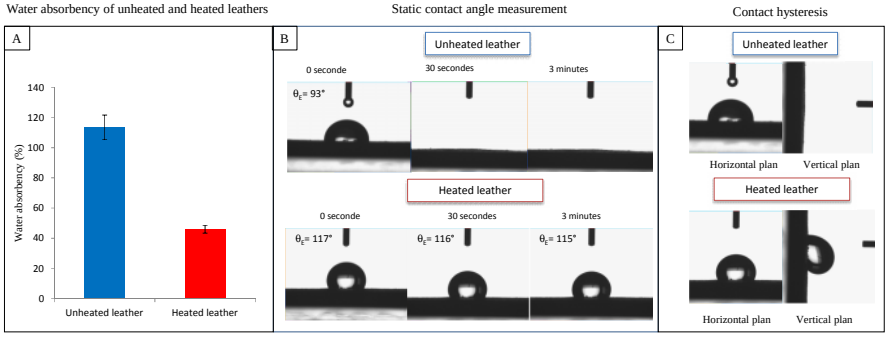

Figure 2. Water absorbency [A], hydrophobicity measurement and wettability in static mode [B] and contact hysteresis observation in dynamic mode [C] of unheated and heated leather

Results highlight that water absorbency has decreased in the heated leather compared to the unheated one (fig.2[A]). Contact angle measurement and wettability, show that after exposure to heat leather becomes more hydrophobic, with θE> 90°, and also non wettable, as the droplet does not penetrate into the leather within 3 minutes (fig.2[B]). This variation could be due to heat-induced chemical modifications of leather components, i.e. heated proteins are usually more hydrophobic than native ones (Baldwin, 1986). These results could also be attributed to the increase in rugosity after heat exposure; as shown in figure 2[C]. The droplet does not flow from the vertical surface, this "lotus effect" is due to a large rugosity at the nano- or micrometric scale. This result also predicts probable difficulties for our restoration treatment to penetrate inside the heated leather.

As largely reported in literature, heat creates protein aggregation (Wallace *et al.*, 1986). Our observations of the macroscopic properties of heated leather are in good agreement with such hypothesis. Moreover, sequential extraction experiments have revealed that some proteins (i.e. fibronectin) cannot be solubilized, even in denaturing solutions (urea, sodium hydroxide) after heating, while they are extracted before heating (data not shown). This indicates that exposure to heat leads to a rearrangement of the leather proteins.

Restoration Attempts

The restoration treatment is based on the use of enzymes to hydrolyze protein aggregates formed after heat exposure. Such approach is a challenge since, water is necessary for ensuring enzyme activity by allowing it to preserve its active three-dimensional structure and the flexibility necessary for the catalytic process. However water has also a dramatic damaging effect on leather having been exposed to heat : the shrinkage, the stiffness and the darkening observed after exposure to heat are getting worse in contact with water as shown in figure 3[A].

Thus, the treatment should provide water for the enzyme while limiting the amount of water interacting with leather.

First, the direct action of an enzyme in aqueous solution was attempted. It was expected that the enzyme action on protein aggregates could be fast enough to counteract the effects of the water on leather. The enzymatic solution in buffer (water as control, data not shown) represents the optimal environment for the enzyme as pH value can be chosen and enzyme regulators added. Moreover, in such media thermodynamic water activity (a_w) reflecting the water availability for the reaction is close to 1 which is optimal for protease hydrolysis activity (Clerjon *et al.*, 2003). Nevertheless, with this method, a very strong shrinkage of the heated sample (fig.3[B]) was observed showing that the enzymatic activity, in aqueous solution, does not permit to avoid the leather retraction.

To reduce the water addition to the leather surface, restoration tests were undertaken by the use of enzymatic polysaccharide gels. In this case, water is largely present but the gel network limits water penetration within the leather. The water activity is not lowered (from 0.91 to 1) but water is "locked" by the polysaccharides, being both physically contained in and having strong interactions with the biopolymer network. Thus gels represent a suitable media for enzymes. Nevertheless, once again, results show again a considerable shrinkage of the heated leather (fig.3 [C]), but to a lower extent than in the previous attempts.

To further reduce the water content, a water soluble co-solvent (glycerol), acting as a thermodynamic water activity depressor was used to prepare new enzyme solutions. In this case, interaction between the two solvents reduces water availability toward the leather. Water activity is strongly reduced, decreasing from 0.96 in 1 M glycerol solution to 0.61 in 10 M glycerol solution. The counterpart of this phenomenon is the large decrease in enzyme activity. For a protease, the enzymatic efficiency is reduced by 35% for 1 M glycerol solution and up to 99% for 9 M glycerol solution as compared with the usual buffer medium (Hertmanni *et al.*, 1991). Several concentrations of co-solvent were tested (fig.3[D]). At low co-solvent concentration (1M glycerol) shrinkage is still observed but to a lower extent than with polysaccharides gels. At high co-solvent concentrations (10 M) no retraction of the heated leather is observed, but no significant gain in flexibility occurred, probably due to the low or none enzyme activity. Moreover, this treatment causes a color change of the unheated leather.

The last approach consisted in the use of an enzymatic emulsion made of an aqueous phase, in which the enzyme is introduced, and a hydrophobic phase to facilitate the introduction of the enzyme on the hydrophobic leather surface. In this particular phase structuration, water is available (a_w varies from 0.91 to 0.95) for the enzymatic reaction, but due to the medium compartmentalization, it is in low contact with the leather surface.

The first trials with enzymatic treatment (fig.3 [E]) do not induce color change of the unheated leather neither shrinkage of the heated leather were observed.

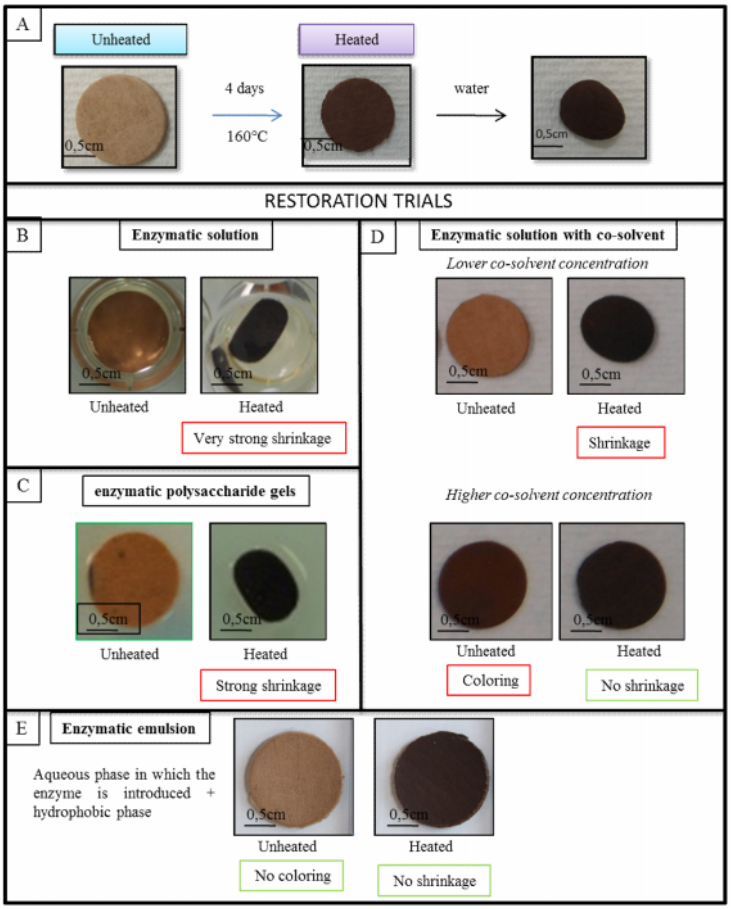

Figure 3. Degradation of a leather exposed artificially to heat, and then placed in contact with water [A]; enzymatic restoration treatments tested on heated leather and on unheated leather as a control, [B] to [E]

Moreover dynamic mechanical analysis (DMA) performed on heated leather treated with the enzymatic emulsion highlighted a flexibility gain. Three days after the enzymatic treatment, a lower storage modulus was measured corresponding to about 30% of flexibility gain as shown in figure 4[A]. This flexibility gain is well due to the enzymatic reaction since without enzyme, the gain reaches is much lower (about 5 %) as shown in figure 4[B].

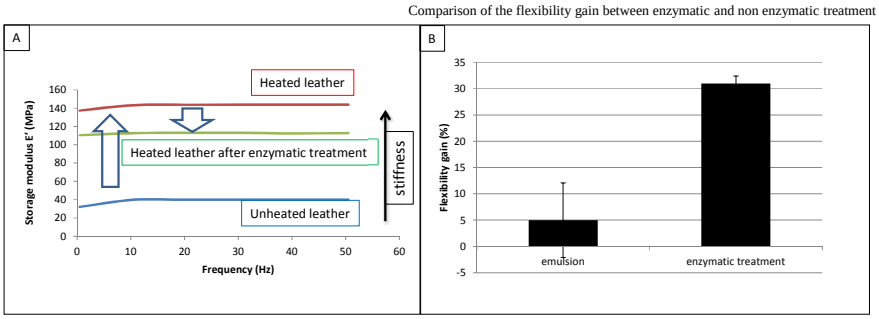

Figure 4. [A] Storage modulus as a function of frequency for unheated leather, leather after exposure to heat and the same sample after enzymatic treatment; [B] Flexibility gain (%) after application of emulsion without enzyme and enzymatic treatment

CONCLUSION

The characterization of heated compared to unheated leathers highlight changes of the leather at various scales. At a macroscopic scale, exposure to heat induces a darkening combined with a stiffness increase and changes in the surface hydrophobicity, wettability and rugosity as well as in water absorbency. All these parameters are consistent with a protein aggregative process. Restoration trials aim to break the protein aggregates by using hydrolases. Various approaches were attempted in order to bring the enzyme to the leather. The results of restoration tests highlighted the need to limit the water in contact with the leather. Since water is necessary, we applied an emulsion that allows enzyme activity but limits the risks of water damage. No color change of the leather was observed while a gain in flexibility was noticed. The various tests carried out show the feasibility and efficiency of this restoration technique which seems promising. Work will continue in this direction.

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GARMENT OBJECTS - ARTISTIC EXPERIMENTS IN FASHION

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In the contemporary context of interferences between different ways of creative expressions and the approach of new technologies, fashion reaches a new position by modelling, interpreting and providing the garment with a new expression, when connected to its historical past. Thus, besides the usage of conventional techniques and materials, it often uses new and revolutionary materials and techniques resulted from the scientific research.

Keywords: garment object, experiment, materials, new techniques

Nowadays fashion is also included in the large perimeter of contemporary art trying to find, like any artistic approach, a new artistic language – the idea of creation is doubled by the one regarding continuity and becoming, as Raymond Federman also used to state "*... each creator is interested in finding his own language* and, in the postmodern context, the *plurality of languages justifies a whole artistic experience*". As a result, what the development of future creators in the domain is focused on is the exercise of research and experiencing – as the research exercise is fundamental to be released from any constraints; what is also important is the approach of any elements to become individualized as a creator.

Related to global art and contemporary frames, visual art is complex as it has both an experimental and multi-valent component through its multiple power of communication, from consumption to direct spiritual participation.

For the evolution in the artistic creation the mixture of contexts represents the support which can define much more, which can express any idea or concept more suggestively. Therefore, regardless of the nature of materials we use to express ourselves, the perceived image must emphasize an impression – in order to awaken senses.

The pluralism of artistic languages, the inventions, deconstruction, multiplicity, games, parodies, interpretations, fragmentation, the collage are only some of the main concepts defining postmodern creation in general, while also comprising creative clothing.

Regarding the work methodology, in the interpretations suggested by MA candidates, I emphasise especially the detail and fragments – as they subsequently integrate in the general composition.

The detail is always related to the whole, it catches our attention and is sometimes underlined by means of simplicity, accomplishment or conjugation, some other times through complexity, multiplying, deconstructing or interpreting a fragment, providing the created object with personality; by using details, the creator is influencing its work, becoming more different than the others, making the receiver look at that detail attentively. An art historian, Daniel Arasse, analyses the modality which makes the difference among creators, being a deliberate act, a personal interpretation endowed with a certain signification while explaining that a creator of details aims to catch one's look, making him curious, to impress or create a certain state of mind.

We use the experiment a lot as a method to develop the students' creativity.

While experimenting, we often use the inductive method – from a fragment, through associations, overlappings, repetitions, reaching a whole, to systems, ideas or concepts,

to a world of forms or, some other times, the deductive method – through which the fragment is mutually dependent on the whole; fragmenting an object or material one can reach an idea or concept which you haven't even thought of. These methods are frequently used in the practice of contemporary art. The spontaneous association of fragments helps you to create your own recognizable and unique identity, thus any imitation or interpretation can be easily recognised.

As a playful manifestation, fragmentarism is celebrated during the postmodern period of time suggesting: exploration, the definition of new ideas, forms, concepts, facilitating the interference (mixtures) thus eclecticism, postmodernism promote the parody, interpretation, metamorphosis, fragmentation, deconstruction, collage and the pluralism of languages aiming to multiply the artistic messages and contexts.

The development of the fragment's ideology and mixtures by using the pluralism of visual languages: assemblages, intertextuality, intermediality etc., actually define contemporary art which implies a continuous creative act that also allows decodings, through artistic experiments, to analyse world and Romanian archetypes, symbols and motives.

The works I have coordinated are garment objects, artistic compositions suggesting different expressive forms, from decorative objects to characters, focused on the sculptural architectural perspective of works. Sometimes the works become organic representing a philosophy, an interpretation and actual modality of fashion to express different artistic structures through the promotion of the balance between man and nature, structures that are integrated in the environment and develop like a living organism.

The garment objects made with students prove the fusion between different ways of artistic expression which conspire and complete each other: fashion, sculpture, architecture, painting, scenography, the arts of performance etc.; this approach is one of the contemporary ways of creative expression.

Living in the era of images, all the offers are influenced by the image, from widely distributed publications, printed or generated virtually, to the image which becomes the constitutive element of creations, no matter if it is about utilitarian and artistic objects or clothing creations. The image influences us each time when we share a visual communication, no matter if we look at a person, choose a garment, a work of art or only a fragment which we find interesting.

The level of actual visual culture is extremely advanced thus, given the techniques used today (e.g. CGI-computer generated images, 3D mapping, or holograms) to express a concept, no matter if it is about an art exhibition, the presentation of a costume or a performance, the real and the imaginary can be transposed into an overlapping zone which can be hardly differentiated.

The important mobile of the project realized with students in different materials and techniques is represented by the development of creative abilities (interpretation, metamorphosis, decoding), their integration in more complex projects (exhibitions, performances, presentations of costumes etc.), as well as by the participation in relevant contests organised both nationally and internationally (e.g. The fashion contest at Sibiu, the participation in the International Inter-University Contest, initiated and organised by the Triumph firm for two consecutive years, or the International Inter-University Art of Fashion Contest from San Francisco etc.)

Figure 1. Master's students - Fashion Department, National University of Arts, Bucharest

Figure 2. Raluca Mandita

Figure 3. Sergiu Chihaia

STUDY ON MECHANICAL PROPRIETIES OF GAMMA IRRADIATED LEATHER AND PARCHMENT

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Cultural heritage is ineffably degrading due to physical, chemical and biological factors. If physical and chemical degradation can be delayed by controlling the storage conditions, the biological attack, once installed, can be stopped only by a drastic intervention. Among others, ionizing radiation treatment has the advantages of: the certainty of biocide effect, fast treatment, mass treatment, no harmful chemicals and residues. Although, because of the complexity and diversity of the constituents of cultural heritage items, there is always a question: if the radiation induces a supplementary degradation in the material. Literature reports show an increase use of radiation treatment for microbial decontamination of wood, painted wood and paper. Few experiments were conducted on leather and parchment. The purpose of this study is to test several mechanical proprieties of irradiated leather and parchment. Samples from leather and parchment were irradiated at doses from 10 kGy up to 50 kGy. For doses below 10 kGy we can consider that changes in mechanical strength of both parchment and leather samples is insignificant (lower than uncertainty of the measurement) but this should be confirmed by other analytical methods. Generally it is known that crosslinking is the predominant effect of irradiation in case of collagen. In our experiment an increase of the mechanical strength it was observed only in case of leather, for doses of 25 kGy and above. The absence of crosslinking in case of parchment can be explained by the lack of the sites which can support crosslinking.

Keywords: gamma irradiation, parchment, leather.

INTRODUCTION

Cultural heritage is ineffably degrading due to physical, chemical and biological factors. If physical and chemical degradation can be delayed by controlling the storage conditions, the biological attack, once installed, can be stopped only by a drastic intervention. Among others, ionizing radiation treatment has the advantages of: the certainty of biocide effect, fast treatment, mass treatment (large quantities), no harmful chemicals and residues. Although, because of the complexity and diversity of the constituents of cultural heritage items, there is always a question: if the radiation induces a supplementary degradation in the material. Literature reports show an increase use of radiation treatment for microbial decontamination of wood, painted wood and paper.

The Radiation Processing Centre of Horia Hulubei National Institute of Physics and Nuclear Engineering in Romania (IFIN-HH), achieves the preservation of artefacts by applying small doses of gamma radiation to destroy microorganisms and insects. The ongoing PN-II-PT-PCCA-2011-3-1742 research project “Improvement of occupational environment quality in cultural heritage deposits. Validation of gamma radiations treatment of textile and leather cultural goods (TEXLECONS)” coordinated by IFIN-HH intends to expand the results obtained in 3 previous projects on the radiation treatment of polychrome wood and paper to leather and textiles items (Stanculescu *et al.*, 2012).

The ionization induced by high energy photons leads to breaking of molecular bonds. Then, two mechanisms are competing in macromolecules: the chain scission (which is associated to the degradation of a material) and the formation of new

molecular bonds. Crosslinking is a well known effect for certain synthetic or natural polymers and collagen is one of the natural polymers which are cross-linked under irradiation (Cataldo *et al.*, 2008).

The study of the degradation of cultural heritage items needs a variety of investigations with different analytical methods (Badea *et al.*, 2008). The mechanical properties are on highest interest but because of lack of availability of samples and large non-uniformity of the materials of natural origin, the studies are oriented for obtaining correlations with other physical and chemical properties.

The objective of the present study is to determine if there are any changes of mechanical properties induced by radiation treatment for leather and parchment and if so, how big they are.

MATERIALS AND METHODS

The mechanical tests were conducted on leather (fig. 1) and parchment (fig. 2) provided by INCDTP-ICPI. Where tested two types of leather: goatling-mimosa and sheep-quebracho and two types of parchment: goatling and goat.

Figure 1. Piece of leather for testing

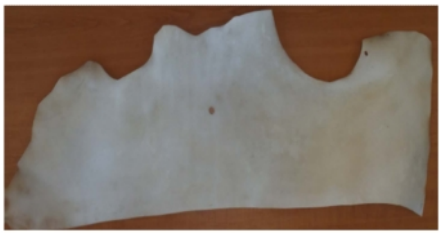

Figure 2. Piece of parchment for testing

We used a Zwick Roel Universal Testing Machine with a 5 kN cell force. For determination of the tensile stress, the tensile strain and the modulus of elasticity we followed ISO 527-3 (film and foils) (1995) with 100 mm/min constant load speed for parchment and 200 mm/min constant load speed for leather. The samples were cut in a bone shape according to specimen number 5 of ISO 527-3 (6 mm in width and 80 mm the testing length). The reason in using this specimen was the ability to determine the modulus of elasticity. Thickness of the samples was measured with a general purpose micrometer.

The samples were irradiated at IRASM department of IFIN-HH with Co-60 radioactive sources at room temperature in a GC-5000 type irradiator. We applied doses of 9.9±0.6 kGy, 24.8±1.0 kGy and 49.6±1.4 kGy at a medium dose rate of 6.2 kGy/h with a dose non-uniformity DUR=1.276.

RESULTS AND DISCUSSIONS

We measured the thicknesses for both leather and parchment. The results are shown in table 1 for parchment and in table 2 for goat.

Table 1. Thickness values for parchment samples

Thickness	Goatling	Goat
Mean (mm)	0.31	0.46
Standard deviation (mm)	0.016	0.04
v (%)	5.1	8.7
No. of samples	11	15

Table 2. Thickness values for sheep samples

Thickness	Goatling	Sheep
Mean (mm)	1.01	0.74
Standard deviation (mm)	0.091	0.038
v (%)	9	5.1
No. of samples	11	15

The results for parchment show that goatling has lower thickness and better uniformity. One of the major problems when working with natural materials is the non-uniformity of the samples. This is caused by the animal constitution but also because of the manufacturing process, as it can be seen in figure 3 (the material is stretched with yarns and it alters the natural formation of material) (Hansen *et al.*, 1991).

In order to avoid some of the mentioned errors, all the tested samples were collected from the middle section, close to the back bone of the animal. In conclusion, we are looking to non-uniformities due to place of cutting the sample caused by the animal constitution and manufacturing process. Also for this case, the variation coefficient is rather high reflecting the non-uniformity of the samples.

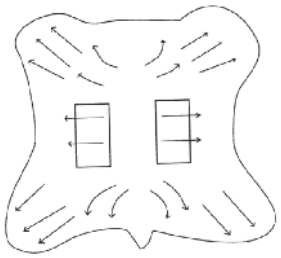

Figure 3. Stretching directions during the manufacturing of parchment (Hansen *et al.*, 1991)

The results obtained for maximum force and for elongation at break are shown in figure 4 and figure 5, respectively.

Comparing leather and parchment for maximum force (F_{max}), it can be observed that parchment has much higher values than leather. For parchment, the tensile strength apparently decrease with the increase of the irradiation dose but this decrease is inside de limits of the uncertainty of the measurement. In figure 4 the error bars represent the standard deviation for 5 samples in case of goatling parchment, 4 samples in case on goat parchment and 3 samples in case of leather. The number of samples was limited because of the available material from each type. A value of 7% is the usual uncertainty of the measurement for mechanical tests.

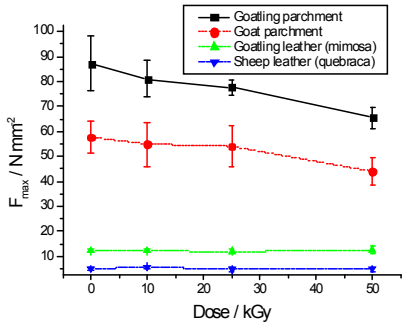

Figure 4. Tensile strength chart

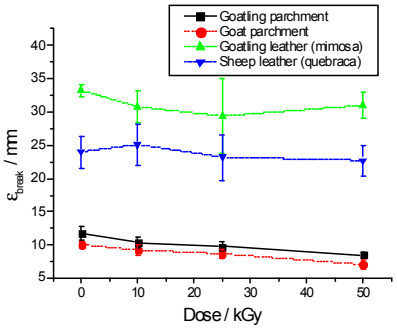

Figure 5. Elongation at break chart

For leather, the behaviour of tensile strength may suggest that crosslinking overcomes the chain degradation of collagen but this is still in the limits of the uncertainty of the measurement.

For the elongation at break (ε_{break}), it can be observed a very similar behaviour for the two parchment types. For leather, the results indicate crosslinking but also in the range of the uncertainty of the measurement.

The elasticity modulus was calculated according to the formula:

$$E_t = \frac{\sigma_2 - \sigma_1}{\varepsilon_2 - \varepsilon_1} \quad (1)$$

where: E_t –tensile elasticity modulus (MPa); σ_1- stress at 0.05% strain (MPa); σ_2- stress at 0.25% strain (MPa).

The values for σ_1,σ_2, ε_1 and ε_2 where read from the curve recorded by the Zwick TestExpert software.

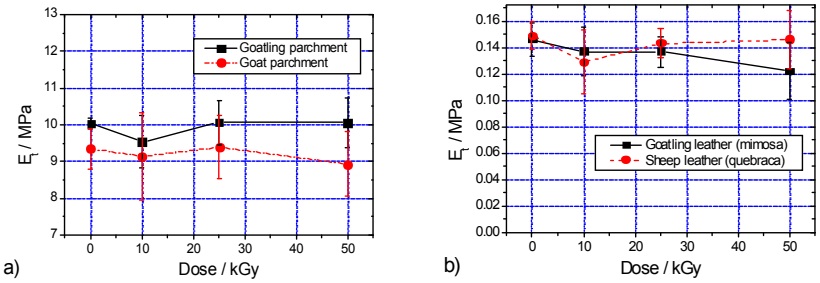

Figure 6. Modulus of elasticity: a) parchment, b) leather

In the figure 6 (a) it can be observed, for both parchment material, that the changes induced by radiation are relatively small, within the area of uncertainty of measurements. Both of materials behaved in the same way, goatling having higher values. At 10 kGy, the results indicated a slight crosslinking for all samples; for parchment, above 10 kGy, it seems that the elasticity module increases which may

suggest some deterioration within the material. However, a similar behaviour was obtained for historical parchment in Portugal, by another method (texture test) which is also dependant on the elasticity modulus (Nunes *et al.*, 2012).

CONCLUSIONS

In the literature there are very few data on the behaviour of the physical and mechanical parameters of leather and parchment under irradiation with ionizing radiation an even fewer on historical (aged) leather and parchment (Nunes *et al.*, 2012).

Taking into account the non-uniformity of the historical materials and the size and number of samples required for mechanical testing, it is practically impossible and useless to test really old samples. We choose to test the effects of irradiation on new materials (not-aged) in order to avoid the non-uniformity induced by ageing degradation. The changes induced by ageing can be further evaluated by accelerating ageing methods. It is expected that the radiation induced degradation to be lower in case of aged samples (Nunes *et al.*, 2012). Generally it is known that crosslinking is the predominant effect of irradiation in case of collagen (wet state) (Cataldo *et al.*, 2008). In our experiment an increase of the mechanical strength it was observed only in case of leather, for doses of 25 kGy and above. The absence of crosslinking in case of parchment can be explained by the lack of the sites which can support crosslinking. For doses below 10 kGy we can consider that changes in mechanical strength of both parchment and leather samples is insignificant (lower than uncertainty of the measurement) but this should be confirmed by other analytical methods.

However, an extended comparative study for aged and non-aged samples irradiated at multiple doses, it is recommended to be performed with a method requiring less quantity on samples (thermal analysis or vibrational spectroscopy).

Acknowledgements

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STUDY OF GAMMA IRRADIATED OIL PAINTING SAMPLES BY FTIR AND FT-RAMAN SPECTROSCOPY

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Gamma irradiation treatment is an efficient means of mass decontamination of art objects such as oil paintings. The investigation of possible physical and chemical changes induced by gamma irradiation in the materials used in oil painting increases the confidence of conservators/ restorers in this still exotic decontamination method. The aim of the present work is to evaluate by different spectroscopic techniques changes induced by gamma irradiation in oil painting samples prepared with pigments of historical importance. In this respect we have used four series of samples: untreated, γ-irradiated, thermally treated, thermally treated and γ-irradiated. Characterization of molecular structure was performed by FTIR and FT-Raman spectroscopy.

Keywords: pigments, Gamma irradiation, FTIR and FT-Raman spectroscopy

INTRODUCTION

Infrared spectroscopy is wide spread amongst within the museum art conservation laboratories, mainly because of the scientific contribution to the organic material characterization in art and archaeometry (Derrick *et al*., 1999). FTIR and FT-Raman spectroscopy may be used to characterize genuine and fake artifacts, to obtain information for art restorers and museum conservation scientists, to quantify effects of environmental degradation on exposed artwork and to identify pigments (Edwards, 2009; Saverwyns, 2010; Kaminska *et al.*, 2006). The use of Raman spectroscopy and microscopy is a developing trend in art objects analysis due to the non-distructive investigation and sensibility (Vandenabeele *et al.*, 2008; Kriznar *et al.*, 2011). In order to increase the confidence in irradiation technologies applied for restoring of biologically contaminated artworks, artificial ageing experiments are necessary to estimate the impact of the irradiation process on the organic and inorganic materials (Rizzo, 2002; Feller, 1994).

In Romania, the IRASM facility commisioning (2001) opened the access for cultural heritage objects decontamination by gamma irradiation (http://www.irasm.ro). Our group developed a FTIR, FT-Raman and reflectance spectroscopy study before and after the radiation treatment of the powder pigments/painted wood panels (Negut *et al.*, 2007; Negut *et al.*, 2010; Negut *et al.*, 2012; Manea *et al.*, 2012; Ponta, 2008).

The purpose of the present work is to evaluate by FTIR and FT-raman spectroscopy the gamma irradiation and thermally treated induced changes in the supramolecular structure of the painting layer of the oil painting samples.

MATERIALS AND METHODS

Materials

Experimental models (four series of samples) consisted of pieces of canvas covered by pigments in mixture with oil, there are:

a) untreated;

b) γ-irradiatied in a SVST Co-60/B irradiator, average dose of 20.4 kGy, dose rate of 0.6 kGy/h (γ);

c) thermally treated at 70˚C for 48 h (t);

d) thermally treated at 70˚C for 48 h and γ-irradiated: average dose of 20.4 kGy (dose rate of 0.6 kGy/h (t+γ).

Pigments manufactured by Kremer Pigmente were chosen as they are typically used in Romanian devotional painting (Fig. 1):

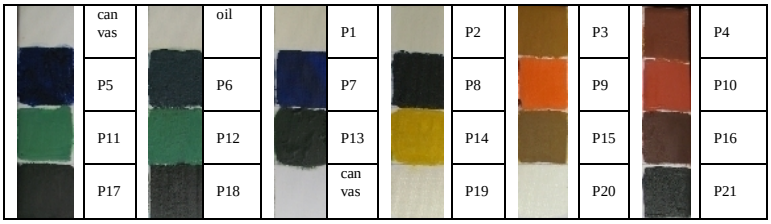

Figure 1. Experimetal model: flake white historical (P1), marble dust (P2), yellow ochre (P3), red ochre (P4), smalt (P5), azurite natural (P6/P13), ultramarine blue (P7), Prussian blue (P8), minium (P9), cinnabar (P10), malachite natural (P11), green earth (P13), cobalt yellow (P14), raw sienna (P15), burnt sienna (P16), bone black (P17), furnace black (P18/P21), titanium white (P19/P20)

Methods

Oil painting samples were irradiated in air at a temperature of 25˚C, by using the National Institute for Physics Nuclear Engineering Co-60 IRASM irradiator facility at a constant dose rate of 0.6 kGy/h. Absorbed doses were evaluated by means of an ethanol-chlorobenzene dosimetry system with oscillometric read-out method, and expressed as absorbed dose in water (ISO/ASTM 51538:2002).

The non-destructive and non-contact vibrational spectroscopy techniques are the ones accepted by the conservators and curators communities. Samples structure characterization was performed with a FT-IR/Raman Bruker Vertex 70 instrument equipped with two mobile probes: a MIR fibre, with a LN2 cooled detector and a RAMPROBE fibre with a LN2 cooled Ge detector, Nd:YAG laser of 1064 nm and 1-500 mW. FT-IR spectra were recorded between 650 and 4500 cm^{-1} with 1000 scans (~ 7.5 min) and FT-Raman were recorded between 50 and 3500 cm^{-1} 500 scans (~ 15 min). Resolution in all cases was 4 cm^{-1}.

RESULTS AND DISCUSSION

Structural changes were monitored by vibrational (FTIR with MIR probe and FT-Raman) spectroscopy on every type of pigment. FTIR and FT-Raman spectra were recorded for pigments' characterization and to monitor the changes induced by the treatments.

FTIR Analysis

FTIR spectra were very sensitive to the organic content of the oil paint. Table 1 gives FTIR bands' position (cm^{-1}) and assignment of oil unirradiated.

Table 1. FTIR bands' position (cm^{-1}) and assignment of oil unirradiated

No.	FTIR bands' position (cm^{-1}) / intensity	Assignment
1	877 / w	$-CH_3$, $-CH_2-$
2	1019 / w	$-CH_2-$, $>CH_2=CH_2<$
3	1109 / w	$-CH_3$, $-CH_2-$
4	1222 / m	C-O-
5	1468 / s	$-CH_3$, $-CH_2-$
6	1589 / m	>C=C<
7	1745 / m	-C=O
8	2860 / w	$-CH_3$, $-CH_2-$
9	2927 / m	$-CH_3$, $-CH_2-$
10	2963 / m	$-CH_3$, $-CH_2-$

m-medium, s-strong, w-weak

Fig. 1 gives the FTIR spectra of oil unirradiated, irradiated, thermally treated, irradiated and thermally treated.

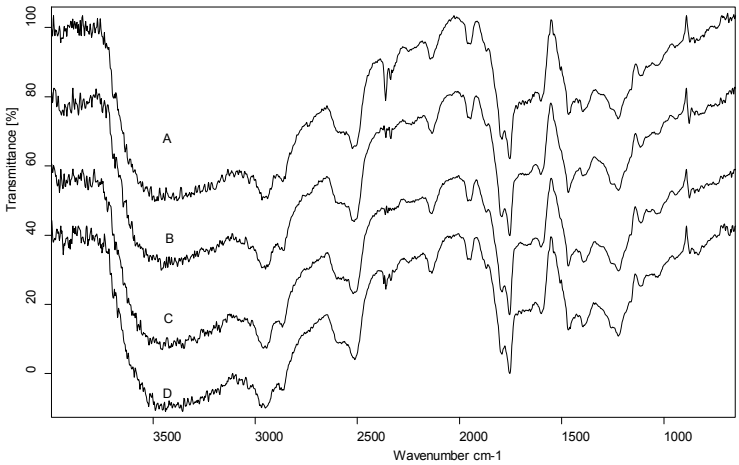

Figure 1. FTIR spectra of oil unirradiated (A), γ-irradiated (B), thermally treated (C), thermally treated and γ-irradiated (D)

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In general, very small band variations are observable in the FTIR spectra of oil (very small variations of oxidation peaks intensity were observed at 1420 cm^{-1} and 1507 cm^{-1} in spectra of oil).

FT-Raman Analysis

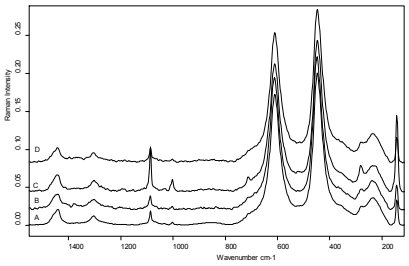
FT-Raman spectra offered better information on the pigment.

Fig. 2 gives the FT-Raman spectra of some pigments of oil painting samples.

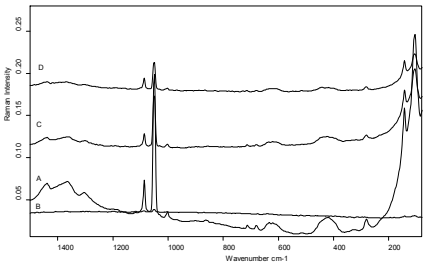
For every type of pigment four samples (unirradiated, irradiated, thermally treated, irradiated and thermally treated) were analyzed and pigments peaks were assigned as follows: titanium white 610s, 448s, 234m, 143w; flake white historical 1439w, 1365w, 1305w, 1087m, 1050vs, 1002w, 633w, 420w, 281w, 143m; marble dust 1438w, 1303w,

1087vs, 1002w, 713w, 634w, 447w, 281m, 144s; azurite natural 1578s, 1455s, 1431s, 1302s, 1095s, 935w, 836w, 762w, 539m, 399s, 292w, 248m, 197w; ultramarine blue 1613m, 1443m, 1297m, 1185w, 1086s, 1002m, 713w, 632w, 548vs, 438m, 362sh, 279m, 145vs; Prussian blue 2150s, 2120m, 2092m, 537m, 274m; minium 549s, 474br, 456w, 391s, 314m, 228m, 151m, 120vs; cinnabar 344m, 285w, 254vs; red ochre 1607m, 1441m, 1288m, 1087m, 615w, 465w, 408w, 292m, 240m, 225w, 145m; cobalt yellow 1398m, 1327vs, 1257w, 837m, 821s, 304s, 276m, 180m, 111w; yellow ochre 1086m, 1002w, 633w, 462w, 405w, 279w, 146m; raw sienna 1086w, 147w (s, strong; m, medium; w, weak; v, very; sh, shoulder; br, broad).

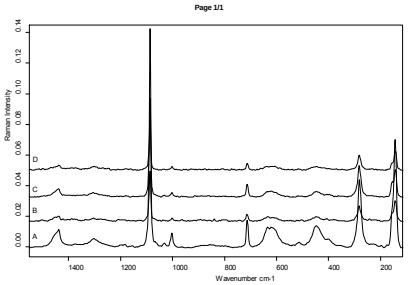
Pigments smalt, malachite natural, green earth, raw sienna, burnt sienna, bone black, furnace black were not detectable by Raman spectroscopy using 1064 nm excitation (Castro *et al.*, 2005; Burgio and Clark, 2001).



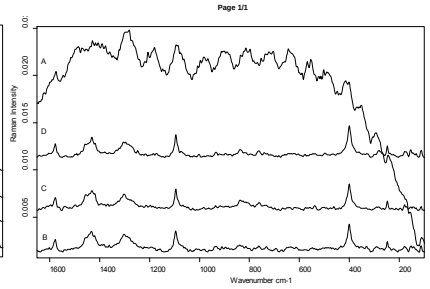
titanium white



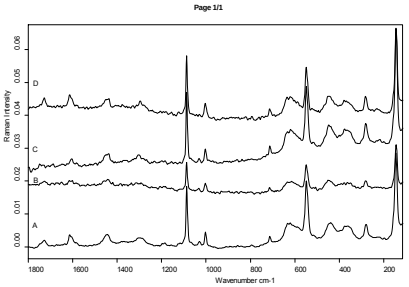
flake white



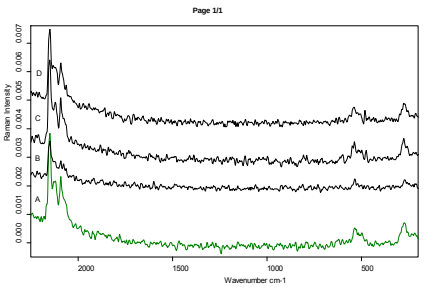
marble dust



azurite



ultramarine



Prussian blue

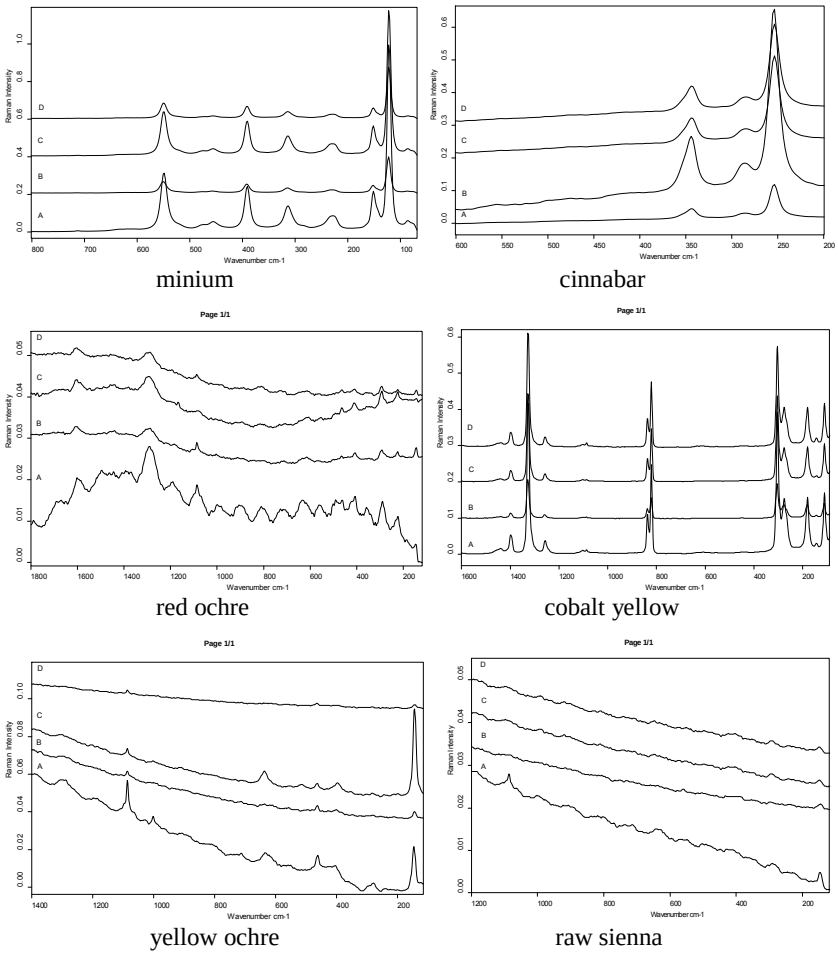


Figure 2. FT-Raman spectra of pigments: unirradiated (A), irradiated (B), thermally treated (C), irradiated and thermally treated (D)

CONCLUSIONS

FTIR and FT-Raman spectra of pigments do not present significant changes regardless of applied treatment.

The negligible structural changes recommend the use of gamma irradiation in the disinfection of oil paintings.

Acknowledgements

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AUTOMATIC DETECTION OF COLLAGEN FIBRES SHRINKAGE ACTIVITY USING Σ-Δ FILTERING

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The thermally-induced structural collapse of collagen fibres in collagen-based historical materials and artefacts such as leather, parchment and skin is currently measured through the Micro Hot Table (MHT) method. This method, widely used in conservation-restoration for characterising historical materials' deterioration, is based on a combined thermal and microscopic technique which evaluates the motion behaviour of the collagen fibres dispersed in aqueous milieu and heated at 2°C·min inside a thermostatically controlled heating cell. The collagen fibre motion observed by a stereomicroscope and digitally-recorded with a camera is called shrinkage activity and has been defined by a sequence of five temperature intervals. The intrinsic main limitations of this method, i.e. time consuming and human eye assessment variability causing high errors and making it impossible the inter-laboratory comparison, can be overcome by the use of image processing techniques for the automatic detection of shrinkage intervals. An improved MHT method incorporating image analysis will deliver a really objective and faster analytical technique and enable for effective diagnosis and conservation decision in museums, archives, libraries, public and private galleries.

Keywords: historical parchment and leather, shrinkage, Σ-Δ filtering

INTRODUCTION

In intact fibres, the helical structure of collagen with the twisting of micro fibrils and minor fibres is easily visible at microscope. During ageing and deterioration, fibres usually evolve through flattening, splitting and/or fraying which lead to fragmentation and end by formation ofa gel-like substance that melts by contact with water or even in a very moist environment. In some cases the end product is represented by small hard fragments that do not react any longer by contact with water even on heating. The vital importance of collagen as a biomaterial for documents and artefacts demands a manifold of essential characteristics such as thermal stability, and mechanical strength. These properties are derived from the fundamental structural unit of collagen, the triple helix. The thermal stability can be evaluated by inducing the collapse of the triple helix by progressive, controlled heating. In fact, when progressively heated in water, the collagen triple-helical structure converts to random coil disordered structures over a defined temperature interval which characterises its thermal stability. This process is called thermal denaturation and its macroscopic manifestation can be observed through a stereomicroscope as the shrinkage motion of the collagen fibres. Collagen shrinkage activity associated with thermal denaturation has been defined as a sequence of five temperature intervals (Larsen *et al.*, 2002):

no activity – A_1– B_1– C – B_2– A_2– complete shrinkage (Figure 1)

In the first two intervals, A_1 and B_1, shrinkage discretely occurs in individual fibres and displays higher activity (namely higher amount of shrinkage per unit of time) in B_1 interval. Then, the majority of the fibre mass shrinks in the main interval C. The starting temperature of this interval is the shrinkage temperature, T_s. Generally, the shrinkage activity levels off through B_2 and A_2 intervals. T_f is the temperature at which the very first motion is observed and T_l the temperature of the very last observed motion. While the shrinkage of collagen fibres from new materials runs through all these intervals, for some historical materials neither of these last two intervals has been observed. In some cases the main shrinkage interval C was even absents illustrated in Figure 1 (ASTO 2.1).

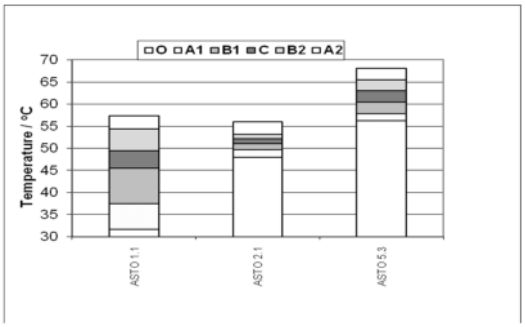

Figure 1. Bar diagram displaying shrinkage intervals for three parchment rolls from the State Archives of Turin (*Le Castellanie* Collection, XIV – XVI century)

The MHT method was established and then improved in the compass of four European projects, STEP (TS967-S82-1994), ENVIRONMENT (EV5V-CT94-0514), MAP (SMT4-96-2101) and IDAP (EVK-CT-2001-00061), in which the end-users have played an active role. The success and the extensive use of the MHT method rely on one hand in its micro-destructiveness (only few corium fibres are required) and on the other hand on the easy interpretation of the results (the more deteriorated the sample fibres are, the lower the T_s). In addition, the simple, easy-to-use and inexpensive equipment composed of a stereo microscope with a camera and a thermostatically controlled heating cell, has certainly contributed to the proliferation of the method in the conservation laboratories across Europe, USA and Canada. Provided that the assignment of the various temperatures to A, B and C intervals is not made in real time but during subsequent observations, the precision of the method may be as good as 0.1 °C while the accuracy was claimed to be 2 °C (Larsen, 2000).The other side of the coin is that, as a simple test of shrinkage temperature measurement, the MHT method has some limitations: (i) low ratio of information obtained over the level of destruction, (ii) lack of detail, (iii) risk of error in the evaluation of deterioration. Correlation with complementary analytical techniques (Badea *et al.*, 2008; Badea *et al.*, 2012b; Mühlen Axelsson *et al.*, 2012; Možir *et al.*, 2012) as well as identification of further shrinkage parameters which well correlate with both the level and type of deterioration such as T_f and T_l temperatures, C and A_2 intervals length, as well as the total shrinkage interval length enables a much more detailed and reliable evaluation of deterioration (Badea *et al.*, 2012a). Furthermore, by analysing the results of the observations obtained by different operators on the same samples, a significant error due to the human factor has been detected, hindering the ability of inter-laboratory comparison. The average

duration of a measurement of about 45-60 min adds a further limitation in terms of the high human resources involvement required by the campaigns of damage assessment of large collections. A technological improvement is thus necessary for delivering a truly objective and in-real time analytical technique.

Σ-Δ FILTERING METHOD

The Σ-Δ filter was used for the automatic detection of the shrinkage temperature of collagen fibres obtained by using the MHT method. Most background estimation techniques use only the difference between grey scale values for considering changes in the illumination (Atkociunas *et al.*, 2005). The process of changing the grey scale values in a background image sequence is described as a signal processing system for each pixel. The Σ-Δ filter is used for the nonparametric motion estimation and can adapt very quickly to illumination variations. The key feature of this method is the robustness given by the nonlinearity of the recursive mean compared with the recursive linear mean that is more computational efficient (Manzanera and Richefeu, 2007).

The Σ-Δ background estimation is a simple and efficient method to detect pixels that substantially change in the static scene, considering a time constant that depends on the number of grey levels. It is a temporal processing that can only offer detection at pixel level.

The Σ-Δ background estimation algorithm was tested on a set of 38 image sequences obtained by MHT method for artificially aged leather samples. The values of shrinkage temperature T_s estimated by the human specialist and by using the Σ-Δ algorithm are shown in Figure 2. Similar results were reported for new and historical parchments (Badea *et al.*, 2013).

The mean absolute error of T_s approximated by the use of the Σ-Δ filter is 1.8°C, less than the accepted error in the case of the traditional MHT analysis, i.e. 2 °C.

A further improvement is represented by the automatic detection of all the shrinkage intervals that is in progress within the COLLAGE project. Such a comprehensive profiling of shrinkage activity can support improved preventive care and conservation treatment of parchment and leather collections. The dramatic reduction of the time required for a measurement and increase of the reproducibility of results will help to introduce MHT as a routine evaluation test in preventive conservation practice.

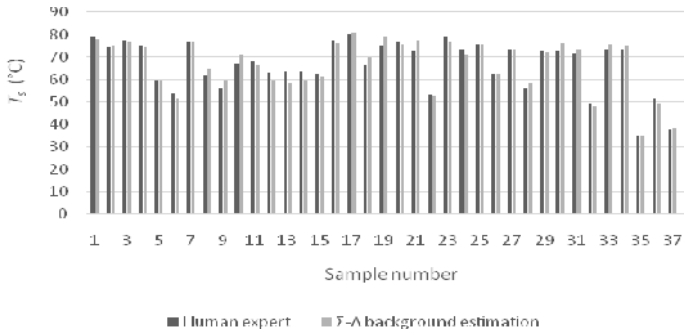

Figure 2. Comparison between the T_s values measured by the human expert and by using the Σ-Δ background estimation algorithm for 38 artificially aged leathers

CONCLUSION

An automatic method for the reproducible and rapid estimation of the shrinkage temperature *T*s of collagen fibres has been developed and tested for a number of leather and parchment objects. The employment of the automated MHT method offers the advantage of providing information on specific alterations at surface and on sample mass, as well as at fibre levelorganisation and thus prevent partial, improper evaluations made by traditional visual assessment.

Acknowledgements

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FREQUENT RETURNS TO ETHNIC GROUNDS IN FASHION DESIGN

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As part of the history and civilization of the Romanian people, the Traditional costume constitutes a living document which lasted for centuries and sent to the generations the message of an authentic artistic creation. The folk costume is a precious artistic document, social and historical. The Traditional Romanian costume is a continuous source of inspiration in fashion design. The analysis of these forms of cultural expression supports the conclusion, according to which the reuse of these decorative motifs can create products with a great effect in contemporary fashion. It is obvious, in this context, the importance of the source of inspiration, but also the presence of a relevant manner of reapplication and reinvention of these elements. Although the contemporary designers are working in accordance with a vision, appealing to a wide area of styles and methods using current technology, cyclical they return to traditional techniques and ethnic folklore motifs, which converts and resize them, integrating them in their contemporary space. In terms of product design, is very important tot take into account some intrinsic elements of the creative process, such as trends and artistic values present in traditions, customs, crafts and design. The current research in fashion related to national folklore motifs developed by INCDTP is to define the anthropometric characteristics of the population and the ethnographic features of the folk costumes from different regions of the country and use it as a source of inspiration for the fashion collections.

Keywords: fashion, Romanian costume, inspirational source

INTRODUCTION

Romanian folklore is the best preserved, most varied and traditional in Europe. The tasteful beauty of the regional costumes can be seen throughout Romania. The costumes reflect ethnic identity and document the historical and artistic values of the Romanian people. As part of the history and civilization of the Romanian people, the Traditional costume constitutes a living document which lasted for centuries and sent to the generations the message of an authentic artistic creation. The folk costume is a precious artistic document, social and historical (Doaga, 1978).

The national Romanian costume is also an inspirational source in fashion design because of the multitude of forms particular to each region of the country and decorative elements that can always be reinterpreted.

THE TRADITIONAL ROMANIAN COSTUME

The traditional Romanian costume as general features, has the same resemblance across the country, with certain differences of details, changes of shape, cut, decoration and color. They differ by region: Banat, Transylvania, Bukovina, Moldavia, Crisana, Maramures, Dobrudja, Oltenia and Muntenia (Mocenco *et al.*, 2013).

The structure of Romanian traditional clothing has remained unchanged throughout history and can be traced back to the earliest times. The basic garment for both men and women is a shirt or chemise, which is made from hemp, linen or woollen fabric. This was tied round the waist using a fabric belt, narrow for women and wider for men. The cut of this basic chemise is similar for men and women. In the past those worn by women usually reached to the ankles while men's shirts were shorter and worn over trousers or leggings made from strips of fabric. Women always wear an apron over the chemise. This was initially a single piece of cloth wrapped round the lower part of their bodies and secured by a belt at the waist, as is still seen in the east and south east of

Romania. In Transylvania and the south west of Romania this became two separate aprons, one worn at the back and one at the front.

Figure 1. Traditional Romanian Costumes

Men's traditional clothing throughout Romania comprises a white shirt, white trousers, hat, belt, waistcoat and or overcoat. Local differences are indicated by shirt length, type of embroidery, trouser cut, hat shape, or waistcoat decoration. In most areas shirts are worn outside trousers, which is the older style. This is a basic Balkan man's costume largely uninfluenced by fashions from west or east. Hungarian and Saxon men living in Romania wear trousers with a more modern cut often made of dark material rather than white. This reflects their closer ties, and more frequent communication, with the west.

The outer garments worn by both men and women are similar, the main differences being in cut and decoration which depend mainly on the region of provenance. These garments are usually made of sheepskin, or felted woollen fabric, and decorated with leather appliqué and silk embroidery. Traditional clothing worn on workdays and festivals used to be similar, the main difference being that the festive dress, especially those worn for weddings was more richly embroidered. In the past the headwear worn by the bride was especially ornate with specific local styles. In poorer areas basic clothing with little or no embroidery has always been worn (Stefanuca, 1990).

Figure 2. Outfits from the collection “Influence Mix”

In Maramures the popular art is preserved in its original form and the predominant colour of the folk costumes is green. The traditional costume from Moldavia is characterized by simplicity and sobriety. Specific for Oltenia are the bright cheerful colours of the embroideries and the splendour of the decorative compositions made with precious materials - gold and silver metal threads. In Bucovina, the basic piece of the female costume is the flax blouse with hems and the chromatic composition is dominated by red, yellow and orange. The traditional costume from preserves the vigour of some ancient clothing pieces: women's zadii (rectangular woollen skirts) and guba (a long white coat, woven with long wollen treads introduced into the filling to obtain afur effect) worn by both men and women. The folk costume of Transylvania is characterized by a unitary morphological structure of the basic clothing pieces. In Banat, there are some unique pieces - opregul cu franjuri lungi (catrinta) and the conci (bonnet) worn by women-, and on the other hand, to the sumptuous embroideries and alesaturi made in gold and silver threads. The popular costume from Dobrudja consists of: the shirt (camasa dreapta) with two aprons with "peak" for women and a shirt with wide trousers, wrinkled (in dark colours) and red belt for men.

Figure 3. Outfits from the collection "Influence Mix"

The various pieces of costume have gone out of use at different times during the 20th century. The first item to disappear in many areas were leather peasant sandals (*opinci*), although these could be seen in poorer villages again in the years just after the communist regime fell. In most rural areas men's traditional trousers were replaced by modern factory made trousers by mid century and in the post communism years jeans has become universally common. Traditional over garments became an expensive luxury, new garments only being purchased by people living in the very wealthy villages. More recently the traditional jacket makers in many areas have died with few new artisans being trainer to carry on their craft.

FASHION COLLECTION WITH INFLUENCES FROM ROMANIAN FOLKLORE

The Traditional Romanian costume is a continuous source of inspiration in fashion design. The analysis of these forms of cultural expression supports the conclusion, according to which the reuse of these decorative motifs can create products with a great effect in contemporary fashion. It is obvious, in this context, the importance of the source of inspiration, but also the presence of a relevant manner of reapplication and reinvention of these elements. Although the contemporary designers are working in accordance with a vision, appealing to a wide area of styles and methods using current technology, cyclical they return to traditional techniques and ethnic folklore motifs, which converts and resize them, integrating them in their contemporary space (Olaru *et al.*, 2014) In terms of product design, is very important tot take into account some intrinsic elements of the creative process, such as trends and artistic values present in traditions, customs, crafts and design (Mocenco *et al.*, 2013).

Figure 4. Outfits from the collection“Influence Mix”

The current research in fashion related to national folklore motifs developed by INCDTP is to define the anthropometric characteristics of the population and the ethnographic features of the folk costumes from different regions of the country and use it as a source of inspiration for the fashion collections. In the initial stage of one project, financed through national founds, a documentary study on ethnographic characteristics

of the popular costume from different regions of the country was elaborated. Based on this study it was developed a fashion collection inspired by the Romanian folklore.

The fashion collection entitled "Influence Mix" is inspired by the traditional Romanian costume and its decorative motifs. The dress forms and the decorative motifs specific to traditional costumes from the regions Oltenia, Muntenia and Dobrudja are reinterpreted in a modern way, with refined proportions and volumes restructured. The traditional motifs taken from the Romanian folklore are digital printed on the clothes, arranged in various artistic compositions. The fabrics used in making the collection are made from natural fibers, cotton, flax and hemp, treated to retain their natural properties. These fabrics have so many properties and they are environmentally friendly (Dabija *et al.*, 2014).

Figure 5. Outfit from the collection "Influence Mix"

The mix of folk influences of the three regions of the country - Oltenia, Muntenia and Dobrudja are combined in a versatile, current collection. The clothes are printed with Romanian traditional motifs, updated and converted into ingenious decorative compositions. For achieving these folklore-inspired prints digital print was used on textiles from natural fibers (cotton, linen and blends of natural fibers). The clothing shapes and details refer to the Traditional Romanian costume. Also, each outfit of the collection is versatile, being composed from several pieces of clothing. The color palette is limited, consisting in tone of beige, red, burgundy and non-colors black and white.

The collection "Influence Mix" stands out due to the clean lines, asymmetries and unexpected cuts.

CONCLUSIONS

The Traditional Romanian costumes reflect the ethnic identity and documents the historical and artistic values of the Romanian people. It has a great and artistic value because of its spectacular cut and decorations. The traditional costumes differ by region: Banat, Transylvania, Bukovina, Moldavia, Crisana, Maramures, Dobrogea, Oltenia and Muntenia. In fashion, nowadays, is an important inspirational source.

INCDTP has developed a fashion collection inspired by the Romanian folklore entitled “Influence Mix”. The clothes are printed with Romanian traditional motifs, and their shapes are asymmetric with unexpected cuts.

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HUMANISTIC EPISTEME IN SUSTAINABLE DEVELOPMENT OF CREATIVE INDUSTRIES

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Sustainability is based on the requirement to make all economic systems less dependent on resource use and to make products last longer, thus requiring a slowdown of serialization, production and consumption and an increase in the added value and customization of products. This can be done by building a cultural paradigm with different rates of application and development, and transfer the entire arsenal of tools, processes and creative productions to the creative and cultural industries. In this context it becomes important to formulate the humanistic episteme of sustainability of creative industries, as it is the fundament of any concept in fashion, design, advertising, media, etc. This step is an obvious one because creative industries are not manufacturing or construction industries, but conceptual industries that are defined by the prior existence of a practice and history of knowledge and creation. The paper aims to outline a sketch of humanistic episteme in the sustainable development of the creative industries, with the example of the fashion industry, this theory proving to be a milestone in effectively structuring the culture of an office of style or design, because style offices are nothing but small industrial research centers, without which independent production companies cannot operate.

Keywords: humanities, episteme, sustainability, creative industries, fashion industry

INTRODUCTION

Sustainability of anthropogenic products and processes is analyzed today from the points of view of many sciences involved in their preparation and development: environmental engineering, sociology, economics and humanities. From the humanistic perspective, the product of creative industries is not amorphous, in terms of cultural identity, but an active product, which means creativity, innovation and belonging to a style and theme of ideas, and therefore cultural identity. In the fashion industry this creative process occurs according to man's natural rhythms and is found in what slow fashion means, the opposite of fast fashion, where all processes, from product design to sales are very fast, operating in the rhythms imposed by the computer. Slow fashion means “a design, production, consumption and better life by combining ideas of regeneration cycles and nature evolution with those related to values and traditions” (Fletcher, 2008). In the past decade, through the development of creative industries and slow fashion, it received a new name, *ethical fashion*, which is the opposite of fast fashion, but with an emphasis on quality, it is a different approach, as designers, distributors and consumers are much more aware of the impact of the product on workers, communities and ecosystems. It can be said that the essence of the idea of slow fashion is the balance between the different production and use rates, achieving more expensive clothing items, that reflect the materials, labor and values, as well as the respect for values, people, and the environment, being organized and identified through the following defining aspects: organic, vegan, craftsmanship, customization, ethical standards of production and marketing, recycling, vintage.

Integrating these concepts in product design and constantly oriented towards creativity, the European fashion industry has used cultural paradigm in researching product and brand trends, demonstrating its innovative quality since the 1970s, but has also adapted it to the requirements of sustainability in the last twenty years. The participation of art, psychology, history or anthropology, i.e., the humanities, became

immanent, increasingly focusing on the societal interests of fashion as a major social phenomenon, both in the creation of the fashion product and in the development of creative industries of the twenty-first century. In a possible taxonomy of textile arts (Figure 1) it can be observed where fashion, costume art, and fashion design stand in the panoply of contemporary fine and decorative arts. It can be seen that the majority of textile arts contribute to the cultural development of fashion: costume art (in all its forms of manifestation, from restoration to costume performing), artistic fabrics, prints, embroidery, patchwork, collage, mixed 2D, textile design. This contribution is direct, practical, applicative, through artistic creation. But in turn, the field of any artistic production is increasingly rich if we use the humanistic foundation of the artistic discourse through: elements of art history, anthropology, psychology, semiotics, philosophy, sociology, etc. In particular, the evolution of fashion is determined by studies, research or innovative concepts of a school of thought at a given moment, a humanistic current also noted in socio-economic studies. Hence there is a history, philosophy, sociology, anthropology, psychology of fashion as theoretical points and in the creative and artistic practice of the fashion product (design or decorative arts), there are the art sciences contributing to its development, such as: applied aesthetics, visual semiotics and archetypology. Certainly, any fashion product, either clothing or object, such as clothing or environmental accessories, or the full range of life-style items, including houses, cars, and urban environment come to be sustainable cultural products only in the extent to which proper sustainable development-oriented research studies, both technical and humanistic, are developed in terms of education and research in every industrial field.

In the creative industries of fashion a boundary must be set between the utilitarian and the fashion product in order to correctly target both research and product design. Functionally and emotionally, fashion and clothing are two different concepts: fashion connects us with the cultural period and space of the society in which we live, representing our emotional needs (psychological), social status (sociology, anthropology), cultural needs (art, visual and musical culture, history of places), communication needs (communication sciences, visual language) helping us to express ourselves as individuals, while clothing and shoes meet our physical or functional needs, protecting us. “The function of usual clothing is a material one - to cover us and keep us warm, while the function of fashionable clothes is to indicate who we are, expressing our own cultural values and communicating a message to others - these emotional needs are complex, subtle and inexhaustible” (Fletcher, 2008). Based on this social function and different economic needs, in the last twenty years the fashion industry, which is a creative industry, started to distinguish itself from the clothing industry, which is an utilitarian industry. For a sustainable development of creative industries its specialists are required to understand the importance of humanistic culture in this area and apply it to the concept, product strategy, marketing and advertising of the humanistic mix brand that creates sustainable added value.

It is the role of designers, researchers in fashion and textile arts, of those specialists who integrate numerous humanistic and socio-economic studies in their work to create a product tailored to the needs of an informed consumer in matters of sustainability and increasingly educated; it is then the role of these people in the fashion area to develop creative industries in a sustainable manner, both materially and culturally.

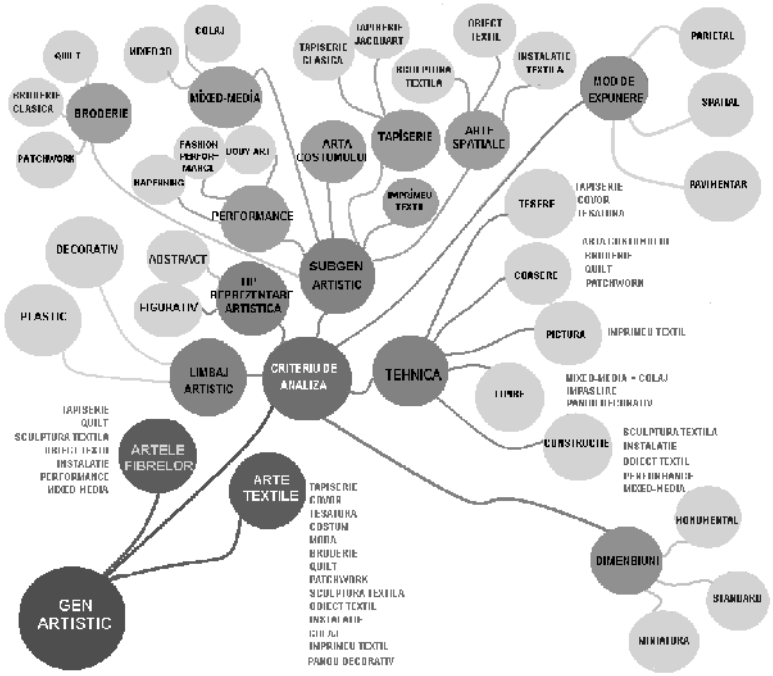

Figure 1. Taxonomy of textile arts

At this point, the role of humanistic episteme can be defined in the sustainable development of creative industries, both in product concept, and in the general plan of strategies and creative, brand or company management, to the knowledge of the one who provides the concept of sustainable product addressed to an informed consumer.

If from the large field of textile arts we extract only the fashion area and attempt a visualization of its structure, again through a taxonomic scheme, we can also view the humanities area directly affecting or pertaining to this creative field.

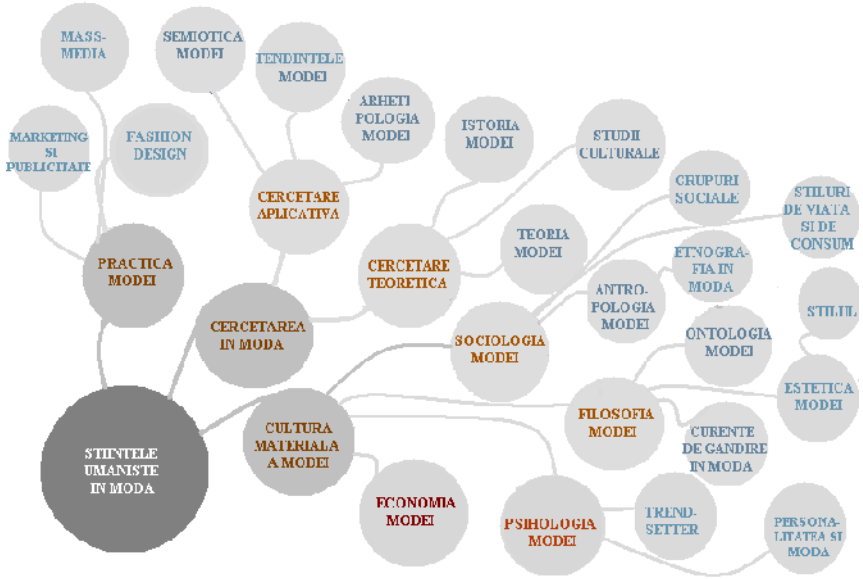

Figure 2. Taxonomy of fashion from the perspective of humanities

The simple reading of the categorised areas illustrates the truth of the statement that the particular humanities, from the history of fashion to the philosophy and psychology of fashion have left their mark on this socio-cultural phenomenon, ever since its inception, and that fashion is basically a field of "art on the human" (Nanu, 2000).

Specific methods and tools of humanities applied in fashion are related primarily to constructing and reading the image: applied aesthetics, visual semiotics and semantics, psychological chromatology to harmonize the sign-symbol of the fashion theme with the color that has value in the buyer's conception of the clothing, the aesthetic style which must be compatible with the lifestyle, etc. Socio-psychological methods used in fashion are integrated in applications for marketing and advertising of brands that aim to sell products with the story and to gain clients using images and subtle psychological arguments. Behind the stories and the image sold and selling the product there is an army of people who conduct interdisciplinary humanities and socio-economic studies, ranging from lifestyle to purchase and post-consumption behavior studies.

The fashion issue thus intersects with that of sustainability and we discover that humanistic elements play not only the role of binding concepts, but primarily serve to promote humanistic values of sustainability relating to: promoting traditions, product customization, ethical fashion and recycling through the vintage product, which is an old product with cultural value.

CONCLUSIONS

Sustainability implies a slowdown of serialization, production and consumption and an increase in the value and customization of products, therefore, a greater emphasis on cultural trends and values. The great style offices around the world demonstrate their

concern for cultural sustainability and ecodesign assertion, providing product designers around the world enough themes of inspiration for sustainable fashion. A slow fashion involves better design, production, consumption and life, using natural values along with the traditional cultural ones. However, metropolitan life and urban dominance of the 21st century means adapting sustainability to other rhythms. Therefore slow fashion is not the antithesis of speed, but a different approach, focusing on quality, where people are more aware of the impact of the product on workers, communities and ecosystems. Only in this context, eco-design, as practice of sustainability of fashion, reduces the environmental impact of clothing throughout the product's life cycle, and given that the concept of the product is always improved, the design becomes sustainable.

Thus, *sustainable fashion* means better aesthetics, philosophy of beauty, design, production, consumption and life by combining ideas of regeneration cycles and the evolution of nature with those related to societal values and traditions. The growing demand for sustainable products will increasingly put companies in a position to seek creative professionals with the expertise to integrate sustainability in the entire business of fashion brand, so that humanistic expertise in sustainable fashion would become a new profession.

Transforming the fashion industry into a sustainable industry is an ongoing process, which is carried out through actions based on the humanities:

- a new anti-consumerism philosophy, promoting natural and organic values, as socioeconomic studies have proven the harmfulness of consumerist philosophy and practice to the human species;
- promoting an experiential philosophy as a cultural obligation to experience everything and to buy products according to this experience in order to be part of a continuous process of individual and hence group and community identity formation;
- a new psychology of lifestyle, because changing the diet and adhering to a anti-consumerism philosophy involves adopting other values that can only be cultural, from reading books to group psychology practice;
- appreciating eclecticism, both in its cultural variant, of steering the use of products specific to different styles or cultures, and in its multifunctional variant by appreciating clothing with several features, or by combining existing fashion pieces with organic or vintage ones;
- using traditional elements on several levels: both in the cultural one, of aesthetic appreciation of traditional symbolic signs, as well as by integrating original traditional pieces in current clothing or by adapting urban products to the needs of a traditional life, as well as using vintage pieces as a creative cultural component;
- the hardest way to integrate humanistic values in sustainable fashion is to adhere to an ethical fashion, because this means fairness, ethical production processes and sale of products, payment of labour and creation of civilized jobs. If in the fashion industry or offices of style, design, promotion, advertising and PR agencies, economic mechanisms are easy to check, in the garment industry, the one that materializes product concepts, these things are hidden, knowing that over 30% of this industry is a “black” or “gray” industry, and therefore having great socio-economic ethics issues.

In order for sustainable fashion industry, which is a creative industry, and therefore an industry oriented towards the use of individual creative potential, to be a profitable industry in the context of working with the garment industry and the textile industry, which are commercial industries, organized just for economic profit, specialized clusters must be set up. In this respect, using the principle of cooperation for communication

and creativity, people working in these areas can create centres of interest both in the business, culture and art design of the fashion textile and leather field, through those systems of innovative services that are creative clusters. Potentiation of young professionals' access to cultural, scientific, financial and technological resources, promotion of regional and national traditional culture through cultural, sustainable, smart and ethical products, as well as creating the framework for initiation of a market for cultural products, both innovative and traditional, in this area, restored to modern standards, are ways through which a creative cluster in the fashion industry can be profitable, can create jobs and can be sustainable for a long time.

This is the only way that the humanistic episteme in sustainable development of creative industries may find its integrated, functional applicability, generating new values to develop the epistemological background of this field.

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UNILATERAL NMR FOR DAMAGE ASSESSMENT OF VEGETABLE-TANNED LEATHER. CORRELATION WITH HYDROTHERMAL PROPERTIES

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Unilateral NMR has proven to be a valuable tool in the field of collagen-based cultural heritage where non-destructive analyses are highly demanded. Old leather is a collagen-based biomaterial made from animal hides chemically treated by vegetable or mineral tanning to increase chemical and physical durability and confer desired handling and working characteristics. In this study unilateral Nuclear Magnetic Resonance (NMR) combined with shrinkage temperature measurement by the Micro Hot Table (MHT) method were applied to evaluate the conformational, structural and stability changes of variously vegetable tanned leathers exposed to accelerated ageing by heating at 70 °C in controlled atmosphere at 30% relative humidity (RH) and irradiated with 4000 lx in the visible light region for 8, 16, 32 and 64 days. Longitudinal relaxation time T1 values, measured by NMR MOUSE portable equipment using a saturation recovery sequence, showed specific variations depending on both animal species and tanning agent, and ageing time. Collagen fibres' shrinkage temperature Ts values evaluated using the home made MHT equipment available at INCDTP-ICPI, Bucharest, complemented the hydrothermal information on fibre level.

Keywords: Vegetable tanned leathers, NMR-MOUSE, MHT method.

INTRODUCTION

Historical and archaeological leather objects and artefacts are an infinite source of information of historical and cultural interest. They illustrate the evolution of social customs, habits, aesthetics and technology, but also the perpetuation of popular and religious traditions. It is vital therefore that these materials and artefacts remain well preserved. The aim of our study is to bring together non- and minimal invasive investigation techniques for collagen fibre characterisation as practiced by conservators, i.e. shrinkage activity measurement, with the current nanoscale measuring systems, i.e. unilateral NMR, to relate information at the fibre level to that at the collagen fibril level. Early detection and identification of deterioration by using qualitative tests as unilateral NMR and MHT method can highly extend the life of the objects/artefacts.

Unilateral NMR has been developed after 1990, when the first portable equipment NMR MOUSE (Mobile Universal Surface Explorer) was built (Casanova *et al.*, 2011). NMR MOUSE is a relatively small and compact device design to perform noninvasive and nondestructive analyses, highly valued in the field of cultural heritage. Objects like mummies (Rühli *et al.*, 2007), paintings (Presciutti *et al.*, 2008), frescoes (Proietti *et al.*, 2005) and parchments (Badea *et al.*, 2008; Masic *et al.*, 2012) were successfully analysed using NMR-MOUSE.

The hydrothermal stability of collagen fibres is currently evaluated by a micro-destructive diagnostic technique based on the combined use of optical microscopy and thermal analysis and called MHT method. The shrinkage temperature T_s of collagen

fibres characterises the collagen fibres structural collapse and hence the loss of mechanical stability and strength. This parameter is widely used to evaluate degradation level of collagen-based historical materials.

This work concerns with the investigation of the synergetic high temperature, low RH and visible light irradiation effect on the deterioration of calf and sheep leather tanned using various vegetal tanning extracts by correlating T_1 (longitudinal relaxation time) and T_s (shrinkage temperature) values.

EXPERIMENTAL PART

New leather from calf and sheep hides was obtained through traditional using different tannin extracts such as mimosa bark, quebracho and chestnut wood at the National Research and Development Institute for Textile and Leather, Leather and Footwear Research Institute Division (INCDTP-ICPI), Bucharest.

A first series of leather samples were exposed to accelerated ageing treatments inside a test chamber Binder APT Line KBF-ICH, at 70°C, 30% RH and 4000 lx illuminance (visible light region) for 8, 16, 32 and 64 days. It should be mentioned that the visible light exposure corresponds to 60, 120, 250 and 500- year dose, respectively.

Unilateral NMR measurements were performed with a portable Magritek NMR MOUSE, model PM 25, at 13 Mhz frequency. Longitudinal relaxation time T_1 was measured using a saturation recovery sequence combined with Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. T_1 values were measured by directly placing the leather samples on the measurement window of the instrument.

Shrinkage temperature T_s was measured with the home-made MHT equipment available at INCDT-ICPI using the procedure previously described (Badea *et al.*, 2012b). For the *Ts* evaluation a few fibres (~0.3 mg) from the corium side were immersed in demineralised water, covered with a cover glass placed inside the thermostatic cell of the hot table and heated at 2°C min^{-1}. Collagen fibres' shrinkage activity was recorded with the camera connected to the stereomicroscope and then visually evaluated by the operator.

RESULTS AND DISCUSSION

New Leather

Longitudinal relaxation time T_1 values for the new, untreated leather samples are presented in Figure 1, and shrinkage temperature T_s values for the same samples are reported in Figure 2. It can be seen that T_1 values depends on both tannin type and animal species. In general, T_1 values were higher for the leather samples tanned with chestnut wood extract (hydrolysable tannin), while the lowest T_1 values were obtained for the sheep hides tanned with mimosa bark extract (condensed tannin) independently of animal species. Since T_1 value is a measure of the strength of water – collagen fibres bonds we may infer that water interactions with collagen fibres in leather depend on both tannin type and animal species. We can thus assert that the number of leather sites capable of strong interactions with water is determined by both the tannin chemical structure and morphological structure of collagen fibres.

Shrinkage temperatures T_s showed to not depend on animal species. In addition, the chestnut tanned hides presented the highest T_s values. Shrinkage measurements results

indicated higher hydrothermal stability for leathers obtained using condensed tannins, in good agreement with the data in the literature (Larsen, 2000; Cucos *et al.*, 2014).

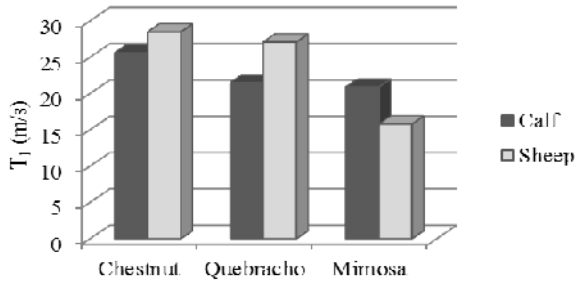

Figure 1. Longitudinal relaxation time T_1 values measured with NMR MOUSE for calf and sheep leather obtained using condensed (quebracho and mimosa) and hydrolysable (chestnut) tannins

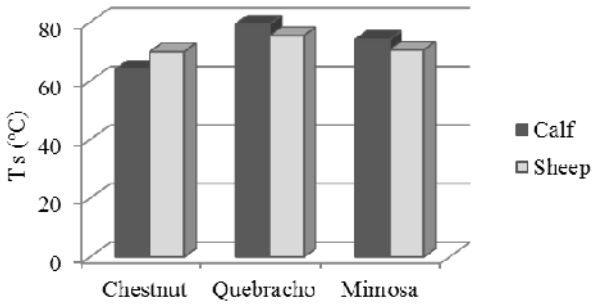

Figure 2. Shrinkage temperature T_s values measured through MHT method for calf and sheep leather obtained using condensed (quebracho and mimosa) and hydrolysable (chestnut) tannins

Accelerated Aged Leather

The longitudinal relaxation time T_1 values for the accelerated aged leather samples are presented in Figs 3 and 4, while shrinkage temperature T_s values for the same samples are reported in Figs. 5 and 6.

Data presented in Figures 3 and 4 indicate that the accelerated ageing treatment did not significantly influenced on T_1 values for calf leather (Figure 3), whereas for sheep leather a considerable decrease with ageing time was observed, especially for mimosa tanned leather (Figure 4).

As far as the hydrothermal stability is concerned we observed a progressive decrease of T_s value with ageing time for both calf and sheep leathers (Figures 5 and 6). Chestnut tanned leather showed to be the less resistant, calf leather being less hydrothermally resistant than sheep leather.

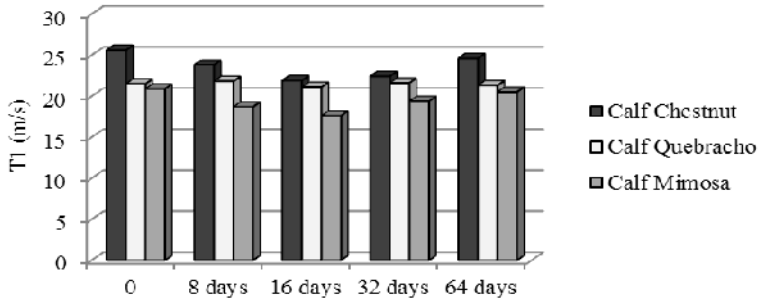

Figure 3. Variation of longitudinal relaxation time T_1 values measured with NMR MOUSE for calf leather obtained exposed to accelerated ageing at 70°C, 30%RH, and visible light irradiation for 8,16, 32 and 64 days

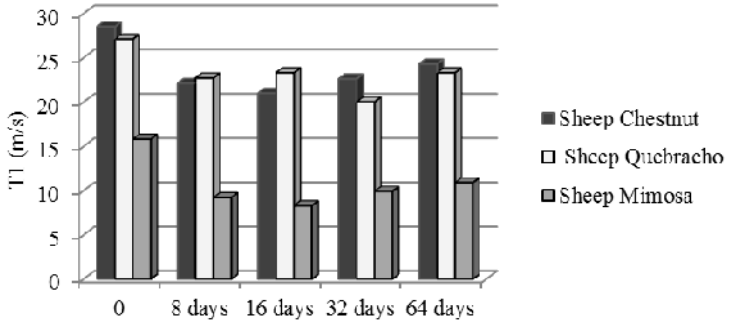

Figure 4. Variation of longitudinal relaxation time T_1 values measured with NMR MOUSE for sheep leather obtained exposed to accelerated ageing at 70°C, 30%RH, and visible light irradiation for 8,16, 32 and 64 days

The behavior illustrated by T_1 and T_s values suggest that thermal destabilization of calf leather may be attributed to the progressive conformational alterations resulting in formation of collagen populations with lower hydrothermal stability without significantly affecting the collagen – water interactions. This well correlates with the deterioration pattern observed for parchment exposed to accelerated ageing at high temperature, low RH and light irradiation (Badea *et al.*, 2012a). On the contrary, for sheep leather, both the hydrothermal stability and collagen – water interactions are influenced by accelerated ageing treatments.

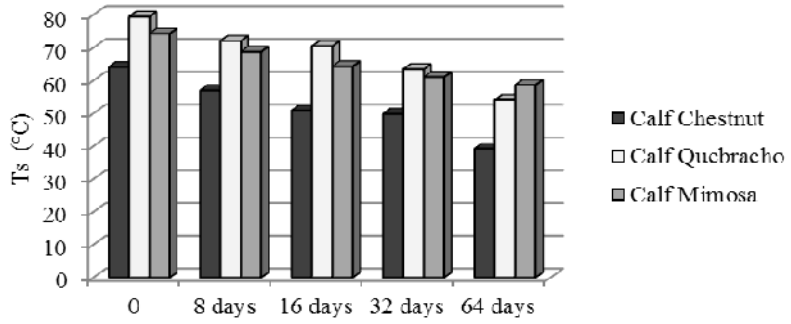

Figure 5. Variation of shrinkage temperatures T_s measured through MHT for calf leather exposed to accelerated ageing at 70°C, 30%RH, and visible light irradiation for 8,16, 32 and 64 days

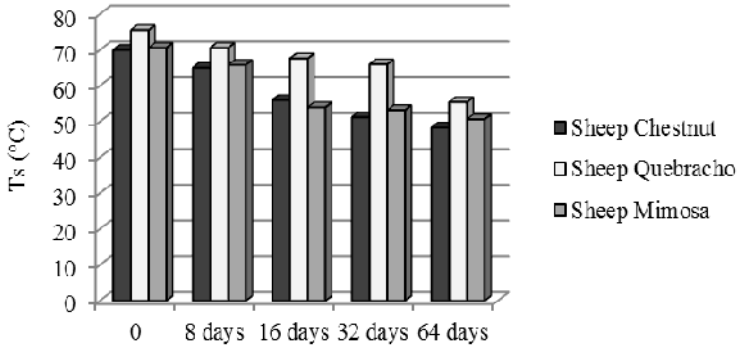

Figure 6. Variation of shrinkage temperatures T_s measured through MHT for sheep leather exposed to accelerated ageing at 70°C, 30%RH, and visible light irradiation for 8,16, 32 and 64 days

CONCLUSIONS

The synergetic effect of high temperature, low relative humidity and visible light iradiation on vegetable tanned leather from calf and sheep was investigated. The interaction between intrinsic water and collagen fibres was evalueted by measuring the longitudinal relaxation times T_1. Both new and accelerated aged calf and sheep leather obtained using both condensed (quebracho and mimosa) and hydrolisable (chestnut) tannins were non-invasively measured using a portable NMR MOUSE equipment. The hydrothermal stability of the samples was evaluated by measuring the shrinkage temperature T_s through MHT method. In summary, the main results of our study are as follows:

- Shrinkage temperature T_s significantly decreased with ageing time independently of animal species and tannin type. Chestnut tanned leathers were the most sensitive to the ageing treatment.
- T_1 values showed to depend on both animal species and tannin type. Moreover, sheep leather was sensitive to the ageing treatment, while calf leather does not show significant changes during accelerated ageing treatment.
- T_1 and T_s parameters are potential indicators for characterising tannin – collagen interactions as well as for assessing conformational, structural and stability changes during ageing and deteriorating of leather.

Acknowledgements

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RADIORESISTANCE OF BIODEGRADATION FUNGI AND ITS IMPORTANCE IN ESTABLISHING THE DECONTAMINATION DOSE

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The decontamination and preservation of artifacts from natural materials like paper, parchment, leather, textile, etc. is a continuous struggle against their colonization with bacteria, fungi or insects. Decontamination of cultural heritage objects by gamma radiation is a better alternative to chemical disinfection. Optimal decontamination dose selection is challenging. For this, the nature of the objects, the bioburden and the radioresistance of the contaminant microbial communities should be considered. Also, when establishing the radioresistance of a microorganism, some physical factors (irradiation support, storage temperature before irradiation) should be considered. These factors, especially the water content, influence the radioresistance, expressed as D10 value. Studies on Aspergillus niger and Trichoderma viride, which are common moulds that colonize and attack a wide range of artifacts, were carried out. The range of D10 value, influenced by the factors mentioned above, was studied. The focus of the study was the isolation of microorganisms from cultural heritage artifacts and their characterization regarding the radioresistance which further represents one of the preliminary steps in preservation/ restoration of cultural heritage artifacts.

Keywords: D10 value, radioresistance, fungi

INTRODUCTION

Fungi have a great impact on the deteriorating of the cultural heritage, due to their ability to grow in different environmental condition. They can produce decaying of very wide types of cultural heritage artifacts (paintings, textiles, paper, parchment, leather).

Remediating the mould growth in a storage place for cultural heritage objects needs to consider the following aspects: remove the mould attacked items, clean the air spaces, control the physical conditions like temperature and humidity. Appropriate conservation measures and restoration treatment to deal with fungal infections include mechanical, chemical and physical methods, which entail effects on the object itself and health hazard for human.

Several techniques have been developed for book and document conservation reducing the threat of biodeteriorating agents, such as fungi. Some of these techniques involve the use of very toxic chemicals, including ethylene oxide, which has carcinogenic properties and is banned in a number of countries, besides being expensive (Flieder *et al.*, 1994; Adamo *et al.*, 2001; Gonzales *et al.*, 2002, da Silva *et al.*, 2006). An alternative is the use of gamma radiation, a promising treatment in the preservation field (da Silva *et al.*, 2006). Studies demonstrated that the damage in mechanical-physical properties caused by gamma rays on paper was not significant. The doses of 10 kGy did not cause any negative effect on the mechanical-physical properties of the paper, even after an accelerate ageing of 12 days (Adamo *et al.*, 1998, 2001; Gonzales *et al.*, 2002).

Fungi have been successfully inactivated from different materials, such as paper, wood and soil with radiation dose ranging from 6 to 15 kGy (Hanus, 1985; Jörg *et al.*, 1992; Pointing *et al.*, 1998; McNamara *et al.*, 2003). In a Brazilian study some fungi from books could not be completely eliminated after irradiation with doses of 20kGy (Tomazello and Wiendl, 1995).

However, it is not necessary or practical to remove 100% of all mould spores from all items. Even if this goal is achieved, the result would only be temporary as new mould spores will continuously settle on items (www.moldservicesgroup.com).

The killing effect of radiation in microorganisms is generally expressed by the decimal reduction dose or D10 value (Thornley, 1963). The D10 value is the reciprocal of the slope of the exponential part of a survival curve. This value may also be obtained from the following equation:

$$D10 = \text{Radiation dose} / \log10 (X0-X) \quad (1)$$

where X0 is the initial number of organisms, and X is the number of organisms surviving the radiation dose.

Consequent treatment of artifacts, at a certain dose can be applied, using D10 value, to provide the desired reduction of microorganisms. The bioburden population is normally reported as the number of colony forming units (or CFU). In order to establish the treatment irradiation dose, aspects like the degree of degradation of the artifacts and the degree of contamination should be taken into account. Establishing the optimum dose is real challenge. The irradiation dose can be established starting from the radiation resistance of the contaminants.

The study aims to establish how the environmental condition like humidity (common condition in the storage place) influence the D10 value and as a consequence, the irradiation dose.

MATERIALS AND METHODS

The present study was focused on the determination of the D10 value of Aspergillus niger and Trichoderma viride, which are common agents of biodegradation of the cultural heritage objects (paper and cellulose textiles) (Mesquita *et al.*, 2009; Meier and Petersen, 2006; Blyskal, 2009; Pangallo *et al.*, 2009; Sterflinger, 2010, Chirila *et al.*, 2014). The influence of humidity on D10 value was than analyzed.

Spores from a biodegradated Orthodox religious book (Fig. 1), from early 19th century “Strastnic”, Blaj, Romania were harvested using wet swabbing method.

Figure 1. Orthodox religious book Strastnic, Blaj, 1817

The spores were directly transferred on Sabourand agar in Petri dish, incubated at 220C and purified by further subcultures on the same agar medium.

Two types of mould were identified based on the morphological features as Aspergillus niger (Fig. 2) and Trichoderma viride (Fig. 3), using microscopy techniques (Zeiss Axio Imager D1m). Trichoderma viride was also characterized by Scanning Electron Microscopy (SEM) (Quanta 200-FEI) (Fig. 4).

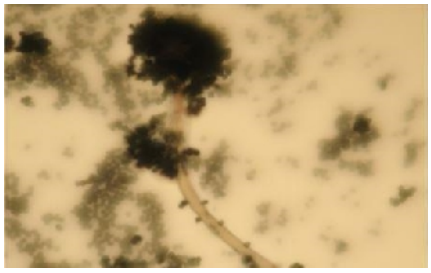

Figure 2. Aspergillus niger (400X)

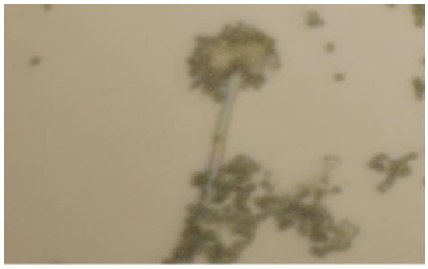

Figure 3. Trichoderma viride (400X)

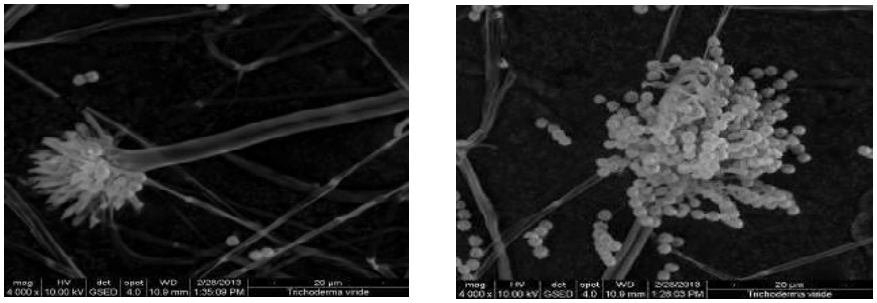

Figure 4. Trichoderma viride: conidiophores with phialides

For both species, pure cultures were made on Sabourand agar, in Erlemeyer flasks and incubated at 22.5°C for 7 days until abundant sporulation was obtained. After sporulation, the spores were harvested by flooding the flasks with 10 ml of Sodium Chloride Peptone Broth with 0.3% (w/v) Tween 80 (APS 0.3%Tw) and by shaking with glass beads. The washings procedure was repeated 3 times. The collected suspension containing the spores was than centrifuged 10 minutes at 3500 rpm, discarding the supernatant each time. At the end the spores were resuspended in APS 0.3%Tw and subjected to vortex in 3 session of 30 seconds each, for a better dispersion. The degree of dispersion of the spores was checked by phase contrast microscopy (optical microscope Zeiss Axio Imager D1m). The concentration of the spore suspension was confirmed by plating dilutions. The final concentration of the suspension was 8x108 CFU/ ml in case of A. niger and 5x108 CFU/ ml in case of T. viride.

The gamma irradiation was made on paper, in order to simulate the natural support of the cultural heritage artifact.

Sterile pieces of paper of 1 cm^2 were evenly contaminated with 0.1 ml of suspension. Aliquots of 0.02 ml were distributed in 5 points of the paper, in order to prevent clustering of spores.

The pieces of paper were then dried in sterile conditions and put into plastic cap tubes. Three tubes were irradiated for each of the following doses 0.25 kGy; 0.5 kGy; 1 kGy; 1.5 kGy; 2 kGy; 3 kGy. The spores were removed by shaking each piece of paper in APS 0.3% Tw diluent, in a stomacher (Bag Mixer), at 300 rpm, for 5 minutes, after 10 minutes of soaking for each. The recovery percent from total matrices mass was not quantified but it was assumed to be equal among all replicates and dilutions. The decimal reduction was calculated by comparison with the recovery from the non-irradiated sample. The surviving microorganisms were enumerated by serial dilutions immediately after irradiation, using Sabourand agar. The regression slope and the D10 value were calculated.

The same experiment was performed two weeks later, for other two sets of identical samples. During this interval, one set of paper was kept in environmental humidity, and the other was kept in humid atmosphere (> 85%) at 28°C, in order to simulate the humid storage conditions for an artifact.

RESULTS AND DISCUSSION

The D10 values obtained in different experimental conditions of time and humidity were compared in case of both fungi. The two fungi have a very different behavior in similar conditions (humidity).

As seen in the table and figures below (Table 1 and Fig. 5 - 6), in case of A. niger, D10 value decreased after two weeks of storage in humidity conditions from 0.86 kGy to 0.51 kGy. Also, after two weeks of storage at environmental humidity, the D10 decrease to 0.66 kGy.

The decreasing trend of D10 value mantained also for Trichoderma viride although the diferences were not so remarcable. In fortheen days of storage, the radioresistance ranged between 0.50 kGy and 0.45 kGy.

Comparing the radioresistance of the two fungi, it can be concluded that A. niger is more resistant to gamma radiation than T. viride. Also, time and humidity have a greater downward influence of the D10 value for A. niger, while decreasing is almost insignificant for D10 value in case of T. viride.

Table 1. D10 value for A. niger and T. viride

Microorganism / Conditions	Paper, 28°C, 2 days	Paper, 28°C, 14 days	Paper, 28°C, 14 days, humidity
Aspergillus niger	0.86 kGy	0.67 kGy	0.51 kGy
Trichoderma viride	0.50 kGy	0.47 kGy	0.45 kGy

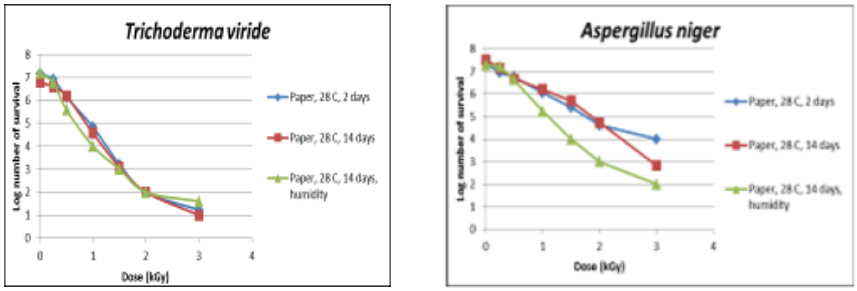

Figure 5. Survival of T. viride and A. niger after gamma irradiation

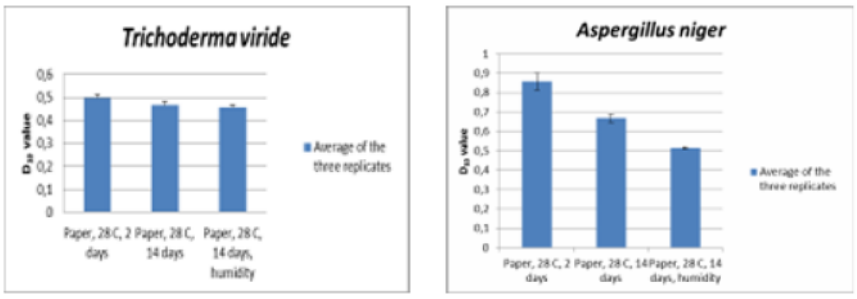

Figure 6. D10 values for T.viride and A. niger after gamma irradiation treatment

CONCLUSION

Taking into account the decreasing of D10 in time and in humidity conditions, we can draw the conclusion that these factors do not influence the radioresistance in a way that the increasing of the decontamination dose should be considered. We emphasize that the decreasing was obtained within two weeks of exposing the spores at high humidity condition and at environmental humidity.

Further investigations are needed to study the degradation and the behavior of different fungi species recovered from the cultural heritage artifacts under gamma irradiation exposure.

Acknowledgements

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TESTING OF ARTIFICIALLY AGED LEATHER IN ACID RAIN

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To assess the resistance of calf leather tanned with quebracho to acid rain, the changes in shrinkage temperature, melting temperature and enthalpy of crystalline zone, and contact angle of a liquid drop as artificially aging in acid rain were determined. Acid air aging of leather was revealed to bring about the weak decrease of shrinkage temperature, melting temperature and absolute value for enthalpy of leather crystalline zone. On the other hand, there is not a significant difference between values of contact angle of initial and aged samples. All these results show that the investigated leather exhibits a good resistance to acid rain.

Keywords: leather, artificial aging, acid rain.

INTRODUCTION

The chemical pollutants play an important role in the deterioration of leathers at all levels of structural hierarchy, from molecular to microscopic levels (Florian, 2006). Gaseous pollutants from wet air, especially CO_2, SO_2, NO_x, combined with water, oxygen and ozone could form acid rain, which has an important aging effect on collagen based materials. Sulphurous acid results by reaction of sulphur dioxide with water. Sulphur dioxide can also react with oxygen and ozone and forms sulphur trioxide, which reacts with water, resulting in sulphuric acid. So obtained mixture of sulphurous acid, sulphuric acid, sulphur dioxide and air could cause hydrolysis of collagen and tannins that determines the deterioration of leather. This deterioration leads to changes of physical-chemical properties of leather. Therefore, it is important to investigate artificial aging of leathers in acid rain. The objective of this work was the determination of the resistance of leather manufactured from calf skin tanned with quebracho to acid rain.

EXPERIMENTAL

Material

The leather was produced at National Research & Development Institute for Textile and Leather, Division Leather and Footwear Research Institute (INCDTP-ICPI) by an original procedure, using calf skin as raw material and quebracho as tannin.

Aging Conditions

The preparation of artificial acid rain: 0.04 mL H_2S0_4 (98%), 0.06 mL HCI (36-38%), 0.02 mL NH_4OH (25-28%) were dissolved in 80 mL distilled water and then

0.0296 g Ca $(OH)_2$ was added in the solution. The solution had a constant volume of 2L. The pH of float was determined to 3.25.

Artificial acid rain aging: each quebracho tanned leather sample was soaked in a float of artificial acid rain solution in a sealed beaker at room temperature (about 25°C) for 48 hours. Then the soaked samples were covered by sealed bags and put in a sealed box for 0, 5, 10, 15, 20 and 25 days at 100% relative humidity and 50°C respectively. The treated samples were dried 48 hours in the air. And then the samples were placed in desiccators to adjust to a constant weight over a week for further tests.

Determination of Shrinkage Temperature

The shrinkage temperature was determined by the following methods:

- the standardized method for determination the shrinkage temperature described in SR EN ISO 3380 – 2003 and TEST IUP 16 of the International Union of Leather Technologists and Chemists Societies (Williams, 2000), using Leather Shrinkage Temperature Tester GIULIANI, IG/TG, 2001;
- Micro Hot Table (MHT) method by the procedure described by Larsen *et al.* (1993) using a MHT apparatus produced by Caloris – Romania.

DSC Analysis

The DSC curves were recorded using DSC 204 F1 Phoenix apparatus produced by Netzsch – Germany in the following conditions: sample mass 2-3 mg; nitrogen flow (purity of nitrogen is higher than 99.999%; 20 $mL \cdot min^{-1}$); heating rate of 10 $K \cdot min^{-1}$, and the temperature range 25^0C … 280^0C.

Contact Angle Measurements

Contact angle measurements were made using an equipment which allows analyzing static or dynamic phenomena which take place when a liquid drop interacts with a solid surface (VCA OPTIMA). For each sample a 5 µL water drop was used and maintained in contact with the leather surface for 180 seconds. Five pictures per second were made and the results were processed by using a special soft called VCA Optima XE.

RESULTS AND DISCUSSION

Determination of Shrinkage Temperature

The values of shrinkage temperatures (T_s) determined by standardized and MHT methods are listed in Table 1. The inspection of this Table shows:

- For a given t there is a difference between the values of T_s determined by different methods. These differences are due to different shapes of samples used in the two methods of shrinkage temperature determination, namely a rectangular shape for standardized method, and few fibers for MHT method.
- The increase of aging duration determines a weak decrease of T_s value. However, for a given method and $t \geq 10$ days, the differences between T_s values are smaller than 3^0C, which could be due to non-homogeneity of samples and/or to inherent experimental errors.

Table 1. The values of shrinkage temperature (T_s) determined by standardized and MHT methods

Method → t/day*	Standardized method T_s/^{0}C	MHT T_s/^{0}C
0	80	70
5	75	71
10	74	67
15	73	68
20	73	69
25	71	69

* t = duration of aging in acid rain

DSC Analysis

DSC curves associated to thermal transitions which typically occur in leather samples measured in open crucibles and nitrogen flow display a broad endothermic peak followed by smaller endotherms (see Figures 1 and 2). The broad peak in the temperature range (50 – 120) °C is associated with thermal dehydration of the sample. The one or two endotherms that occur at T > 220°C were ascribed to thermal melting (denaturation) of the crystalline collagen embedded in the amorphous matrix (Popescu *et al.*, 2008). It was shown that temperatures corresponding to these peaks are well correlated with the thermal stability of the crystalline (rigid) fraction of collagen in collagen based materials.

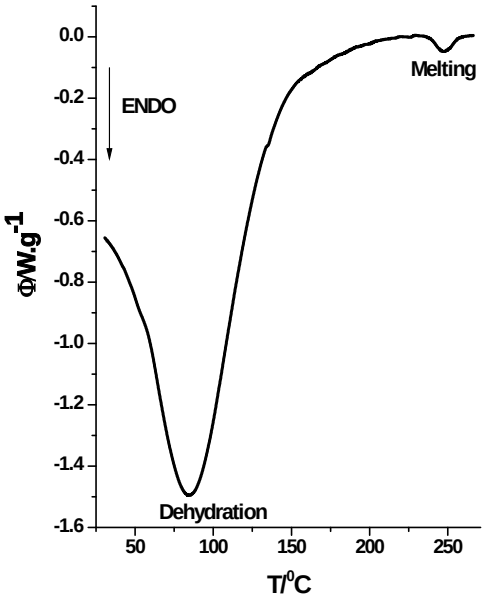

Figure 1. DSC curve obtained by analysis of initial (unaged) leather

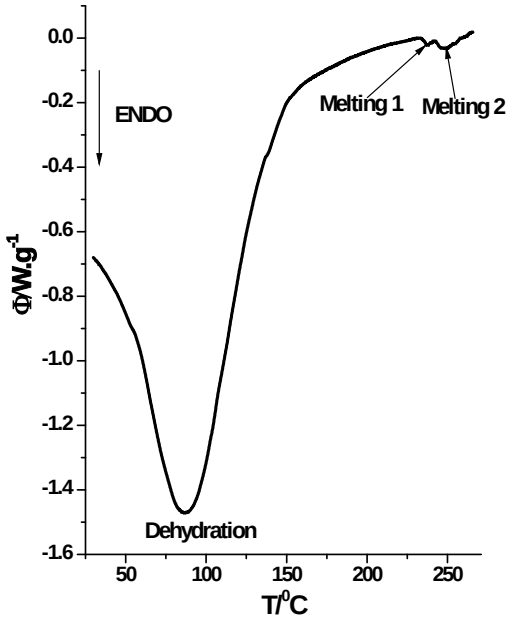

Figure 2. DSC curve obtained by analysis of leather aged in acid rain for 10 days

Dehydration process is characterized by temperature corresponding to the first minimum in DSC curve (T_{min}), while the melting process is characterized by melting temperature (T_m) and corresponding enthalpy (ΔH). The values of these parameters are listed in Table 2.

Table 2. The values of characteristic parameters of dehydration and melting processes for initial and aged samples

Process →	Dehydration	Melting of crystalline zone	
t/day	T_{min}/^{0}C	T_m/^{0}C	$-\Delta H$/J.g^{-1}
0	84.5	247.4	3.2
5	83.8	248.4	4.2
10	86.8	237.8	0.4
		249.0	1.7
15	83.8	251.8	2.7
20	82.6	252.0	2.2
25	79.1	252.5	2.1

According to data listed in this Table, the increase of aging duration determines a weak increase of melting temperature of crystalline zone of leather. This means that the aging in acid rain determines a weak increase of rigidity of crystalline zone.

The absolute value of ΔH can be correlated with the relative content of crystalline zone. According to data listed in Table 2, the relative content of this zone increases after 5 days of aging, and is lower and practically constant after 10 days of aging. The sample

aged for 10 days exhibits two distinct melting processes. Such behavior shows that this leather exhibits two kinds of crystalline zone.

Contact Angle Measurements

The obtained results indicate that the samples are hydrophilic (the contact angle is <90°) (see Figure 3). The values of contact angle are listed in Table 3. The differences between the blank and the treated samples are insignificant, meaning that the treatment does not affect the surface properties.

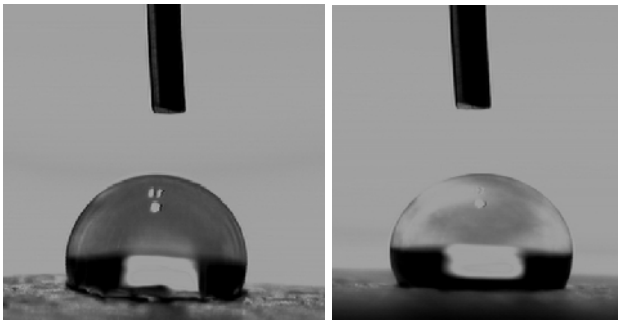

Figure 3. Water drop on leather surface

Table 3. The values of contact angle for initial and aged samples

t/day	contact angle
0d	23.07
5d	22.31
10d	22.58
15d	18.33
20d	18.75
25d	21.56

CONCLUSIONS

- The changes in shrinkage temperature, melting temperature and enthalpy of crystalline zone, and contact angle of a liquid drop corresponding to calf leather tanned with quebracho, as the result of artificially aging in acid rain were determined.
- The application of standardized (TEST IUP 16) and MHT methods have shown a weak decrease of shrinkage temperature when the aging duration increases.
- The use of DSC analysis in nitrogen flow has shown that both melting temperature and absolute value of enthalpy corresponding to crystalline zone of leather exhibit a weak decrease when aging duration increases.
- The results obtained at determination of contact angle of a liquid drop have shown that the values of contact angle are not practically changed as a result of artificially aging.

- All obtained results highlight that the investigated leather has a good resistance to acid rain.

Acknowledgments

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VI.
INNOVATION

A MODERN APPLICATION FOR CUSTOMIZED FOOTWEAR DESIGN

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The aim of this article is to present a modern application for customized footwear design using MindCAD software. These CAD systems are the next generation of design solutions and engineering for the footwear industry. Designed entirely for use with modern operating systems and environments, it provides a full range of instruments, intuitive and easy to use. 3D computer aided design techniques (3D CAD) enables direct modelling of footwear on the last, so even before the product is fabricated, it can be analysed in terms of visual, functional, industrial and financial criteria. Starting with a shoe last (digitized, scanned or from database), it can quickly be designed a complete footwear model, in any colour or material combination. The result is a 3D realistic view of the product, ideal for presenting it to the buyers, customers or producers.

Keywords: computer aided-design, shoe last, footwear design, 3D modelling

INTRODUCTION

The foot is the interface between the body and the ground, opposes both static and dynamic compression and considerable shear forces. Its protection is assured by the particular architecture. Plantar aspect of the heel and foot arches are the main weight-bearing structures (Biga, 2009).

During walking, the foot plays the role of a flexible shock absorption system, deforming on uneven surfaces before undergoing through a series of biomechanical changes that allows it to act as a rigid lever of force (Dawe and Davis, 2011; Kitaoka, 2008).

Elements such as toe cap shape, sole and heel shape and dimensions should be taken into consideration in order to solve the main problem of static and dynamic foot pathologies.

CAD (Computer Aided Design) systems should be as discrete as possible so that an engineer can concentrate on what it designs and not on the interface or system procedures that are to be used. For many years, the main purpose of CAD software manufacturers was growth and development of parameters and geometric characteristics. But lately, it has been introduced an intuitive approach called direct modelling. It was originally designed for conceptual design and architectural planning, but direct modelling successfully integrates commercial packages for various industries. Direct modelling allows the user to intuitively select and manipulate real-time geometric entities (Fiorentino *et al.*, 2010).

Consumers' requirements focus more on comfort and functionality in terms of shoes, making these features to be important considerations for modelling, designing and evaluating the footwear (Rupérez *et al.*, 2010).

The shoe last, a 3D instrument used to design and produce the footwear, influences the shape and size of the shoe. Current software design or process focuses mainly on reverse engineering and modification of existing lasts (Drsicu and Costea, 2013; Costea and Mihai, 2013; Vasilescu *et al.*, 2012).

3D scanning technologies and CAD solutions, recently developed, allow reconsidering the design process of lasts, and providing useful tools for designing customized footwear (Sikyung *et al.*, 2007).

Product performance can be assessed based of its functions (the product works as designed), its shape (aesthetic) and its fits (purpose). The shape follows function in case of bare feet, but for many consumers, the dimensional comfort can govern function and therefore, being an important criteria. In traditional applications of mechanical engineering, there are different types of matching according to function.

METHOD

Footwear role in ensuring correct body posture and balance condition during static or dynamic phase requires the study of ergonomic factors in product design. It is applying a new design concept, footwear product should reflect the normal anatomical and functional state on the foot and gives a natural feeling of barefoot walking.

In shoe modelling and design activities a series steps have to be followed, which must fulfil the footwear criteria, namely: aesthetic, functional, economic and technological. Current 3D CAD systems enables direct modelling of shoe last, so that even before the producers made the product, it can be analyzed in terms of these criteria.

MindCAD is the perfect solution for the product designer and engineer, offering a balanced mix of creative and technical 2D and 3D CAD tools (Mindtech).

The unique and innovative features of MindCAD solutions contribute decisively to your effectiveness and productivity.

MindCAD SOLUTIONS

- 3D Design & Engineering for Footwear
- 3D Viewer
- 2D Design & Engineering for Footwear
- 2D Design & Engineering for Luggage
- 2D Design & Engineering for Automotive
- 2D Design & Engineering for Furniture

Main features of MindCAD 3D Design & Engineering for Footwear are:

- Last digitizing and editing (see in figure 1)
- Sketch style lines (see in figure 2)
- 3D upper part modelling (see in figure 3)
- Sole modelling (see in figure 4)

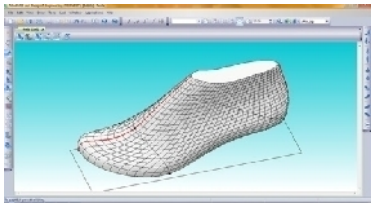


Figure 1. Last digitizing and editing

Figure 2. Sketch style lines

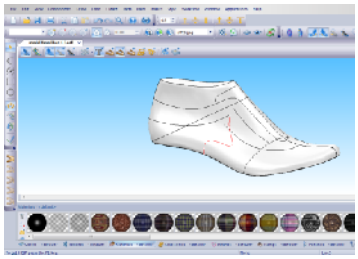
Figure 3. 3D upper part modelling

Figure 4. Sole modelling

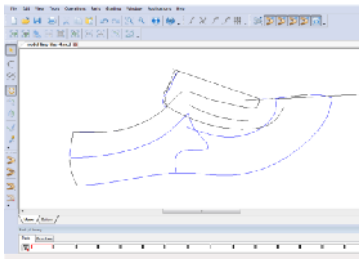
Starting with a last, it can be rapidly designed a complete footwear model, in any colour or texture combination (Pastina *et al.*, 2011, Pastina and Mihai, 2011). The result is a 3D realistic view of the product, ideal for presenting it to the customers, buyers or producers.

The main advantages of MindCAD compared to other software applications are:

- An intuitive interface that allows the user to work in a productive way
- The interaction in real time with the design (see in figure 7)
- A realistic representation of the product (see in figure 4)
- The integration between 3D and 2D applications: for example, a change made in 3D reflects in 2D in the same time and also form 2D to 3D (see in figure 5 a and b)
- The instruments, the way of using it, the interface, are similar from 3D to 2D, so the user doesn't have to learn how to use 2 applications (see in figure 7)
- The software producers stay in touch with all their partners and clients form industry and schools, so the software benefits from their feedback
- A precise method of patterns grading and a full range of size numbers that can be obtained (see in figure 8)



a



b

Figure 5. a, b. Integration between 3D and 2D

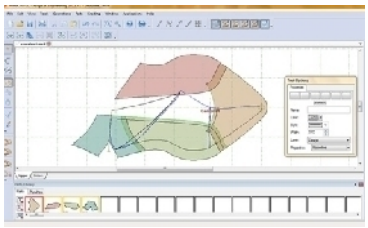


Figure 7. 2D patterns design

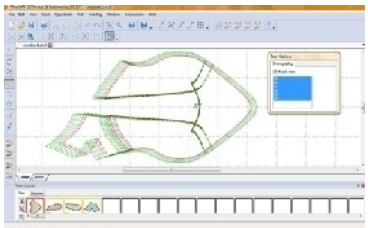


Figure 8. Pattern grading

CONCLUSIONS

CAD / CAM revolutionary systems solutions represent the next generation of computer-aided design and engineering in the shoe industry. Unlike manual methods for retrieving foot measurements and shoe design, the development of current systems allow designing different models according to feet structure. There are used procedures and techniques that were not possible in the case of manual methods, such as 3D scanning and 3D modelling, 3D viewing, automatic analysis of forms, extracting and interpreting patterns.

Dimensional correspondence of the foot with the shoe size (length, width, circumference, height) is a very important requirement to ensure dimensional comfort. To ensure this requirement, the shoe must be designed and produced in order to allow the foot to function normally without severe constraints, both in static and dynamic conditions.

The main advantages of using MindCAD: reduces the number of physical samples; a fast way to design a product; modifying operation directly on the model; applying or eliminating new components; model visualization from different angles by interactively rotating the last.

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*** http://mindtech.pt/

INNOVA-LEATHER - INNOVATIVE TECHNOLOGIES FOR LEATHER SECTOR

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Leather industry has to cope nowadays with major environmental problems because of the polluting processes (a World Bank report has placed the leather industry in the ninth place when considering the environmental impact). Therefore, increasing the environmental efficiency in the leather sector is the major aim of leather, auxiliary materials and equipment manufacturers. The development of new tanning agents and new technologies is required to cope with the increasingly higher environmental pressure on the current tanning materials and processes such as tanning with chromium salts. This paper presents the main results obtained in the framework of INNOVA PROJECT. The original contribution of this project in solving the above problems has involved the use of solid titanium wastes (cuttings) resulting from the process of obtaining highly pure titanium (ingots) in the preparation of new tanning compounds intended to increase the environmental efficiency of the leather sector. Also, is within the above line, aiming to obtain wet-white leather by an organic tanning process in order to reduce chromium in tannery effluent. Other main objective of the project is valorization of wet white leather waste as raw material for obtaining new biodegradable auxiliaries with application in agriculture, cosmetics, industry.

Keywords: tanning agents, wet white, FOC leather, waste valorization, cosmetics, soil remediation, sustainable development

INTRODUCTION

Chrome tanning is the most common type of tanning in the world. Chrome tanned leathers are characterised by top handling quality, high hydro-thermal stability and excellent user properties. Chrome waste from leather processing poses a significant disposal problem. It occurs in three forms: liquid waste, solid tanned waste and sludge. In most countries, regulations governing chrome discharge from tanneries are stringent. Today, all tanneries must thoroughly check their waste streams. Chrome discharge into those streams is one of the components that has to be strictly controlled.

Conventional chroming process generally involves in pickling, chroming and basifying, and there are several defects existing in the process (Sykes, 1981; Germann, 1995): i) 8-10% salt and 1.0-1.2% sulfuric acid were used in pickling, which results in higher contents of chlorides, sulfates and chemical oxygen demand (COD) in the effluent; ii) The uptake of chromium in conventional chroming is lower (70-80%), a considerable amount of chromium left in the effluent may result in environmental problems (Ludvik, 1997); iii) A great deal of chrome containing solid wastes such as splittings and shavings are produced, which is certainly difficult to be degraded and harmful on the environment if discharged directly.

Much criticism has been directed towards the use of chromium salts in leather tanning, but it has to be borne in mind that chromium can occur in different oxidation states and its compounds behave differently. Most chromium(VI) compounds are highly

toxic and classified as MAK III A 2 carcinogens, but chromium(III) is an important trace element in man and animals. Chrome is mentioned in list 2 of the Annex to Council Directive 76/464/EEC of 4 May 1976 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community. Tannery wastes containing chromium are not included in the European Hazardous Waste List on the basis that the wastes do not possess the characteristics necessary for classification as a hazardous waste (COTANCE, 2002).

The Main Objective of the project was to **develop a new "clean", eco-friendly tanning technology, alternative to chrome tanning (wet blue)** and **valorization of leather wastes** obtained through this system. This new system includes obtaining of new tanning agents **(*Knowledge/based Tanning Agents / KTA),*** of a new tanning system, and obtaining of new type of leather, so called **"wet white"**.

S&T Objectives of the project were:

(*) **synthesis the new tanning agents (*Knowledge/based Tanning Agents / KTA)**;

(*) development of **new eco-technologies** for leather pretanning/ tanning;

(*) development of new type of leather, so called **"wet white"**;

(*) development of **new conversion procedures of "wet/white" leather wastes** into **by-products with increased added value**;

(*) **transformation / functionalization of different peptides** (obtained from "wet/white" leather wastes) by coupling/reticulating chemical reactions, into raw materials for obtaining new, biodegradable auxiliary materials destined for various applications: industrial, agriculture, cosmetics etc.;

(*) development of **new biodegradable auxiliary materials**;

(*) **LCA - Life Cycle Assessment Studies** for the new developed processes.

RESULTS AND DISCUSSION

The main results obtained in the project are:

Tanning Agents KTA-M Based on Ti and Al

Exploring the valorisation of solid Titanium metallurgic end wastes, as a low cost raw material has yielded new tanning agents for the replacement of Cr(III) tanning salts, a hitherto unthinkable or non technically feasible mission. In turn, as demonstrated here it is plausible to: increase of eco-efficiency in the leather manufacturing sector by making use of solid wastes, which cannot be recycled in the industry that generated them:

i) total or partial replacement of chromium salts in the tanning process with cheap to produce and easy to apply in rapid full substance bovine leather manufacture, that, in turn required minimum process rationalisation or modification; moreover, the new mineral tanning agents are free of restricted or regulated metals Cr, Pb, Cd, Hg and Ni;

ii) increase in articles diversity (Crudu *et al.*, 2014).

Figure 1. Tanning agents KTA-M

Tanning System KTA-S Based on Resorcinol and Oxazolidine

Combinations of oxazolidines with resorcinol can replace chrome tanning without sacrificing the physical and thermal properties of the tanned leather. Finally, with the use of oxazolidine, a more effective salt-free pickling process can be achieved and the environmental impact within leather manufacturing can be further reduced.

Since there was no chromium existed in the splittings and shavings, the wastes could be treated and reused more easily (Deselnicu *et al.*, 2012).

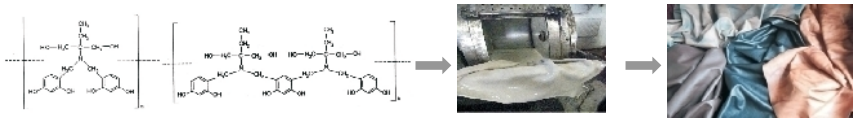

Figure 2. Tanning system KTA-S

Obtaining of New FOC (Free of Chrome) Leather Assortments: Bovine Upper Leather, Bovine Upholstery Leather, Clothing Leather

Chrome-free leather tanning system has many **demonstrable advantages**:

* no chromium in the effluents;
* solid leather wastes which can be recycled into value-added products (fertilizers and / or chemical auxiliaries used in leather industry and other industries, cosmetics);
* solid wastes (sludge resulted from purifying waste waters) without chromium;
* no risk of Cr(VI) – (causes cancer) formation from Cr (III);
* excellent shrinking behaviour;
* brilliant dyeing, especially for fashion items;
* heavy metal free leathers for allergic persons;
* improved sorting leathers for various destinations, as early as the pretaning stage;
* the possibility of storage and marketing of wet - white leathers;
* materials economy by sorting and assigning hides before tanning, to optimal sorts;
* reducing the depollution costs;
* more biodegradable leathers (Chirila *et al.*, 2014).

Valorization of Wet-White Leather Waste

Studies have been made for transferring the solid wet-white leather waste into raw materials which can be used for development of novel bio-composites (fertilizers and / or chemical auxiliaries used in leather industry and other industries, cosmetics) (Figures 3-5) (Albu *et al.*, 2014; Deselnicu, D. *et al.*, 2014; Zainescu *et al.*, 2014).

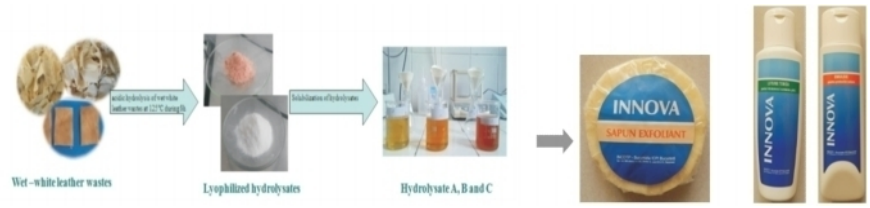

Figure 3. Obtaining of new cosmetics

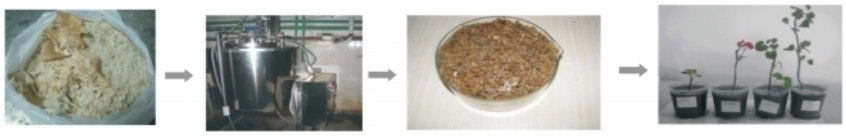

Figure 4. Obtaining the soil remediation product for agriculture

Figure 5. Obtaining Pigment paste for leather finishing and adhesive for footwear sector

LCA Study (Deselnicu, V. *et al.*, 2014)

Application of new tanning systems and transformation of the solid leather waste into **new value-added products** lead to remarkable life-cycle-improvements of the starting materials and close loops in terms of sustainable utilization of former wastes, i**ncreasing the eco-efficiency** and **economic efficiency** of leather sector. LCA comparative study between chrome tanning (Cr) and tanning leather with KTA-M system (FOC) revealed the results presented in Figures 6 and 7.

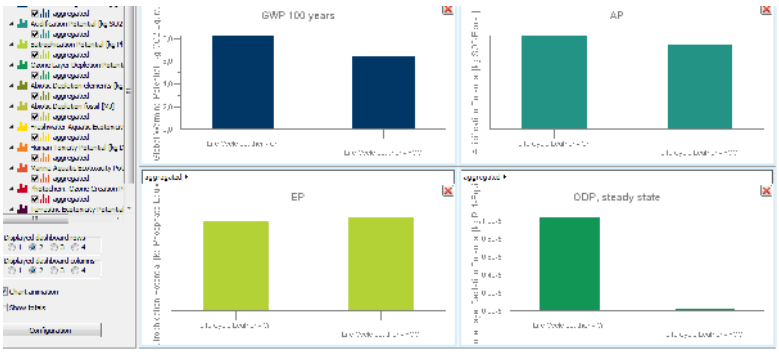

Figure 6. Global Warming Potential (GWP), Acidification Potential (AP), Eutrophication Potential (EP) and Ozone Depletion Potential (ODP)

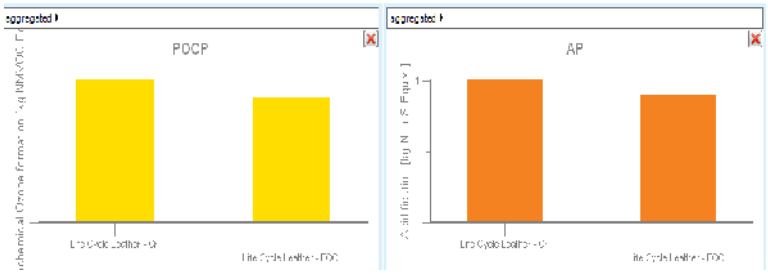

Figure 7. Photochemical Ozone Creation Potential (POCP), Acidification Potential (AP)

The Carbon footprint obtained for KTA-M -Ti-Al technology system was 9,7250 kg CO_2 equiv. and for chrome tanning system was 11,4848 kg CO_2 equiv.

The main conclusion of the study is that **the new KTA-M overall tanning technology developed in INNOVA LEATHER project generates a 15% lower environmental impact measured as Carbon footprint (Global Warming Potential indicator) than the classic Chrome tanning technology.** The other calculated impact category indicators have comparable values between the two technologies.

New Data Bases (Macovescu *et al.*, 2012)

Five new data bases were developed regarding: DB1 - *Environmental legislation database for the leather and footwear industry*, DB2 - *Clean technologies for leather manufacture*, DB3- *Specific analysis for leather*, DB4 - *Information about Romanian companies from leather- footwear sector* and DB5 - *Project results.*

Patents and Awards

The innovative solutions made the object of **5 patent applications** and were awarded with **7 Gold medals** and **3 special awards** at important international fairs.

Beneficiaries

The main beneficiaries of the project results are:

Direct Beneficiaries

1) INCDTP - Division ICPI by:

- The implementation of the project created a **scientific and technologic competence core** by **research – development** – **innovation** within INCDTP Division ICPI in the leather processing field at European standards, having as a result **the development of new products, technologies and services**, with high added value and market demand, as a basis for technological transfer in the industrial sector and production application;

2) Civil society by living in an environment less pollutant for a healthy life;

3) The leather industry in Romania (tanneries), mostly SMEs;

4) The Association of Leather and Fur Manufacturers in Romania and **The Owners Organization in the Leather Footwear Industry,** which are interested in the project realization and are directly capitalizing its results, thus contributing to the project implementation process.

Indirect Beneficiaries

1) Related industries: footwear, leather clothing confections, morocco goods, fashion;

2) The chemical industry in Romania/Europe, because, in the project, methods of sustainable use of wet white leather wastes will be elaborated, supporting thus the use of renewable resources other than oil;

3) Food industry (butcheries) from which result significant quantities of inedible wastes, which can be recovered by similar procedures, after adaptation (the elaboration of a new project is taken into account for recovering these wastes);

4) Cosmetics – by making new products with animal protein content;

5) Agricultural sector by applying the fertilizers obtained from wastes;

6) Education system - pupils, students, teaching staff, benefiting from the new information acquired by elaborating the project.

CONCLUSIONS

By application of new innovative tanning systems **significantly reduces the environmental impact** (generating solid wastes and effluents without chromium), **and people safety impact** (leather without chromium). The solid wastes without chromium can be valorized as **by-products with increased added value,** leading to favourable economic and environmental benefits by increasing their life cycle (as compared to *incineration* which is currently practiced in EU, and *disposal* which is currently practiced in Romania).

The Project's results contributes to the **development and validation of a sustainable production system** for the leather sector in Romania.

Acknowledgements

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PROCEDURAL ASPECTS ON THE APPLICATION MAINTENANCE CONCEPT BASED RISK AND RELIABILITY CENTERED IN THE CASE ASSESSMENT STRUCTURAL INTEGRITY OF EQUIPMENTS FOR INDUSTRIAL PROCESSES

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The essential aspects are presented and discussed specific application of the national concept of risk based maintenance (*RBM*), focused on reliability (*RCM*) and focus on the performance of equipments and facilities in the process industries. Currently, this type of maintenance is one of the most modern and innovative conceptual models which is based on control, monitoring and risk based inspection (*RBI*) and using a specific application procedure with great benefits and superior performance on improving the safety, integrity structural reliability of equipment and industrial installations and reduce costs by eliminating operations diagnostics, control, monitoring and inspections ineffective and increasing the availability of basic technical equipment.

Keywords: risk of failure, matrix of risk, structural integrity.

INTRODUCTION

Although still exists in current practice at many national organizations in the process industries, tend planning and use of preventive control activities, monitoring, inspection and maintenance oriented state-based and prescriptive rules and experience, however, the necessity of and application of existing and conceptual procedures mederne risk based maintenance and reliability centered (*RBM / RC*M) has become almost imminent.

But, actually, the concept of risk based maintenance (*RBM*) is the subject of the present paper has in view the use of a specific application procedures and assessment of the reliability and structural integrity of the equipment and industrial installations and the risks that manifested (RIMAP). This type of maintenance is all the preventive maintenance works based on a large volume of monitoring, knowledge development parameters of major equipment, knowledge and performance characteristics of equipment components, replacement costs of equipment knowledge itself and its components, knowledge and associated costs. This type of maintenance assumes a database on:

- performance of equipments and installations;
- the evolution of in operation parameters;
- monitoring and diagnostic equipments;
- record the events every basic equipments;
- interruptions cost in supply of utilities.

Choosing this type of risk based maintenance and reliability centered feature is dependent on the facilities and equipments that are new, refurbished, ongoing refurbishment or have a normal life to the limit allowed by manufacturers or regulations.

METHODOLOGY OF APPLICATION PROCEDURE

Applying the concept of risk based maintenance and reliability centered (*RBM / RCM*) in the of equipments and installations for industrial processes requests that all related work specific activities (inspection / control, maintenance, repairs, etc.) to be executed by experienced personnel at all levels. Usually, it is recommended that

organizational management to consider the composition of complex multidisciplinary teams with expertise in inspection, maintenance, manufacturing materials research, engineering fracture mechanics (mechanisms of damage / degradation, safety and structural integrity), operation and processing equipment and facilities, reliability and risk assessment. In Figure 1, is the procedure for application of risk-based maintenance, which consists of five basic technical steps (RIMAP):

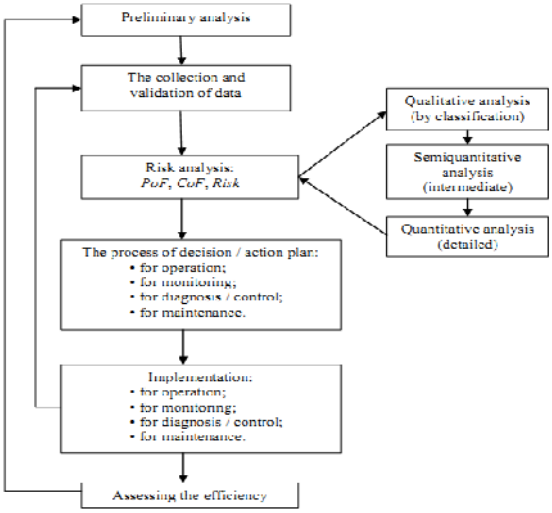

Figure 1. Representation procedure for applying the risk based maintenance (RIMAP)

Additionally the 5 basic technical stages presented above, add a further a technical-organizational stage, defined by evaluating the efficiency. One of the six specific stages procedure for applying the risk based maintenance, with the largest dimension, is the risk analysis on multiple levels.

Preliminary Analysis

This first stage characterized technologically the location (equipments, facilities, components, etc.), establishes objectives and system boundaries considered for analysis (components, main degradation mechanisms, possible failure scenarios and time selected for risk analysis).

For example, in the case of a pipeline technology system within the installations and equipments for industrial processes, defining the limits for technical system analysis shall consider the following:

- components in the system: main pipelines for technological steam (material / steel 12H1MF; pressure P = 150 bar; the working temperature t = 550 ºC; the number of operating hours 141.000; the number of start-stop 142; dimensions of pipe Ø 325 x 38; without incident in operation);
- the main deterioration mechanisms, primarily for simple mechanical stress of creep and mixed, to fatigue-creep;
- the main failure scenario possible: breaking of creep; the secondary, cracking creep-fatigue at due to vibration;

- time selected for risk analysis is for technically system considered 100.000 operating hours, established by the designer (7,5 years) and 200.000 operating hours, the target-objective established for the analysis (15 years).

The acceptability criteria are established by the holder of activity through the regulatory requirements (ISCIR, TRD etc).

The Collection and Validation of Data

The target - purpose of this stage is represented by collection and organization of all relevant data and the information necessary for the analysis. For example, in the case of industrial technological pipeline system analyzed, shall collect the following types of data:
- geometrical characteristics (inside diameter, projection thickness of the wall etc);
- operating parameters (temperature and pressure of design);
- the characteristics of material (average of breaking strength of creep, fatigue strength at a given temperature);
- operating time, in hours;
- parameters monitored (temperature and pressure);
- results of previous test (nil ductility temperature, *NDT*; transition temperature at break with generalized plastic deformation, *FTP*), including the records previous inspection;
- preliminary data calculating (for example, the code ASME, the code TRD/EN 14952).

Risk Analysis

The purpose of a risk analysis is to reduce workloads for the objects with low risk and enhance efforts for the ones at high risk.

The result of this stage is the establishment of a category for the probability of failure (*PoF*) and of another categories for the consequence failure (*CoF*), the corresponding of each component equipment examined. Based on the *PoF* and *CoF*, results of the risk assessment could represented graphically in separate matrix for each type of risk (of technical risks, of OHS, of environmental, of economic and financial etc). Since the creep and fatigue are the principal mechanisms of degradation specific installations and equipment of process industries, determining failure probability (*PoF*), in case of the example considered, is based on exhaustion of creep and on exhaustion of fatigue.

In the present paper is approached first level for qualitative risk analysis (by classification) that uses available data of design of the component under analysis technique, and as additional data, actual number of hours of operation. Through use German technical rules *TRD* (EN 14952), is calculated the working voltage and exhaustion factors (exhaustion of creep, e_z; exhaustion of fatigue, e_w). Definition of classes corresponding for failure probability (*PoF*) and for failure consequence (*CoF*) is utilized for realization of failure scenarios with the help diagram type "bow-tie", is presented in Figure 2, through an example of application for industrial technological piping system analysis. Thus, for the definition of class *PoF* following are used notation: *PoF Ez* – failure probability based on exhaustion of creep; *PoF Ew* - failure probability based on exhaustion of fatigue; *PoF E* - failure probability combined for *PoF Ez* and *PoF Ew*.

Similary, it is proceeding to defining classes *CoF*, using the following effects and economic consequences of failure: the additional cost of replacement; the typical cost for repair; production loss due to failure; the total cost of replacement; combined costs of repair / loss of production; costs due to additional degradation of the equipment; costs due to replacement / degradation combined; value of replacement; costs breaking of through global degradation.

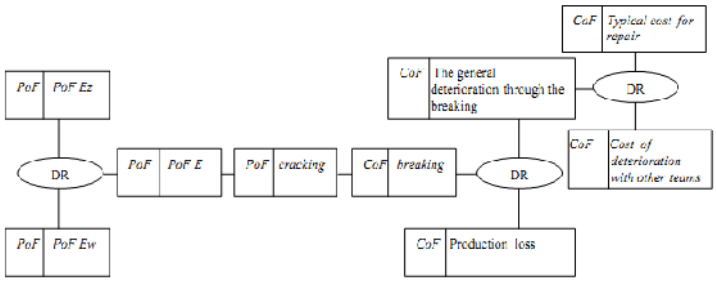

Figure 2. Diagram type "bow-tie" for the technical analysis system

Definition stage aforementioned of the classes is succeeded by the calculation of values their, respectively for *PoF* and *CoF*. In the qualitative analysis of the classification stage 5 classes defined themselves for *PoF* (class 1 - practically impossible, class 2 - highly improbable, class 3 - improbable, class 4 - somewhat probable, class 5 – very probable) and 5 classes for *CoF* (class A - Repair / loss of time, class B - repair or replace the pipe with financial consequences, class C - the breaking and the financial and environmental consequences, class D - breaking and shutdown instalation/ financial and environmental consequences / loss of reputation, class E - additional to the previous class - human casualties / deaths, injuries).

The consequence of failure (*CoF*) was evaluated by examination / diagnostics combined with existing information from service and maintenance history (Heerings *et al.*, 2003; Brear *et al.*, 1994). Having regard *POF* and *COF* values and using the scenario previously defined through the diagram ,,bow-tie" corresponding to each compenent, it is proceeding to determining of risk through it is proceeding to determining of risk through risk matrix corresponding to this first level of qualitative analysis, by using the corresponding classes for these values. In the case of example considered, the risk matrix is presented in Figure 3.

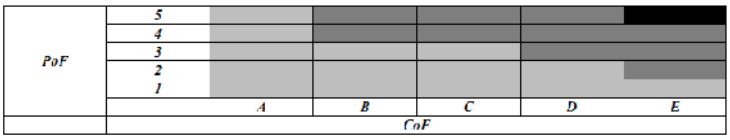

Figure 3. Risk matrix for the qualitative analysis

Since calculated duration for 100.000 operating hours was overcome to this level of risk analysis, is considered that there a probability of the maximum failure and a consequence of the maximum failure, also, probability of failure *PoF* is in class 5, also consequences of failure *CoF* is situated in the class E.

EVALUATION STRUCTURAL INTEGRITY FOR THE TECHNICAL SYSTEM ANALYZED

The evaluation of the deterioration (degradation, destruction) the of the pipe material analyzed in the present paper, in the case mixed request creep-fatigue, in the absence of national regulations is done according code ASME, Case N - 47- 29, Annex T (1990). Thus, according (ASME, 1990; Gusenkov, 1983; Mahutov *et al.*, 1987; Jinescu, 2011), cumulation of deterioration at the mixed request creep-fatigue must satisfy the relation:

$$\sum\left(\frac{n}{N_d}\right)_j + \sum\left(\frac{\Delta t}{T_d}\right)_k \le D \quad (1)$$

in which, $D = 1$ is total deterioration creep-fatigue; $(n)_j$ - number of repetitions applied to the type of cycle „j"; $(N_d)_j$ – admissible number of cycles at projection for type of cycle „j", determined from the curve of fatigue (thermal) corresponding to the maximum temperature of the cycle; q – the number of time intervals necessary unique for length of service at the request of creep; $(T_d)_k$ – duration of admissible time determined by extrapolating the curve for technical resistance of duration of creep; should be used maximum strength in pipe by the factor K', according Table T 1411-1 (ASME, 1990). For the deformation ε_t = 0,2% from the curve of fatigue (Jovanovic *et al.*, 2003), resulting value of $(N_d)_j = N_{fmedp}=50\%$ = 37520 cycles.

Considering safety coefficient for the extent of deformation n_e = 2 (Heerings *et al.*, 2003; Brear *et al.*, 1994) and safety coefficient for the number of cycles n_N = 10 [7] for $\Delta\varepsilon_a$ = 0,2/2 = 0,1%, resulting durability $N_{fmedp}=50\%$ = 26000 cycles. Applying safety coefficient n_N = 10, resulting admissible number of cycles $(N_d)_j$ = 26000 cycles. By doing the report between the number of cycles starting-stopping expected (142), resulting the quota of deterioration of fatigue (thermal):

$$\sum\left(\frac{n}{N_d}\right)_j = 0{,}00546. \quad (2)$$

The values for technical resistance of duration determined by extrapolating through method Larson-Miller for 20.000 operating hours, according Table 1, are represented in Figure 4, below:

Table 1. The values for technical resistance of duration determined by extrapolating

Method	$R_{r\,med.}$			$R_{r\,min}$		
	10.000	20.000	30.000	10.000	20.000	30.000
Larson-Miller	99,00	93,9	91,1	79,2	75,12	72,88
Scherby-Dorn	97,3	91,7	88,7	77,84	73,36	70,96

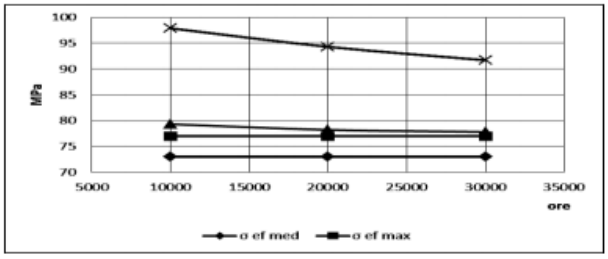

Figure 4. Variation technical resistance time through *Larson-Miller* extrapolation method

The quota of deterioration of creep is determined with the relation:

$$\sum\left(\frac{\Delta t}{T_d}\right)_k = \frac{20.000}{50.000} = 0{,}4. \quad (3)$$

Taking account of the common action creep-fatigue, the sum for quota of deterioration calculated with the relation (1) is 0,40546, inferior the value of 1 (the corresponding for linear summation of deterioration).

In the end, it has been found that besides consumed lifetime for 141.000 operating hours, pipe - thoroughfare can also operate the safe approximately 20.000 hours, according Figure 5.

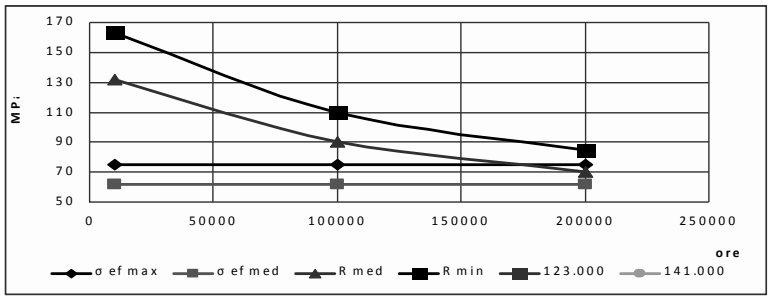

Figure 5. Diagram the life-time

Thus, the probability of failure *PoF* can be framed in class 2 and the consequence of failure *CoF* in class E. That is why, it is recommended non-destructive examination after about 10.000 hours after the consummation 141.000 operating hours, for the detection faults possibly of type fissure, which are very dangerous for such application. Therefore, risk matrix is almost identical to with that of Fig. 3, above.

RESULTS AND CONCLUSIONS

Given that present paper represents a procedural approach for applying the concept of risk based maintenance within the equipment and installations in process industries, is highlighted practical character of it for risk assessment and consequences on the safety and integrity of installations, people, the environment and financial costs.

From the analysis of qualitative risk it follows that technical system studied is characterized by an high risk, which places in the critical area of major risk matrix, also assessment of the state of deterioration of the pipe material analyzed confirm of the one part, the possibility operating safe on the its remaining life and of the other part, the need to apply specific procedures of planning the operations / work monitoring, control and maintenance based on risk generated by the probability of failure in the period remaining life.

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ENHANCING THE ECO-INNOVATION CONCEPT IN LEATHER INDUSTRY BY CAPITALIZING THE PROCESS MODELING THINKING

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The increasing peace of globalization and the high growth of consumerism have created a significant pressure on leather tanning sectors to reduce the environmental burdens envisage by conversing the raw materials into leather. The modernization of the leather producers counted for the concerns of environmental protection, waste reduction, recycling and recuperation of secondary raw materials. The paper takes an interdisciplinary view on how the business engineering thinking leverages the enhancement of the sustainable development and the growing recognition the eco-industry and the green growth. Consequently, it has applied the process modeling thinking on the air pollution measurement process with the aid of the business process modeling notation – BPMN, since this process represents a key concern for most of the leather and footwear manufacturers. The results capture the workflow of the air pollution measurement process, and also data about the steps in the process which can be used for process improvements based on what-if analysis. Using the BPMN diagraming technique to map out flows and process relationships is facilitated the process understanding that help document and communicate to all parties involved how the process should be performed. Finally, the findings illustrate the benefits of the process modeling thinking in modernization the activities related to environmental protection goals from the leather sector. By designing, adopting, and leveraging process models for environmental protection, the manufacturers of leather and leather products are stimulating the sustainable development, and also the concept of eco-efficiency and eco-industry.

Keywords: business engineering, process modeling, eco-industry.

INTRODUCTION

The manufacture of leather and leather products is a global industry that is exposed to increasing competition from a large number of low-labor costs countries. To remain competitive in this global marketplace, the leader producers are expected to exploit more efficiently the raw materials, adjusting their production operations towards higher quality outputs and high fashion content leather products.

During the last decades, the major concern on creating a sustainable future by reducing the problems arising from human impact on the environment has determined the key European actors to develop a significant and diverse range of environmental measures.

Although there are no specific environmental EU Directives for the leather tanning sector, two main directives affect the leather tanning industry, directly: the Council Directive 96/61/EC (1996) concerning Integrated Pollution Prevention and Control (IPPC) aiming at reducing emissions of the air, water and land, and the new Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation that ensures a high level of protection of human health and the environment from the risks posed by chemicals. According the official information of European Commission, the impact of environmental regulation on the leather industry is considerable, being estimate that environmental protection costs amount to 5% of all operational costs.

As environmental legislation is a necessity and the enforcement of the legislation is considered a very important aspect so that all enterprises are effectively required to

comply with it, Danish Technological Institute has studied the key issues encountered by the SMSs in dealing with the environmental challenges. According to the Report on SMEs and the Environmental in the European Union (2010), the average environmental impact from SMEs in the EU27 is 82% from DC sector (manufacture of leather and leather products) at NACE classification subdivision level. This reality suggests a high environmental impact of the leather industry, since it followed the NACE - HA sector (transportation and storage) with an average of 86% impact and NACE - FA sector (construction) with 85% impact.

The high impact sectors are those with a high environmental impact on at least one of the indicators: energy use, greenhouse gases, air emission, waste or hazardous waste. Moreover, the analysis of total sector impact per country shows a usually high environmental impact of the enterprises from the NACE – DC 19 sector comprising tanning and dressing of leather, manufacture of luggage, handbags, saddlery, harness and footwear with an 11% impact, the percentages being calculated for CA10-KA74 sectors at NACE subdivision level.

It is worth to notice that the Report on SMEs and the Environmental in the European Union (2010) estimates that a 60% - 70% of the environmental impact originates from SMEs and it would be too complex to determine the detailed contribution made by the SMEs to air pollution in terms of different types of pollutants. However, the core idea of the impact analysis emphasizes the huge business potential for enterprises acting in DC sector in reducing environmental damage through innovative cost-effective solutions.

Under these situations, the pressure to remain competitive requires enterprises from leather sector to invest in environmental protection, to modernize the working context for greater resource productivity and better logistics. These require new approaches were engineering must function together with environmental focus in an integrated manner.

According to OECD (2009), the eco-innovation concept is seen as the developments or the implementation of a new or significantly improved products, processes, marketing methods, or organizational structures which lead to environmental improvements compared to relevant alternatives. Therefore, the key enabler for gaining competitive advantage from eco-sustainability is to innovate the workings through the process thinking that ensures the coherent alignment of business goals to business processes.

From these views, the research issue was related to the increasing need of leather producers to invest in their operating activities in order to minimize or correct the problems related to environmental damage to water, air, soil, and eco-systems. Thus, the scope of the research was referring to the cross-disciplinary business challenges of leather producers arisen from the necessity to permanently innovate their internal processes in a way that allow the achievement of environmental protection objectives. Consequently, the research objective was limited to the planning process with focus on the process of air pollution measurement, as a means of accomplishing a high level protection of the environment.

According to Wil and Kees (2002), the advent of information technologies has moved the focus on the organizational process modeling based on process workflows, an important modeling concept for business engineering that aims to ensure the optimal convergence between the enterprise′s resources and the strategic direction required to create added value to the customer.

As result, the actual interest of enterprises in adopting and using business process modeling methods and techniques is essential for a properly understanding of internal

processes and for a more readily communication of steps and decision points to all stakeholders envisage.

METHODS

In order to fulfill the research objective, the authors have applied the process modelling concept on the air pollution measurement process, based on the Event-driven Process Chain (EPC) technique. The EPC diagram uses graphical symbols to show the control flow structure of a business process as a chain of event and functions. The model resulted captures not only the visual picture of the process but also data about the steps in the process which can be used for process analysis based on what-if technique. This technique allows business analyst to evaluate various aspect of process, to uncover inefficiencies or other issues and to determine way to improve it (Sharp and McDermott, 2009).

According to the Business Process Modeling Notation v.2 (2010), the standard notation for describing a business process, the process flowchart or workflow diagram visually depicts the flow of work and information within the process. The BPMN diagraming technique allows modeling the workflow using activities, events, and logical connectors as syntax elements which determine the conceptual mainstream of the information system design (Mendling, 2008).

The core function of Event-driven Process Chain (EPC) technique is to answer to the question ″what should be done″, facilitating the design of the necessary activities, the corresponding events, and also the possibilities to create a modular framework based on the process interface (Pascadi and Tutunaru, 2011).

In this regard, the key syntax and related semantic aspects needed for mapping out flows and process relationships are as follows: activity (A) - defines the action to be perform, clearly and concisely; event (E) - defines pre-conditions and post-conditions of functioning; process interface (PI) - points the necessity of executing the process between the flowchart′s predecessor and the flowchart′s successor.

Worthy to be mentioned, the EPC diagram connectors are as follows: SPLIT connectors with one incoming and multiple outgoing arcs and JOINT (J) connectors with multiple incoming arcs and one outgoing arc. The SPLIT connectors comprise the following types: AND – split (A) triggers the execution of all subsequent branches in concurrency, OR – split (O) triggers the execution of any combination of the multiple subsequent branches, based on the condition of at least one branch execution, and XOR – split (X) represents a choice between one of several alternative branches and requires the execution of selected branch.

Modelling the air pollution measurement process has drowned valuable knowledge from environmental management science as referred in Wright (2008). Likewise, the flow of work for air pollution measurement is in accordance with the approach to developing an environmental system, and also with the engineering aspects of ISO 14001 requirements from environmental management standard (Morris, 2004). The clauses in ISO 14001 standard are defined in a general way so that the enterprises from a wide range of manufacturing sectors with high environmental impact such as the NACE HA, FA, and DC 19 classification subdivision level can adopt and apply the recommendations, to successfully minimize the environmental damage caused by their operations and activities.

RESULTS AND FINDINGS

The EPC diagram for air pollution measurement process illustrated in figure 1 depicts the flow of work and information required for measuring the emission level so that the variables of manufacturing leather process are maintained within the acceptable limits.

The essential activities in capturing the flow of the process start with establishing the points of emission measurement based on the technological flowchart related to the core operating activities of the enterprise such as tanning and dressing of leather, manufacturing of luggage and handbags, or manufacturing of footwear.

Secondly, it is required to define the air quality measurement parameters in terms of particulate matter content or concentration of polluting gaseous products, because the measurement technique depends on whether the particles are lighter than or heavier than air. The ISO 14001 standard highlights that there are several necessary conditions in achieving a high-quality measurements such as setting up the level of measurements: accuracy and frequency. In doing this, it is required to consider the range of expected reading values and the measurement frequency.

To achieve the specified level of measurement accuracy, the air pollution measurement technique used must be carefully analyzed and chosen. In this regard, the technical specialist is expected to consider for analysis the wet chemistry laboratory technique or online sensors technique (Morris, 2004).

The air pollution measurement flow goes on to designing and/or choosing the suitable measuring instruments with respect to the static and dynamic characteristics that are appropriate to the needs of the measurement situation. The static characteristics comprise accuracy, sensitivity, linearity and the reaction to ambient temperature changes, and dynamic characteristics describe the behavior between the time that a measured quantity changes value and the time when the instrument output attains a steady value in response. However, all relevant static and dynamic characteristics are expected to be recorded in the data sheet format for any particular measuring instruments.

Furthermore, in assessing the relative suitability of different instruments with the measurement situation it is expected to consider the tradeoffs between the cost, durability, and maintenance of the measuring instrument.

The next step consists of ensuring the correctly calibration of the measuring instrument chosen in which the instrument is tested over its whole range by repeating the comparison procedure for a range of inputs. This activity requires the national reference standard for calibration chain and may trigger the calibration services subcontractor accredited by National Testing Laboratory.

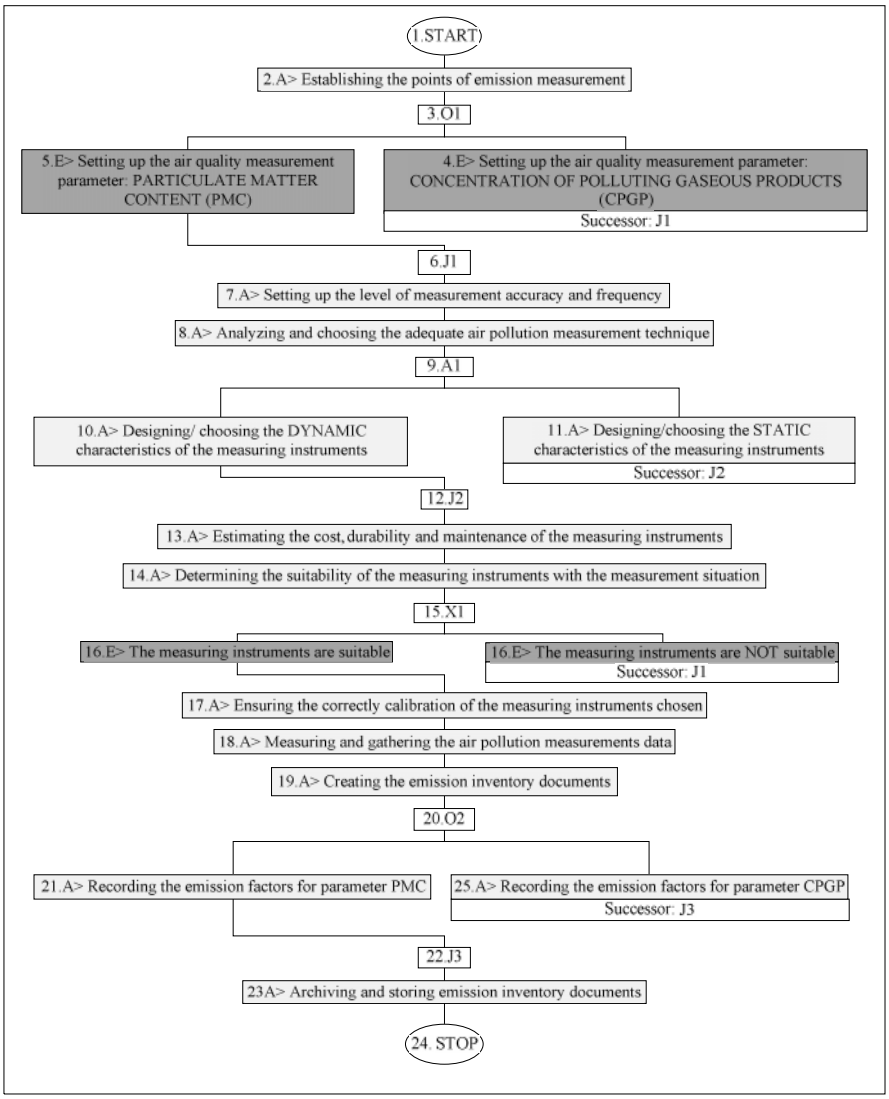

Figure 1. The air pollution measurement EPC diagram

The flow goes on to measuring and gathering the air pollution measurement data documented on the measurement record sheet for parameter: particulate matter content or concentration of polluting gaseous products, followed by the activity of creating the emission inventory documents.

Finally, a thoroughly designed air pollution measurement process requires recording the emission factors for parameter: particulate matter content or concentration of polluting gaseous products, and archiving and storing emission measurements inventory documents.

As alluded to earlier, mapping out flows and process relationships is facilitating the process understanding and help document and communicate to all parties involved how the process should be performed. Hence, the air pollution measurement EPC diagram accounts for a bridge document between business/technical user that can understand the basic elements and IT/business analyst that fill in the details so that the process should be automated, allowing users to collaborate on and work together on models in real time.

Executing the process is generating the acquisition of useful air pollution measurement data which allow decisional factors to analyze opportune, accurate and structured information in order to make managerial decisions, in a flexible and cost-effective manner.

CONCLUSIONS

Using innovative management methods and techniques for designing and implementing the necessary processes correlated with the areas of environmental damage is a form of innovation resulting in demonstrable progress towards the goal of sustainable developments through reducing environmental impacts, enhancing resilience to environmental pressures or achieving a more efficient and responsible use of natural resources.

The findings emphasize that the interest on innovating the workings based on designing, adopting, and leveraging process models for environmental protection helps enterprises from leather industry to profitably improve their internal culture, working processes, materials and related supply-side issues, stimulating the sustainable development, and also the concept of eco-efficiency and eco-industry.

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IMPLEMENTATION OF INNOVATION POLICIES THROUGH RESEARCH AND DEVELOPMENT PROJECTS

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This paper analyses types of policy instruments responsible for the success of policy implementation through projects. Based on comparative case studies, the paper provides an analytical perspective from real practice about how and why various types of instruments lead to either successful or unsuccessful projects. Particularly, the key finding is that, in order for projects to implement successful policies, policy instruments must be designed based on composite thought systems related to flexibility. Results provide the key direction, but without the holistic conceptual component of developing the implementation theory, which needs to go beyond conceptual fragmentation and polarization.

Keywords: innovation policy, implementation instruments, thought systems, innovation projects, flexibility

INTRODUCTION

Facing increasing demand and capacity-related constraints in national research systems, the EU has set policies regarding innovation, which, due to the size and complexity of research systems, are best implemented by means of multiple projects and multi-national programs (Jamieson and Morris, 2007). The most important limitations within the existing empirical research are associated with the endogenesis of the public support for research and development and biased selections, which is apparently involved in program implementation (Lee, 2011).

In addition, lacking generalization, implementation theory also suffers from polarization, because research studies examine implementation in both top to bottom and bottom to top approaches. Top to bottom approaches ignore the local agency and focus on control of actors, by coercive and normative mechanisms, while bottom to top models are based on the fact that implementation agencies will fit, due to the existence of remunerated and normative mechanisms (Sabatier, 2007). Most studies generally prefer a top to bottom approach, which provides legitimacy for policy elaboration, is easier to use when research is undertaken and simplifies the tool design process (O'Toole and Meier, 2004).

The aim of this paper is to approach this decisive conceptual gap in the policy implementation theory, explaining the cause-effect relationship between policy instruments and project performance. The argument in this paper is that this causality can be investigated by analyzing real examples of the way in which different types of instruments influence the management of similar innovation projects. In this regard, evidence has been gathered to answer this question: what types of policy implementation instruments contribute to successful projects (why and how)?

CONCEPTUAL FRAMEWORK AND RESEARCH HYPOTHESES

Most implementation studies analyze a public research and development program specific to an industry or a country and those dealing with data on multi-project, multi-

country are almost non-existing (Lee, 2011). This study approached this vacuum by selecting two integrated comparative cases that incorporate several case studies (Yin, 2003). The two policies in the SSH/FP7 program (innovation, competitiveness and labour market policies / economic structures and productivity) were chosen to be incorporated in two projects from two different coordinating countries. The program was chosen because its main goal was to implement innovation in the socio-economic area, in similar periods and political contexts, but the main difference was that they used different implementation instruments. The reason behind this choice was that the two cases incorporated had many things in common to facilitate minimization of variations, but their significant difference allows comparison of effects that various instruments have in the implementation process.

METHODOLOGY

The Research Instrument

The undertaken process involves first of all, the examination of policy and project documentation, such as official publications (legal documents, etc.), prior and subsequent assessment reports, official websites which have been used to build the background for each of the two studies. Secondly, a total of 31 semi-structured interviews, of which 13 were conducted with managers of project 1, 18 interviews with managers of project 2, and with project participants.

Sample and Method Description

The analysis was performed according to techniques suggested by Miles and Huberman (2002) regarding a first process within the case and a second one comparing the cases. Data regarding project management tasks were classified as planning, communication and control/coordination task (Tables 1 and 2); implementation instruments were grouped into two categories (conventional and systemic); performance was classified into results and output (Table 3) and project success was classified into the actual implementation of technology and management of unforeseen situations (Table 4). Subsequently, grouped data were transferred to comparative matrices and by means of data reduction techniques, the common categories were identified in the incorporated cases. The final set of models was transferred in causality chains to discover the cause-effect factors between project instruments and results.

A summary of resulting causal factors is presented in Tables 1-4.

Table 1. Corroboration of models for tasks of project manager 1 (based on empirical data)

Project management tasks	Method	Explanations
Planning	Planning as it is	Project manager provides output reports with aggregated data regarding project results, not output processes. Plans are flexible with certain focused goals and then there is the flexibility to decide upon the course of action.
Communication	Frontier management	The main tasks of the project manager throughout the project. Formal and informal. The project

Project management tasks	Method	Explanations
		manager communicates through frontiers with the government (national and EU), the network, peers and promotes the laboratory network (3 interfaces).
Control and coordination tasks	Loses output-oriented control task	Low at operational level, because project managers did not have the lever effect to impose data management standardization regarding laboratories or control over resources.

Table 2. Corroboration of models for tasks of project manager 2 (based on empirical data)

Project management tasks	Method	Explanations
Planning	Top to bottom prescriptive plans	The project manager meets the requirements of the contract/compromised tasks to match WBS plans, project managers have limited negotiation power with both EU and Consortium partners.
Communication	Info-cracy (standardized communication procedures)	Focus on external communication with customers - limited communication with other projects – various partner objectives – distance between participants/dictated by contract and participation rules. Other project interfaces (for instance, users) are marginalized.
Control and coordination tasks	Loses output-oriented control task	Low at operational level, because project managers did not have the lever effect to control project teams – fulfilling the contract – various partner objectives inhibit task control

Table 3.Comparison of results from both incorporated cases (based on empirical data)

Policy	Strategic objectives	Implementation instruments	Project management	Output	Results
Project 1	Stimulates implementation of innovation through collaborative projects	Systemic-providing resources, organizing collaboration through interaction and monitoring of results, flexibility to manage, learn, communicate, experiment and network	Systemic-focus on mediation between various limits by interested parties to meet project objectives	Compromise and efficiency objectives	Medium development (differs depending on the project)
Project 2		Traditional-financial and management instruments – monitoring the management	Normative-focus on managing borders for a positive assessment	Compromise and efficiency objectives	Ceremonial development*

Policy	Strategic objectives	Implementation instruments	Project management	Output	Results
		process by participation rules and operational criteria	from the sponsor		

* Ceremonial development refers to the ritualic-symbolic trend where project results (as in meeting project performance criteria) have been presented in evaluations, but these results do not really represent real large-scale market implementation results.

Table 4. Comparison of successful completion of projects in the two programs, defining success as technology implementation on the market and the nature and management of change (based on empirical data)

	Result is implemented	Nature of change in plans and activities	Facing change
Case studies Project 1			
1	Yes, frequently	Plans are increasing, specific for this situation	Adaptation-asks for assistance from other professionals
2	Yes, little	Plans are increasing, specific for this situation	Cost and diversity of professional practice
3	No-very little	Plans are increasing – rather formally – but not frequently, borders of management and laboratories	Cost, diversity of professional practice and various strategic plans
Case studies Project 2			
1	No	Incremental development – no major deficiencies	Does not use change
2	No	Technical-trivial	"Ineffective" management (which has led to problems with technical completion) as well as lack of emergency planning or efficient management of change
3	No	3-month extension for results	
4	No	6-month delay, had to change project manager in the first nine months	
5	No	Bad WP planning and loss of technical objectives	
6	No	6-month extension, the technical component did not work	Communication procedures and sponsor procedures have not been flexible enough
7	No	Bad planning – unrealistic expectations	
8	No	Many delays – problems with hospital administration	
9	Yes, partially	Generally successful plan – minor changes	Market changes have not allowed full development, but the product is launched on the market

Validity of Content

The role of project managers in all cases has proven to be that of mediator who negotiated between the policy and limits of the laboratory and made adjustments to implementation activities making compromises between the practical needs of network stakeholders and program objectives. In fact, project managers have worked systemically, managing and adjusting both activities and relationships (Table 1).

Project managers had to prioritize between network participation level, quality of reported data, reliability of reports and software protocol development.

Managers of project 2 have frequently focused on the idea of filling a position and anything that deviated from the plan was avoided or ignored. In other words, managers have focused on showing that the project was developed efficiently and that evaluation objectives were met (Table 2). In some cases, it was reported that managers were marginalized by their own team and in two projects they were selected particularly to deal with administrative tasks and not to "intervene" in work packages.

Essentially, instruments of implementing the socio-economic program did not allow project managers to manage: this lack of flexibility in management activities has led to weak leadership, group coordination problems and loss of focus.

CONCLUSIONS

This study aims to identify effects of policy implementation instruments on project performance. The research question was what types of policy implementation instruments contribute to successful projects (why and how)? To answer this question, two multi-project multi-country EU programs were chosen as incorporated cases, each corresponding to a FP7 policy of implementing innovation in the socio-economic area. The main objective of these programs was to implement innovation, both having similar periods and political contexts, with the main difference that they used different implementation instruments. The objectives and structures of each policy had common goals: to stimulate change and develop innovation in the socio-economic area by the users. Moreover, both policies have had similar structural problems: they were related to subsidiary and targeted the fragmented, diverse or underdeveloped national infrastructure, with extended capacity. It was found that both policies had objectives that were either optionally or partially in conflict. On the one hand, managers of project 2 were given a choice between validation and development, the former objective being by far the easiest choice, while, on the other hand, managers of project 1 had to implement three objectives: network expansion, data collection and development of a new network.

Regarding policy instrument debate, project 1 used systemic instruments, including both performance control (minimum critical specifications) and relational instruments. As a result, collaboration opportunities existed, plus a sufficient margin to manage through project limits with users and within the project team, as well as having the flexibility of dealing with change in plans. By contrast, project 2 used conventional instruments incorporated in standardized evaluation procedures and, as a result, the system was rigid, with little or no evidence of frontier management and efficient provisions for management of change.

In conclusion, project flexibility appears as an essential factor to successfully implement policies. Evidence from the case studies shows that flexibility must be

integrated in designing policies as systemic instruments aiming at reaching policy objectives through successful projects.

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DESIGN AND STYLING – CONTEMPORARY INTERESTS IN HAUTE-COUTURE ACCESSORY FASHION

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Fashion is a symbol. Clothes and accessories are a clue for the social, professional or cultural group an individual is part of. This separation is visible especially among youngsters, who dress according to their favourite music, choosing idols from the popular stars. Starting from the connotation of the graphic symbol, haute-couture product design designates a mental plan, a project, an outline or a sketch, a basic drawing or a model for a work transposed into various materials and aesthetic dimensions, an implication and motivation in individual styling. The relationship between design and styling is really a response of the customised product, meeting an emotional need, leading to stylistic harmonisation and a conception governed by the logic of things. Each individual has a personal style through his aesthetic presence. This style must be harmonised with the design of the products he wears. The style that is an expression of useful objects emerges from their aesthetic shapes, characteristic to a particular period.

Keywords: fashion, symbol, design, styling, aesthetics, customised product.

INTRODUCTION

Accessory design, as the other fields of fashion and art in general, is a means of expressing an artist's vision, a manifestation of beauty through a creative endeavour. Unlike stylism, footwear design does not only deal with the image of the product and image elements of the elaborated stylistic concept, but also with transposing an idea into components and patterns. In design, the ideatic logic is organized based on the principles of classic logic, and in terms of economics, the product concept is organized based on principles of efficiency.

To harmoniously bring together product design and style, the designer proposes to identify, by means of idea sketches, the relationship between object, in our case haute couture footwear and leather goods, and the aesthetics of the perceiver, namely the person wearing it. The themes proposed may become a certainty in fashion design of 2014-2015 and are aimed at the relationship between form (design) and style.

BALLAD'S NOSTALGIA – NON-CONFORMIST STYLE - PRINT DESIGN – GRAPHIC STYLE, MESSSAGE

The non-conformist style is marked by the return to the cusp between the 60s and 70s, The nostalgia of a dreamed but never fulfilled freedom has led to simple aesthetic-artistic manifestations, but which carry an important message (political and social) to the century we are now living in.

Intermingling of colours, the state of dream, of "intentional" oblivion, massive accessories, cufflinks, snaps seem negligent and make up a theme of unpredictability and feeling.

A concept which many fashion critics call "espionage". Whether it conveys what is felt or what is not felt, it is imperative. Whether the graphic message is decrypted or not, the game becomes interesting. A communicative style (apparently) with multiple interpretations.

Accessory design is a lot more masked than that of other components, to induce a state of confusion. Each encounter carries a different MESSAGE.

Figure 1. Ballad's nostalgia, non-conformist style print design

Figure 2. Graphic style, message

OP-ART – SUBLIMINAL STYLE – IMPRESSION OF MOTION - BIKER ON ROAD - SPEED, ADRENALINE, RISK

A style of the 70s that "operates" again on the catwalk. The informed know that subliminal language, apparent movement and dioptric visuals induce a feeling of insecurity to the onlooker.

The contrast between black and white distributed on uneven surfaces is the essence of the motion effect. Materials may be printed or obtained by braiding.

Style inspired from speed sports, motorcycling and extreme sports. It is a re-interpretation of the 60s-70s rock style.

Materials used are vegetable leather doubled by a metallic structure with polyurethane finish. Basic colours are black, red, silver and gold.

Accessories are fitted with safety clasps.

Figure 3. Op-art – subliminal style – impression of motion

Figure 4. Biker on road - speed, adrenaline, risk

POWER OF COLORS – OPTIMISTIC STYLE PLASTIC 60s – TECHNOLOGIC STYLE, SYNTHETIC ART

Strong colours, either spectral or pigmented exert on the individual a significant psychological role, causing impressions, feelings or states full of optimism and confidence.

Primary colours, red, yellow, blue, associated with secondary ones, orange, green, violet, distributed on balanced surfaces give the wearer an optimistic air.

Modern technology contributes to producing trendy materials, substitutes for natural products, which, through colour as well as comfort and hygiene, bring us back to the style created by COUREJE in the era of Plexiglas.

Accessory design is simple, without additional elements that destroy the shape, rigorous and accompanied by ingenious technical solutions. Plexiglas gives the accessories special light refraction effects.

Figure 5. Power of colors – optimistic style plastic 60s

Figure 6. Technologic style, synthetic art

CAMOUFLAGE – WAITING, SAFARI SEARCH – HUNTER, SANGUINE STYLE

Initially a style of "hiding", of not being seen, it has become a style of waiting, of being looked for, of being seen but never too late. Colours and drawings are taken from the vegetable and animal realm and from the environment.

Accessories are massive, usually from patinated bronze, unsophisticated.

The savannah and African desert provides designers with constant inspiration in approaching a sanguine and precious style.

Feline furs with fascinating patterns, from leopard to tiger, cheetah and antelope are the main components in creations that make up this style.

Figure 7. Camouflage – waiting, safari search

Figure 8. Hunter, sanguine style

MASCULINE – STRONG STYLE, DOUBLE PLAY – BRIGHT STYLE, ATTRACTION

This style appears in a new approach by borrowing elements from the military uniform, giving the wearer an air of distinction and power. The double play is given by the contrast between masculine and feminine.

The tie, jabot, starched collars, cufflinks and accessories made of semi-precious materials confer force to footwear, bags and clothing.

Essentially bright and luminous, this style confers both distinction and attraction. Refined restaurants and clubs, casinos are represented by this style.

Interweavings from metallic wires, polymers, semi-precious stones produce light reflexes with unexpected visual effects. The faceted ornaments also cause surprising gleams.

Figure 9. Masculine – strong style, double play

Figure 10. Bright style, attraction

GOLD – PRECIOUS, IMPRESSIVE STYLE - RED PASSION – SEDUCTIVE, INDESTRUCTIBLE STYLE

The resurgence of gold with white gold and bronze appearance keep up with the precious, but unsophisticated style.

Leather with smooth and refined finish and those with geometric embossing, faceted by the play of golden hues, add to the voluptuousness and preciousness, the shiny accessories of this style.

Red has always been the eternal and fascinating colour of the seductive, alluring style, thrilling and elegant. Non-aggressive and imperial, it will be reinvented in creations of designers and style houses.

The matte and precious appearance of velour leathers without special effects, accompanied by those with golden finishes and short hair furs, but full of tenderness are marked by black embroidery and contrasts.

Figure 11. Gold – precious, impressive style

Figure 12. Red passion – seductive, indestructible style

CONCLUSIONS

The design-styling relationship is actually a coherent and professional response, an understanding of the relationship between object and form.

Body anatomy and state of mind help you understand who, when and where you want to be.

Accessory design may develop styles of nostalgia, attraction, arrogance, grandeur, joy and brightness. The search remains the connection between seduction and reality. Design and styling mean knowledge – education – character.

The imaginative transfer to a state that will become reality in the endeavour of creating an object, irrespective of its destination (fashion design by excellence), becomes an artistic composition using the means provided by modern, classic and artisanal technologies.

Acknowledgements

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DESIGN INNOVATIONS ON FOOTWEAR FOR OVERWEIGHT/OBESE PEOPLE

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The overweight/obese population is aggressively growing worldwide due to laxity on physical fitness, sedentary lifestyle and eating habits. The obese individuals, in general, experience instability during locomotion and they are towards the direction of rapid development of musculoskeletal disorders, pain symptoms on the regions like heel, ankle and plantar surface of the foot. The main objective of Design innovation concept is to evolve varied angles particularly on the heel region of footwear to relieve pain and stress posed by obese people. The comfort solution has been attempted pragmatically through adaptation of innovative research in this paper. In the developmental process, the design modifications externally on the heel regions with the varied angles 20 degree, 30 degree and 40 degree have been designed. The slip resistance tester is employed for the estimation of Coefficient of friction between the sole surface and floor surface. The results revealed that the externally designed shoes represented with improved values of Coefficient of friction while compared with standard shoe. The design innovation concepts have resulted in higher coefficient of friction values on the externally modified footwear and the newly designed footwear is referred as an ideal remedy to acquire therapeutic advantages and benefits for overweight/obese individuals.

Keywords: obesity, friction, design innovation

INTRODUCTION

The foot is a structural marvel consisting of 26 bones laced together with many layers of ligaments, tendons and muscles. The foot is built to absorb shock like a suspension bridge and is subjected to pressure at all time of life of human. An average person strides predominantly 5000 to 10000 steps a day and the impact of each step exerts a force about 50% greater than the body weight. In an average day of walking, a force equal to hundreds of tons is placed on the feet .Due to these factors, the occurrence of foot related problems have become frequent and common phenomenon especially to heavier categories.

The overweight/obese population is aggressively growing worldwide due to laxity on physical fitness, sedentary lifestyle and eating habits. The obese people possess wider foot dimensions and lower foot arch index while comparing with normal foot people. The body weight of obese people itself is a major disadvantageous factor which tends to develop all the foot related consequences as well as ill-healthy foot. Hence, the design and development of footwear necessitates the higher BMI individuals to feel comfort as well as foot health friendly.

Those footwear that are in existence for overweight/obese individuals are predominantly possessed with wider fit and dimensional characters. The Sports footwear is suitably meeting these characteristics especially for overweight/obese individuals. This necessitates the footwear specifically to address the foot related problems and evolve external design modifications and innovations in order to gain therapeutic benefits for overweight/obese individuals.

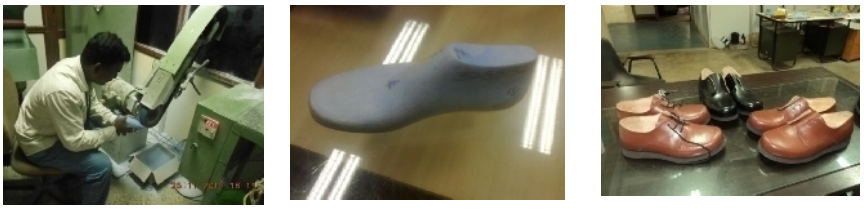
This paper mainly deals on the design concepts of external modifications particularly on the heel regions of footwear thereby rendering comfort for the targeted people. The main objective of this paper is to design varied angles/curvature on the heel

profile of footwear to relieve pain and stress experienced by the overweight/obese individuals. The advantages of externally modified profiles on the heel region of footwear would influence weight diffusion/dispersion between the foot and floor surface. The specific research is aimed at in this paper to render comfort solution pragmatically for the goodwill of the overweight/obese individuals.

MATERIALS AND METHODS

The foot anthropometric parameters of overweight/obese individuals were primarily studied in this paper. There were more than 100 individuals covered under the anthropometric measurement analysis and the varied parameters were collected and presented for modelling suitable shoe lasts exclusively meeting the requirements of overweight/obese individuals.

The Shoe lasts ranging between size 6 and 10 were designed and developed by M/S Shangavi Shoe Accessories P (Ltd) at Chennai, India. The design and development of footwear for overweight/obese individuals were prototyped.



a *b* *c*

Figure 1. Last Making (a and b) and Full shoes (c)

The shoes were constructed over the lasts to meet the targeted beneficiaries. The uppers were designed with Derby model on varied size ranges and subsequently were converted into three dimensional shaped lasted upper in the manufacturing process of footwear. The leather from cowhides was chosen as upper portion as it possesses improved degree of physical strength characteristics and elongation behaviours when subjected to application of force. The bottom material Micro Cellular Rubber (MCR) was chosen and fabricated as a rubber unit sole for bottom application. The shoes developed were provided with wider inside room for the accommodation of broad fit characteristics of foot of overweight/obese individuals. The shoes were reinforced with counter and toe stiffener to maintain its shape and dimension for the users to feel comfort and satisfaction.

In the specialisation of footwear, the external design modifications play a pivotal role in achieving improved comfort features for the beneficiaries. The design modifications externally on the heel regions of footwear with the varied angles 20 degree, 30 degree and 40 degree – figure 2 – were designed and configured on footwear ranging between 7 and 8. The control shoes on these sizes were developed during initial phase of developmental process.

a *b* *c*

Figure 2. External design modifications on shoes for obese

The SATRA slip resistance tester (STM 603) was employed for experimentation of shoes exclusively designed and developed for overweight/obese individuals. This tester is an indicator of conditions encountered during walking when slip is most likely to occur. This device measures the slip resistance between the sole of the shoe and the floor.

Figure 3. Slip resistance tester

The machine – figure 3 – is equipped with a specially-designed control and data acquisition system which provides the coefficient of friction values on each test sample tested. This tester is finally illustrated with a multiple graph showing varied components produced after the test performed. There are four lines displayed on the graph representing vertical load, speed of table movement, horizontal load and coefficient of friction.

RESULTS

The shoes (Size 8) on standard and externally designed categories were subjected for experimentation analysis. These shoes were scientifically assessed for determining Coefficient of Friction (COF) between the bottom surface of footwear and the surface of flooring. The floor Quarry tiles was specified under SATRA TM144 test method and employed for analysis using the shoes. Initially, the standard shoe of size 8 was subjected for evaluation of COF and subsequently, the shoes configured with varied angles such as 20 degree, 30 degree and 40 degree were experimented on two parameters namely forward foot slip and forward heel slip respectively. In the method of evaluation, a vertical load of 500 Newton was subjected for analysis on shoes and each shoe was assessed for minimal five times and the mean value of COF was calculated accordingly.

Table 1. COF of Standard Shoe

Material		Mean ± S.D.
Forward Foot Slip	Left Shoe (COF)	0.552 ± 0.0130
Condition	Right Shoe (COF)	0.514 ± 0.0089
Forward Heel Slip	Left Shoe (COF)	0.532 ± 0.0228
Condition	Right Shoe (COF)	0.526 ± 0.0270

The Table 1 illustrates the mean COF values of left and right shoe of standard design on two parameters namely forward foot slip and forward heel slip condition. The mean values of COF for left and right shoe in respect of forward foot slip are 0.552 and 0.514 and the mean values in respect of forward heel slip condition are 0.532 and 0.526 respectively.

Table 2. COF of 20 Degree heel Modified Shoe

Description		Mean ± S.D.
Forward Foot Slip	Left Shoe (COF)	0.694 ± 0.0195
Condition	Right Shoe (COF)	0.644 ± 0.0152
Forward Heel Slip	Left Shoe (COF)	0.782 ± 0.0444
Condition	Right Shoe (COF)	0.828 ± 0.0630

With regard to Table2, the mean values of COF for left and right shoe of 20 degree modified design in case of forward foot slip condition are 0.694 and 0.644 and the mean COF values in respect of forward heel slip condition are 0.782 and 0.828 respectively.

Table 3. COF of 30 Degree heel Modified Shoe

Description		Mean ± S.D.
Forward Foot Slip	Left Shoe (COF)	0.674 ± 0.0207
Condition	Right Shoe (COF)	0.638 ± 0.0164
Forward Heel Slip	Left Shoe (COF)	0.760 ± 0.0292
Condition	Right Shoe (COF)	0.866 ± 0.0329

The Table 3 refers 30 degree heel modified shoe with their mean values of Coefficient of friction. The values of mean COF for left and right shoe are 0.674 and 0.638 in respect of forward foot slip condition and the values of COF for left and right shoe of forward heel slip condition are 0.760 and 0.866 respectively.

Table 4. COF of 40 Degree heel Modified Shoe

Description		Mean ± S.D.
Forward Foot Slip	Left Shoe (COF)	0.628 ± 0.0228
Condition	Right Shoe (COF)	0.606 ± 0.0195
Forward Heel Slip	Left Shoe (COF)	0.598 ± 0.0363
Condition	Right Shoe (COF)	0.658 ± 0.0383

In respect of Table 4, the mean COF values for left and right shoe of forward foot slip condition are 0.628 and 0.606 and the mean values of COF for left and right shoe for forward heel slip condition are 0.598 and 0.658 respectively.

DISCUSSION

In general, the COF value higher than 0.50 is considered to be good and comfort for the end-users benefits. The coefficient of friction is a prime factor mainly determines the overall stability of footwear over the flooring surfaces during the locomotion phases. The standard shoe represented COF value greater than the prescribed value of 0.50. The externally modified shoes designed with 20 degree, 30 degree and 40 degree represented with higher values of 0.50 for forward foot slip and forward heel slip condition than the standard shoe and it was clearly understood that the externally designed shoes are advantageous on stability and comfort parameter of the targeted beneficiaries due to its highest COF values.

Statistical Analysis test was carried out to ascertain whether there was any significant difference on COF values between Standard shoes and externally designed heel modified shoes of 20 degree, 30 degree and 40 degree. From (ANOVA) Variance test conducted, it was observed that there existed significant differences amongst all categories of shoes as p value is less than 0.05.Also, it was revealed that the left and right shoe of forward foot slip and forward heel slip condition existed with similar pattern of significance. The Bonferroni multiple comparison test was carried out to investigate what the significant differing groups are? It was clearly understood that the COF for standard shoe was significantly different from the externally modified shoes of 20 degree, 30 degree and 40 degree irrespective of left or right shoe of either forward foot slip or forward heel slip condition. It was critically observed that the COF values of modified 20 degree and 30 degree resulted with no statistical significance and difference as the p value is greater than 0.05 and in all other cases (forward foot slip for left and right shoe, forward heel slip for left and right shoe) resulted with significant differences.

The medical professionals recommend externally modified Shoes for overweight and obese individuals so that their expenditure of oxygen would be higher than the general shoes. The shoes designed and presented in this paper are unstable in nature and aid gear-up the pace of walking and subject to expend additional energy thereby achieving waist and weight loss gradually. The bevelled angles developed in shoes render added advantage of stability for the beneficiaries while landing of foot at heel strike point on the ground surface. Hence, the externally modified shoes influence better stability on the ground and also these shoes possessing higher COF values particularly on forward heel slip conditions. These characters minimise the possible occurrence of slip and maximise the stability on the phases of locomotion with comfort and satisfaction for the beneficiaries.

The externally designed shoes of varied angles influence higher COF values than the standard shoe and the interlocking of surface profile of footwear and floor surface was established mainly due to the resultant of higher COF values. The total area of surface beneath the footwear revealed that the standard shoe possesses greater surface profile than other externally modified shoes. The 20 degree modified shoe possessed lesser area of surface than other two externally modified shoes and standard shoe. It was observed that 20 degree modified shoe represented higher COF values on forward foot slip and forward heel slip as well due to its significant design feature and also rendered added advantage of lesser area of surface profile especially on the bottom of footwear. The higher COF values with lesser surface profile are meritorious for rendering therapeutic benefits for the targeted overweight/obese individuals.

CONCLUSION

In this paper, the standard shoe and externally modified shoes with varied angles was experimented using SATRA Slip resistance tester and subsequently, the Coefficient of Friction values were estimated. It was revealed that the externally designed shoes possess higher COF values than the standard shoe. Amongst the shoes experimented, 20 degree externally modified shoe possessed superior Coefficient of friction value than others and its lesser area of surface profile rendered improved landing at heel strike phase of walking. The higher the value of the coefficient of friction, the less is the possibility of slipping: the smaller the value, the greater the danger. It is finally concluded that the 20 degree externally modified shoe deserves meritorious characteristics to provide therapeutic advantages for overweight/obese individuals.

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CREATIVE TRANSFER OF COMPETENCE IN 3D FOOTWEAR CAD

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The Creative Transfer of Competence in 3D Footwear CAD to VET Professionals Project, acronym - INGA 3D, aims to transfer and extend innovative software solutions and 3D technologies for computer-aided footwear design. The project brings together universities, research and training centres, adult education providers and IT companies from four European countries. The project products will introduce innovative solutions for e- learning in order to test and to validate new teaching methodologies and approaches suitable for vocational training in footwear computer-aided design. It will contribute to developing skills and competencies of VET professionals in order to face with the future challenges.

Keywords: footwear CAD, vocational training, virtual environment, practical and theoretical knowledge.

INTRODUCTION

The e-learning methods promise to improve human resource capability by using new technological capabilities, and resulted in improvements in organizational capabilities as well (Brasche, 2013).

Although 3D CAD is a widely used and highly effective tool in design, it also has its drawbacks: mastery of CAD skills is rather complex and time-consuming, e-learning could be used in a successful CAD training (Bodein *et al.*, 2011; Ionesi *et al.*, 2014).

E-learning refers to training initiatives which provide learning material, course communications, and it delivers the course content electronically, through technology mediation (Johnson *et al.*, 2008). Learning systems do not generally adapt to learners' profiles (Paraskevi *et al.*, 2008), proving that the footwear area selected for developing this multimedia tool is an appropriated one. Such a tool has to implement the adaptive self-consistent learning object as visual language, in order to define classes of learners by stereotypes and to specify the more suited adaptive learning process for each class of learners (Gennaro *et al.*, 2008; Avadanei *et al.*, 2014).

A student-centred approach is required for online learning and it can be used to create a community of learners (Ursache *et al.*, 2010; Mihai *et al.*, 2013). A flexible teaching strategy has to be developed and it has to be oriented towards the students' needs for training and learning (Mihai *et al.*, 2013; Dan and Ursache, 2010).

SUMMARY

INGA 3D project aims to transfer and extend innovative software solutions and 3D technologies for computer-aided footwear design, namely ICad3D+, produced by Spain. This will be achieved through four complementary activities:

- by transferring the innovation from Spain to other countries, namely Romania, Portugal, and UK;
- by developing skills and competencies in 3D footwear computer-aided design in VET professionals (teachers, trainers and tutors) so that they can teach ICT based technical courses that support creativity and innovation among their own VET students/trainees;

- by developing new training content and supportive e-learning tools based on units of learning outcomes and competencies. This will ensure effective assessment, evaluation and validation;
- by setting up an Online Learning Platform.

The project brings together universities, research and training centres, adult education providers and IT companies. The consortium has partners with great pedagogical experience in development and evaluation of methodologies for education and technical vocational training. Also, there are partners with experience in vocational training, and research and development for the footwear industry.

BACKGROUND

All over the Europe, one critical problem of VET study programs is the gap between the level of technical knowledge and professional skills that the learners acquire and the required competencies expected by employers (European Commission's Report - 'New Skills for New Jobs: Action Now', 2010). Footwear companies all over the Europe can find it challenging to recruit VET graduates competent and skilled in Computer Aided Design (CAD) of footwear. VET providers for footwear sectors could reduce this gap by widening their existing curricula to new available CAD/CAM technologies and software solutions that are developed through the latest research and commercial developments. At this point one main question appears: Do teachers/tutors/trainers from VET institutions have the right skills and competencies to teach Footwear Computer Aided Design?

A preliminary investigation that was undertaken by partners in the preparatory stage of this proposal (in RO, ES, PT and UK) revealed:

- VET institutions which are running study programs for footwear sector have ICT based content in their curriculum, but it is designed to cover the only key competencies for generic skills (keyboarding, word-processing, desktop publishing and using the Internet for research and communication);

- Working with CAD/CAM technologies are occupational specific ICT literacy skills and VET curricula rarely cover these in detail. The reason for this varies, as:

1) staff do not have right skills and competencies in CAD;

2) there is lack of teaching resources for footwear CAD;

3) the software developers for footwear CAD offer tutorials that do not meet pedagogical needs of VET system;

4) some training centres use their own curricula and methods for footwear CAD and these differ from those in the public VET schools.

RESULTS

The footwear CAD solution Icad 3D was developed by INESCOP (partner P1) and RED 21 (partner P5) is faster and more precise than other commercial products, and gives an immediate feedback both to teacher and to student/trainee. It allows detailed and accurate visualization of footwear prototypes in a virtual space. Through INGA 3D, the knowledge and the skills for developing patterns and footwear prototypes will be transmitted by VET teachers and trainers to their students and trainees in a dynamic and effective way. It will stimulate creative thinking among VET students and trainees, and it will increase attractiveness of VET study/training programs.

In order to develop the structure of the course, a questionnaire was applied in 3 countries, Romania, Spain and Portugal. In Romania, there were 21 respondents to this questionnaire.

The analysis is structured as the 2 parts of the questionnaire:

- General aspects on the training/teaching process.
- Technical dimensions on Footwear CAD.

In Romania's case, further interest for technical aspects of the course is expressed in gaining relevant theoretical knowledge on principles and techniques for footwear CAD, nearly 90% of respondent have chosen "To a large extent" (see figure 1).

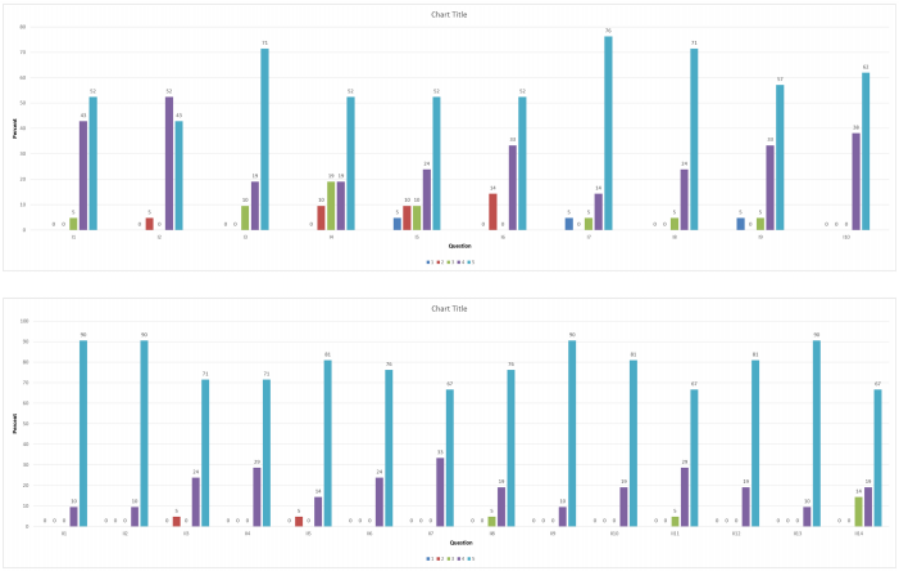

Figure 1. INGA 3D questionnaire's results

"To understand the main differences and advantages of 2D and 3D footwear CAD systems" is also an important topic that the respondents would like to improve. Also, "To produce footwear virtual prototypes by creating panels, adding texture, stitches and decorative elements" and "To draw accurate sketches, panels, boards and technical drawings in order to prepare the collection of models with CAD software" with 90% interest from the participants are aspects to be considered when developing the courses.

Lower values were obtain by the general aspects, like for example "To acknowledge my personal learning needs", "To overcome obstacles, to achieve success in the learning process", "To turn my ideas into action", which demonstrates the fact that these persons already have these knowledge.

CONCLUSIONS

The project products will introduce innovative solutions for e-learning in order to test and to validate new teaching methodologies and approaches suitable for vocational training in footwear computer-aided design. The online platform will integrate various

flexible learning scenarios and supportive tools for learning. The new training content and its supportive guide will be designed, developed, tested and evaluated in line with the best practices identified by partners in their institutions, countries and elsewhere in Europe. It will contribute to developing skills and competencies of VET professionals in order to face with the future challenges.

Acknowledgements

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DEMOULTRAGRIP - OPPORTUNITY TO DEVELOP NEW PRODUCTS FOR THE FOOTWEAR INDUSTRY

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The structure of production units in the Romanian footwear manufacturing industry demonstrates the existence of a production system based on SMEs, 97.1% of companies having less than 250 employees. The design criteria for models currently used by anti-slip footwear sole manufacturers are empirical, often based on intuition and previous experience. The technical problem is the lack of design tools that can be used in the design of shoe models, mainly the footwear's soles, in terms of making a prototype faster and at lower costs, while being more effective in creating an adequate response to the friction between footwear and walking surface during use. With DEMOULTRAGRIP project, the involved shoe manufacturing SMEs will gain a competitive advantage based on the use of new design tools for creating products with high resistance to sliding, speeding the design-prototyping operation, reducing prototyping and production cost, improving the anti-slip properties of products and reducing time-to-market.

Keywords: soles, rapid prototyping, friction coefficient

INTRODUCTION

According to the White Paper of Romanian Leather, Footwear and Leather Goods Industry, in 2010 in Romania there were a total of 1173 companies producing footwear (NACE code 1520) (INCDTP-ICPI, 2010). Depending on the companies' class size, the following structure was registered:

- SMEs:
  - 52.8% having between 0-9 employees,
  - 11.9% having between 10-19 employees,
  - 16.1% having between 20-49 employees,
  - 16.3% having between 50-249 employees,
- Large companies:
  - - 2.6% having more than 250 employees.

This situation, valid before the start of the financial crisis, shows a production structure based on SMEs. Productive units must face particular challenges such as competition from Asian market products, the evolution of CAD-CAM technology for footwear design, the decrease of the "lohn" production system and a need to conquer a place on the European footwear market. In this context, the move towards implementing CAD-CAM technologies for rapid prototyping should represent a key strategic option for SMEs in the footwear industry.

Currently, footwear sole manufacturers are designing the anti-slip pattern using empirical design criteria, often based on intuition and previous experience. The technical problem is the lack of design tools that can be used in the design of shoe models, in terms of making a prototype much faster and at a lower cost, while being more effective in creating an adequate response to the friction between footwear and walking surface during use. To answer this challenge, UltraGrip project (FP7 - SMEs - 2010 - 1.262413) developed a set of guidelines and a specific software solution that can be used as design tools for soles and flooring to optimize performance in relation to the slip phenomenon. Two of the main results of the UltraGrip are a software solution (mathematical model) to predict the behavior of slip and a set of guidelines with recommendations for improving the slip resistance of products.

UltraGrip consortium must conduct demonstration activities to ensure that UltraGrip instruments are ready and appropriate for exploitation and marketing. Consequently, DEMOULTRAGRIP project objective is to reduce the gap between pre-competitive tools developed in the UltraGrip project and a new version of these tools than can be marketed.

In line with this objective new business tools will be marketed. With DEMOULTRAGRIP project, the involved footwear manufacturing SMEs will gain a competitive advantage based on the use of new design tools for creating products with high anti-slip resistance, speeding the design-prototyping operations, prototyping and production cost reduction, improving the anti-slip properties of products and reducing time-to-market. All these issues will potentially increase both market share and the skills of designers and technicians.

INCDTP-ICPI ROLE IN THE PROJECT

Package structure of DEMOULTRAGRIP project activities is shown in Figure 1.

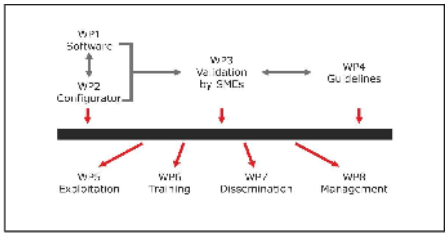

Figure 1. Structure of the DEMOULTRAGRIP project work packages

INCDTP-ICPI has ongoing activities for each of the eight work packages, as follows:

WP 1: Coefficient of Friction, CoF, Predicting-CAD Software

Objective: making the connection between the software solution for predicting friction coefficient from UltraGrip project and a new software solution for designing and predicting the behavior of footwear sole friction, which will be put on the market. INCDTP will manufacture different samples of rubber soles, TR, and thermoplastic polyurethane soles, TPU, and send them for testing to INESCOP which will update the predictive mathematical model of the coefficient of friction.

WP 2: Design Configurators of Soles

Objective: To achieve a configurator for soles that will qualitatively estimate the changes of the CoF due to the change of the sole's material or design. INCDTP will use the beta version of the soles configurator for test design and will send a feedback to the developers. Along with KOPITARNA, INCDTP will validate the online version.

WP 3: Computer DEMOULTRAGRIP Tools Assessment

Objective: To assess the commercial version of the software solution developed in WP1 and of the configurator developed in WP2 by using them in the design of prototype soles with high CoF (UltraGrip line models) obtained from different materials and with different destinations. INCDTP will design and produce a line of thermoplastic

rubber soles TR - UltraGrip TR line - with anti-slip properties for leisure footwear (shoes with anti-slip medium specification level).

WP 4: Guidelines for Commercial Applications

Objective: closing the gap between the recommendations obtained in UltraGrip project and commercial versions for final users. INCDTP will participate in the development of new guidelines along with other members of the consortium.

WP 5: Exploitation

Objective: develop first Market Study and Business Plan and preparation of the Operating Agreement between the consortium partners for marketing-exploitation of the DEMOULTRAGRIP products. INCDTP activity will be oriented to the TR-UltraGrip line.

WP 6: Training of Commercial Software Tools

Objective: training the end users to use the developed commercial software solutions. INCDTP: participation in training and the involvement of Romanian SMEs.

WP 7: Dissemination

Objective: to demonstrate the added value of the project results. INCDTP: participation in specialized footwear fairs.

WP 8: Project Management

Objective: To ensure overall project management. INCDTP: participation in working meetings of the consortium, contributions to reports and deliverables and its own financial management.

OPPORTUNITIES TO DEVELOP NEW PRODUCTS FOR THE FOOTWEAR INDUSTRY

The Market research conducted in the frame of the DEMOULTRAGRIP project has revealed both advantages and competitive disadvantages of micro-production department of ICPI if compared with the main actors in the specialized market (Figure 2). Introducing new materials for footwear sole design represents a constant concern of the Rubber Research Department of INCDTP-ICPI (Alexandrescu *et al.*, 2014).

				Main competitor in each segment		INCDTP		Your company vs. main competitor	
Market segments	% on total market sales	Set of values	% on total company's sales	Strengths	Weaknesses	Strengths	Weaknesses	Competitive advantages	Competitive disadvantages
men	8%	1° price 2° quality	80%	bigger production capacity	poor quality of the equipment	-quality -new materials -new equipment	-reduced production capacity -weak marketing	-new materials	-reduced production capacity
women	2%	1° price 2° quality	20%	much more up-to-date models	poor quality of the equipment	-quality -new materials -new equipment	-old mould models -weak marketing	-new materials -flexibility	-old models -small number of models
Total	10%	-	100%	-	-	-	-	-	-

Figure 2. Analysis of the INCDTP-ICPI competitiveness compared to its main competitors in the local market

In this context, related to the specific objective of the project - namely to develop thermoplastic rubber soles TR-UltraGrip line with high anti-slip properties, based on existing models but using new recipes designed in accordance with this objective - it is necessary to define a new future direction (Figure 3).

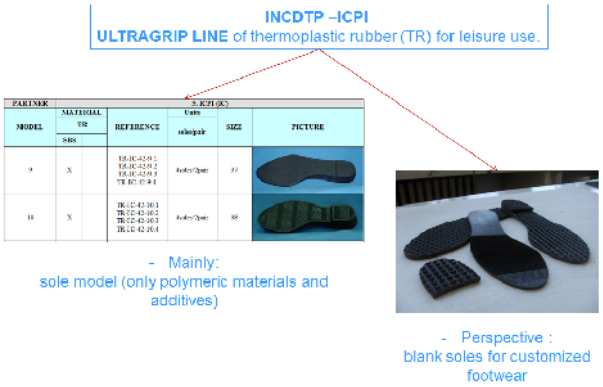

Figure 3. Main objective and development perspectives through participation in the DEMOUTRAGRIP project

The perspective, according to the structure of the production system based on SMEs, but also to the position relative to the main competitors in the market, is the development of a flexible design system of the "blank" plate type soles allowing rapid production of small orders in flexible conditions and in accordance with the specific needs of shoe manufacturing companies. It aims to create a technology for obtaining these plates by injection or by vulcanizing press molding, so the anti-slip design can be quickly changed depending on the beneficiary's request.

CONCLUSIONS

Participation in research projects together with recognized research institutes at European level and with private companies interested in the application of the results in production represents an opportunity to develop new products for the footwear industry.

The development of high added value products using CAD-CAM technology for rapid prototyping is, in the context of product competition from Asian countries, an essential option for the development of SME type footwear manufacturing companies.

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RESEARCH REGARDING ESD GARMENTS DEVELOPMENT

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The electrostatic discharge (ESD) can be defined as a sudden transfer of electrostatic charge between two objects of different potentials. In terms of ESD characteristics, fabrics will quickly dissipate the accumulated charge, but will present a potential risk for electrostatic charging and energy transfer during an accidental discharge; these issues are solved by the fabrics that contain surface conductive fibres. To obtain an ESD garment with superior qualities, the present paper proposes the study of a double layered knitted structure. The fabric were manufacture on a electronic flatbed knitting machine, and tested from the functional characteristics point of view (electrical resistance, shielding factor, discharge time) and from the comfort characteristics point of view (thermal conductivity, thermal resistance, air and vapour permeability).

Keywords: electrical discharge, knitting, protective clothing

INTRODUCTION

The electrostatic discharge (ESD) can be defined as a sudden transfer of electrostatic charge between two objects of different potentials. An electrostatic discharge appears when the charged object comes near to an uncharged object. This is accompanied by a high energy transfer and may cause malfunctions or irreparable damages of sensitive devices. To overcome these issues, ESD protective solutions are developed, which reduce the risk of an electrostatic discharge (Carpus *et al.*, 2014).

In terms of ESD characteristics, fabrics will quickly dissipate the accumulated charge, but will present a potential risk for electrostatic charging and energy transfer during an accidental discharge; these issues are solved by the fabrics that contain surface conductive fibres.

Functional characteristics are defined as follows:

- resistivity 10^4-10^5 Ohm·m;
- distribution of electrostatic charges (EN 1149-1/Protective clothing - Electrostatic properties-Part 1: Test methods for the measurement of surface resistivity);
- provision of instantaneous discharge (EN 1149-3/Protective clothing - Electrostatic properties-Part 3: Test methods for the measurement of charge decay);
- anti-static properties are preserved even after 100 wash cycles.

An ESD protective garment should ideally have the following functions:

• The protective garment should effectively shield the electric field originating from the insulating parts of the operators' normal clothing;

• The protective garment should prevent direct discharges from the operators' normal clothing;

• The protective garment should not itself cause similar problems. That is, it should not generate electrostatic field external to the garment and it should not be a potential source of direct electrostatic discharges.

To ensure the performance of a maximum safety and comfort activity, a two-layer structure with different electrostatic behaviour has been chosen. The outer layer is

mainly dissipative, providing protection against short circuit and limiting the amount of electrostatic energy that can be dissipated to the work environment, while the inner layer is mainly conductive, ensuring controlled drainage of static electricity. An additional requirement for the inner layer is to ensure the user's comfort (Carpus *et al.*, 2014).

In this paper, has been selected a configuration of a bilayer ESD fabric and analyzed by various testing methods.

EXPERIMENTAL PART

Two-layer knit variants were made with plaited structures, with parallel evolution of two or more yarns with strictly determined relative position as a result of their submission at different angles (plaiting yarn V at an angle smaller than ground yarn F). The most used knitted structures are jersey and rib structure. In case of jersey structure, the plaiting yarn V appears on the foreground on the front and the ground yarn F, on the foreground on the back of the fabric. In case of rib structure due to alternating of front-back wales - both the plaiting yarn (at front aspect stitches) and the ground yarn (at rear aspect stitches) will be present on the foreground, on each side of the fabric.

Nega-Stat® is introduced into textile materials to provide protection against a range of risks and hazards caused by static electricity in industrial end-use situations.

Knitting is made on electronic flatbed knitting machines having yarn thread guides with special construction that provide the yarns with different deposition angles under needle head, so that in the stitch forming stage the plaiting yarn (V), in our case a conductive yarn, remains on the front of fabric, while the ground yarn (F), in our case the fabric yarn will stay on the back of the fabric (Carpus *et al.*, 2014).

Within the present paper, from the 21 knitted variants, the variant number 7 has been selected, as it can be seen in Table no. 1.

Table 1. The structure chosen

Structure	F1/Front	F2/Rear	Conductive yarn percentage
plaited jersey	one yarn Nm 50/3, 100% cotton + one yarn 75% cotton + 25% epitropic yarn (Nm 34/1 carbon coated polyester)	one yarn Nm 50/3, 100% cotton + one yarn Nega-Stat P190, 155 dtex, 24f, polyester filament with trilobal carbon inner core	5%

Functional and Comfort Characteristics Measurements

In order to characterize the sample were conducted the following parameters: weight [g/m^2], density (wales/10cm, rows/10cm), thickness (mm), air permeability (l/m^2/s), water vapour permeability (%), thermal conductivity (mW/mK), thermal resistance (m^{2}KW) (Table 2).

Table 2. The results of measurements

Weight [g/m^2]	Density Do	Density Dv	Thickness, [mm]	Air permeability, l/m^2/ s	Water vapour permeability, %	Thermal resistance m^{2}K/W
487	44	86	1.63	484.6	42.5	0.03354

Considerations:

- the figures for weight and density are suitable for this kind of knitted articles considering that the proposed garment are designed for spring-automn season;
- the value for water vapor permeability is placed into optimal zone, due to the presence of the cotton yarn in the structure.
- air permeability causes sensations of warm and cool of clothing products, the value obtained beeing characterized by the presence of cotton yarn and conductive yarn element from nylon filament surface saturated with carbon particles (Scarlat *et al.*, 2014).

Electrical Measurements

Electrical Measurements on Fabrics. Charge Decay Time Measurements

To determine the charge decay time for the knitted samples, a measuring stand using a Charge Plate Monitor (CPM) type 268A-1T manufactured by Monroe Electronics, a discharge electrode, an ESD switch normally open, an oscilloscope and a set of electrostatic insulators was used. CPM has an internal 5 kV power source and an electric field sensor. Through the high voltage power source, the CPM's plate is charged up to a certain potential in regards with the ground. Being in contact with the charged plate, the tested material will also be charged at the same potential. After disconnecting the power supply, the discharge stage is started by connecting the electrode to the ground. The discharge signal is viewed and recorded via oscilloscope and will be used to determine the charge decay time. All insulator items used within the measuring stand were made of polycarbonate. It was intended to separate the charging area from the discharging area for the tested samples.

The charge voltage used for every sample was set to 5 kV. Determinations were made in two different conditions:

- the discharge electrode connected to the sample's dissipative surface (CD);
- the discharge electrode connected to the fibre's conductive core (CC).

Determined parameters which define the discharge process have the following meanings:

- $t_{1/2}$ represents the time after which the 5 kV voltage at which the samples were initially charged decreases by half as result to the discharge (half-time);
- $t_{1/e}$ represents the time after which the 5 kV voltage at which the samples were initially charged decreases to 1/e of its value as result to the discharge (37% time);
- U_{125} represents the voltage recorded at the sample's surface after a period of 125 ms has passed from the beginning of the discharge (residual voltage after 125 ms);
- r_{125} represents the ratio between the residual voltage after 125 ms and the initial charge voltage (Donciu *et al.*, 2013).

The results regarding the charge decay time are presented for the chosen sample in figure 1 and represents the evolution of the discharge voltage. Centralization of data and parameters that characterize the discharge process are presented in table 3.

Table 3. Measured parameters characterising the discharge process

$t_{1/2}$([s]		$t_{1/e}$([s]		U_{125}([V]		r_{125}([%]	
CD	CC	CD	CC	CD	CC	CD	CC
0.0451	0.0278	0.0804	0.0412	1520	480	30.4	9.6

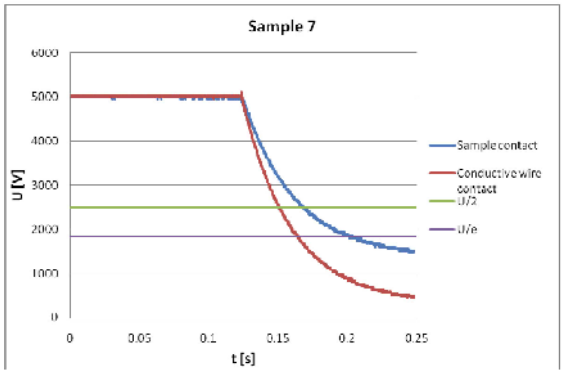

Figure 1. Charge decay times for bilayer sample

Analysing the results it can be noticed that the sample presents good ESD properties. These are strongly enhanced if the conductive core is electrically connected to the discharge electrode.

To determine the fabric resistance, a different applied voltages, V_{appl} = 1-200V was performed to the fabric.

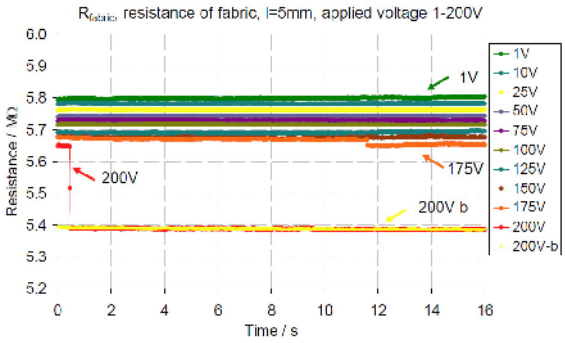

Figure 4. Resistance of the fabric during different applied voltages, V_{appl} = 1-200V

The results of measurements reveal the good electrical behaviour of the fabric of the tested samples. The measured resistances are in the MΩ, the range is sufficient to avoid electrical charge build-up in the fabric.

Electrical Measurements on Yarns

To determine the yarn resistance, a different applied voltages, V_{appl} = 1-200V was performed to a yarn extracted from the fabric.

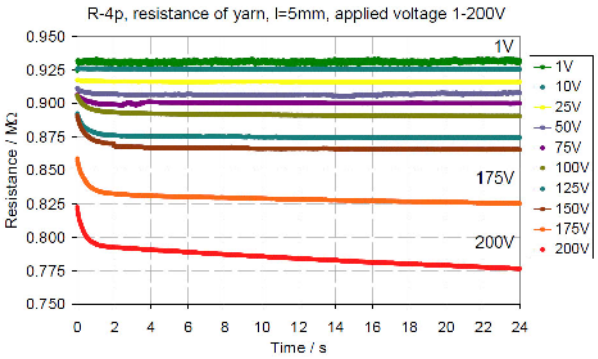

Figure 3. Resistance of yarn

The results of measurements reveal the good electrical behaviour of the yarn from the tested samples, when containing carbon covered fibers. The measured resistances are in the MΩ, the range is sufficient to avoid electrical charge build-up in the fabric that contain the yarn.

CONCLUSIONS

Within this paper, the properties of a knitted fabric with bilayer structure were investigated using various methods. The parameters which were investigated are:

- functional and comfort characteristics: weight, density of the fabric, thickness, air permeability, water vapour permeability, thermal conductivity, thermal resistance.
- electrical measurement on the fabric: discharge time and electrical resistance;
- electrical measurements on the yarns: electrical resistance.

The results of measurements reveal the good electrical behaviour of the yarn and fabric of the tested samples. The measured resistances are in the MΩ, the range is sufficient to avoid electrical charge build-up in the fabric.

- the figures for weight and density are suitable for this kind of knitted articles considering that the proposed garment are designed for spring-automn season;
- the values for water vapor permeability air permeability are placed into optimal zone, due to the presence of the cotton yarn in the structure.

Acknowledgement

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VII.
QUALITY MANAGEMENT AND COMPETITIVENESS

CO_2 EMISSIONS REDUCTION FROM TANNERIES AND FOOTWEAR MANUFACTURE INDUSTRY FROM ROMANIA. REALITIES AND TENDENCIES

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This paper is presenting the objectives and partial results of the project IEE / 11 / 949 / SI2 615946 IND-ECO which is running under the Intelligent Energy Europe – Executive Agency for Competitiveness and Innovation EACI umbrella. A number of 16 entities representing leather and footwear European and national employers' associations, manufacturers, research institutes from Italy, Belgium, UK, Spain, Portugal, Romania and Bulgaria has formed a consortium with the purpose of reducing energy consumption and CO_2 emissions at the level of EU countries.

Keywords: energy efficiency, tannery, footwear

IND-ECO PROJECT PRESENTATION

The European umbrella association and national associations from most relevant countries for leather-footwear industry, technical centres, engineering companies and manufacturers lead by UNIC, Italy join together in a very well balanced and representative partnership.

General objectives of the IND-ECO project consortium are:

- deepening the knowledge on energy performances and CO_2 emissions to define benchmarks for assessing specific energy performances and to identify improvement areas.
- increasing awareness, knowledge and organisational skills of companies on energy efficiency to allow them to seize opportunities, to adopt tools, technologies and financial facilities to access capitals.
- contributing to overcome barriers (those listed in "Energy efficiency plan 2011) to energy efficiency investments (lack of information and lack of access to capital) by working on technical and economic/financial frameworks together with European and national associations and relevant key actors.
- supporting companies to plan and carry out investments in energy efficiency by the end of the project and to plan new investments to be realised by 2020.
- develop a clusters-based approach for investments especially those to be realised by 2020.
- contributing to develop the demand of energy efficient equipment, machines and plants and to increase the turnover of the mechanic industry.
- ensuring a high-quality development, implementation and monitoring of project activities and investments plan through a monitoring and auditing approach.
- making the tools and results widely available through European and national associations, clusters, companies and other stakeholders

Main Objectives of the Project

Main targets of the project are:
- to obtain initial primary energy savings by its end
- to create favourable conditions for much more investments by 2020

Specific objectives of the project are as it follows:

-inventory of energy consumption; benchmark realisation at the level of the leather and footwear manufacture industries;

- verification of energy consumption. Identification of the vulnerable areas which need improvements;

-identification of the financial solutions providers – bank loans for energy investments;

-building a technical and technological solutions database for the energy consumption reduction;

-elaboration of the investments plans;

-project results dissemination and access to the databases created in the project.

The Structure of the Project's Work Programme (WP)

The structure of the IND-ECO work programme (WP) is presented in figure 1.

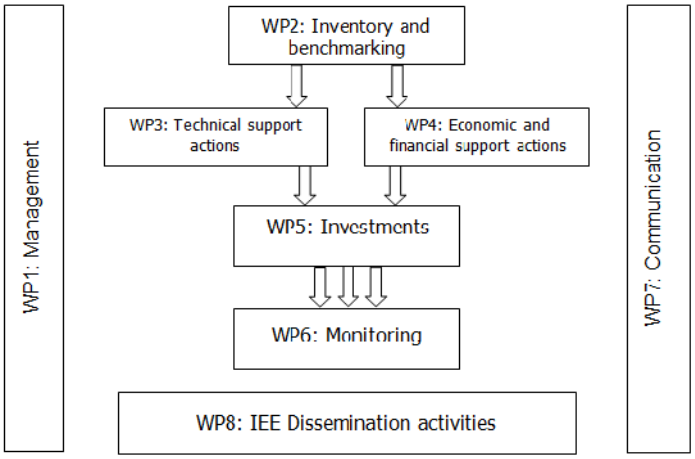

Figure 1. The structure of the IND-ECO work programme (WP)

Benchmark

WP.2 has planned activities which are targeting**:**
- raising companies' awareness of energy consumptions and environmental impacts
- focusing on energy consumption of the involved sectors (tanning, and footwear)
- allowing involved companies to know their consumptions and CO_2 emissions and their position relative to the benchmark
- defining a starting position to evaluate investment opportunities and to assess the achieved results.

Based on the analysis of the inventories received from the companies it was established the next preliminary values for the benchmarking for tanneries and footwear manufacturers (table no.1 – tanneries, table no.2 footwear manufacturers).

Table 1. Benchmark values for tanneries

No.	Type of leather produced	Raw material	Production cycle applied	Energy specific consumption	Unit
1	Finished leather	Bovine hides	Raw to finish	16.47	Kwh/m^2
2	Wet-blue	Bovine hides	Raw to wet-blue	1.8-3.2	Kwh/m^2
3	Finished leather	Bovine	Wet-blue to finish	6.02-9.24	Kwh/m^2
4	Finished leather	Bovine	Crust to finish	4.07	Kwh/m^2
5	Vegetable tanned leather (different from sole leather)	Bovine hides	Raw to finish	6.98	Kwh/m^2
6	Vegetable tanned leather (sole leather only)	Bovine hides	Raw to finish	1.89	Kwh/kgs
7	Finished leather	Calf skins	Raw to finish	8.15	Kwh/m^2
8	Finished leather	Calf	Wet-blue to finish	6.44	Kwh/m^2
9	Fur	Sheep skins	Raw to finish	14.70-16.85	Kwh/m^2
10	Skin	Sheep skins	Pickle to finish	7.94	Kwh/m^2

Table 2. Benchmark values for footwear companies

Indicator	Total production		Cutting and/or stitching partial or totally subcontracted		Cutting & stitching
	Romania	EU	Romania	EU	Romania
Average Kwh/pair	0.5-3.9	0.7-3.9	2.2-5.6	0.8-3.6	-
Kg CO_2/pair	0.3-2.3	0.3-1.9	1.1-2.9	0.4-1.7	-
Maximum Kwh/pair	6.3	0.4	6.3	0.2	2.6
Maximum CO_2/pair	3.7	0.2	3.2	0.1	1.5
Minimum Kwh/pair	0.4	9.3	2.0	6.3	0.4
Minimum CO_2/pair	0.2	4.6	1.0	3.1	0.2
Number of companies	25	108	5	57	5

Energetic Audit

The energetic audit of 67 leather and footwear companies was realized according to the schedule, the results being in the analysis process. Based on these

audits, the benchmark values previously established will be revised. In the same time, based on the results, the further action direction will be established.

Technical Solutions

The **Technical support actions** (WP3), thanks to a scouting activity, also based on WP2 results, will make available knowledge, technological solutions, agreements with technologies providers and their associations and other facilities aimed at making easier the access of companies to technical solutions. An energy efficient solutions data base will be made and implemented with at least 150 recommended solutions identified thanks to the scouting. It will be available on the project site. Till this moment it was realised the database where are uploaded the companies which are offering equipment or technical solutions having as objective energy efficiency increasing.

Financial Solutions

Economical and financial support actions (WP4), aims to improve the access of companies (in particular of SMEs) to capitals. Were identified at the local and European level the financial institutions and economical programs which are offering loans focused on energy efficiency improvement.

Investments (WP5)

Intends to finalise the work done during WP2, 3 and 4 allowing to achieve, by the end of the project and by 2020, the quantitative targets in terms of energy saving and CO_2 emissions reduction. It was realised a model for the investments evaluation from the energetic field, which, starting from the bank's standard, is permiting the simulation of the economical results regarding each specific investment case.

Communication (WP7)

The partners from IND-ECO project will share knowledge, solutions and results with tanning and footwear companies, other industry associations and other stakeholders.

CONCLUSIONS

- In the next period till the end of the project, the European leather industry tanneries and footwear companies will have a functional instrument for the energy efficiency increasing,
- Starting from the energetic audit of the company, it will permit choosing of the best technical and financial solutions, will realize the investment plans which will facilitate an approach according to the state of the art of the technology achieved at the moment,
- Total energy saving of 16,7 mio primary Kwh is envisaged till the end of the project.

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QUALITY IMPROVEMENT IN THE FOOTWEAR COMPANY

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Quality is the key factor in ensuring the competitiveness of an enterprise. In the footwear industry of Moldova there are about 50 enterprises producing shoes for both internal and external market mainly in the LOHN system. This paper shows the results of the case study regarding some aspects related to the footwear quality production at the "Zorile" S.A. based on analysis of data recorded at the final inspection. The Pareto and Ishikawa charts are tools that facilitate analysis and enable the elaboration of offers to improve the existing situation. Based on the actual situation of the footwear quality in the enterprise it is recommended improve the quality of processes and products through the implementation of actions aimed to remove the underlying causes identified and relate to: professional training of workers provided by the employment of graduates and the organization of continuous training; staff motivation by increasing their remuneration accounted from salary supplements for the quality and ensuring the professional growth, organizing the activities in such a way that the employee be able to meet the standard of work produced by staff to improve recognition of the company in terms of continuous improvement quality; revision of the rules of time; organizing activities to prevent occurrence of nonconformities by continuous ensuring with materials needed for technological operations; implementation of new technologies that would require organic substances, total or partial mechanization; providing a favorable work environment, proper lighting, ventilation, workplace policy, workplace convenience.

Keywords: footwear, quality, defects.

INTRODUCTION

Due to the rising competition, operators face with the problem of maintaining the existing customers and also of gaining new customers, along with the need to achieve a certain minimum level of business efficiency. So far, the quality is considered a strategic element of enterprise management, with an important role in increasing the competitiveness of products and services both domestically and internationally.

Quality products are of special importance for the society with favorable effects in all areas of economic activity, but the quality requires continuous improvement. There is no permanent level of quality and the continuous improvement strategy, known as the japanese KAIZEN strategy or the "small steps", directs the efforts of employees to achieve the highest quality levels by increasing the number of quality parameters and always comparing the competition products and the customers' demands.

Continuous quality improvement can be facilitated by the implementation and enhancement of the quality management principles underlying the quality management systems ISO 9000 series standards: focus on the customer, leadership, involvement of people, process approach, system approach, making decisions based on facts and mutually beneficial relationships with suppliers (SR EN ISO 9000, 2001). But compliance is impossible in organizations where management is oriented towards results and immediate profit (Деминг, 2009). Quality and continuous improvement planning should be carried out by trained specialists in quality management (Juran, 2000), which enterprises under the staff turnover is high, it becomes a problem.

QUALITY MANAGEMENT IN THE FOOTWEAR COMPANY S.A."ZORILE"

The S. A. “Zorile” enterprise is the first large company of footwear production in Moldova founded in 1945. In 2009 “Zorile” has released a new enterprise development program according to which was possible the brand “Zorile” restyling, and was created a network of 25 stores focusing on the modern footwear industry. Currently the company aims to achieve a ratio of 40/60 between the own production and the production activities for foreign companies in the lohn system, and to include a local market share of 10% and sales to ensure a level of 400.000 pairs per year of own footwear products.

Product quality is ensured primarily through emphasis on detailed control over all phases of the production cycle: self-control and chain control made by each operator; fixed control made by quality controllers and scutch control made by the foreman, technologist and head of department (Ionescu, 2001; Ionescu, 2002). The "quality" operation is coordinated by the head of the "physical and mechanical testing laboratory and flow control" subdivision, relying on the provisions of the enterprise standard from 02.11.97 called "The complex system of quality control". According to this embodiment, the responsibility lies with each sector of the company, which offers efficiency in taking the necessary measures to address the identified problems.

CASE STUDY

In a case study there were analyzed the defects identified and recorded by the controller during three months at final inspection (table 1).

Table 1. The identified defects in footwear quality control, S.A.”Zorile”

No.	Type of defect	I-st month	II-nd month	III-rd month	Total
0	1	2	3	4	5
1	Vamp sewn incorrectly	18	-	-	18
2	Defectiv enging stitch	17	37	45	99
3	Non-parallel stitch	15	-	-	15
4	Stitching turned 180 ° poor	10	-	-	10
5	Inappropriate manual stitch	6	281	5	292
6	Improprer upper assembly	5	-	-	5
7	Inappropriate sewing of tack	2	-	34	36
8	Improper incasing and decorative fixing	25	-	37	62
9	Excces of uncut lining	55	-	67	122
10	Improper footwear preforming	-	-	93	93
11	Improper lasting of shoes in the tip region	62	65	-	127
12	Improper lasting of shoes in the heel region	15	64	43	122
13	Height caramba different pair	-	-	113	113
14	Holes made provisional elements	12	-	12	24
15	Improper strobel stitch	-	4	10	14
16	Defects stitch the sole to the upper assembly	147	83	202	432
17	Inappropriate refoot (sole) of footwear	53	85	283	421

No.	Type of defect	I-st month	II-nd month	III-rd month	Total
0	1	2	3	4	5
18	Leather defects after dryer procesing	23	-	417	467
19	Improper shoe cleaning	258	461	444	1136
20	Unremoved threads	208	151	142	501
21	Improper application of insole	-	-	59	59
	Total	931	1231	2001	4163

The analysis performed using a Pareto diagram (figure 1-3) showed that in all three months the defect - inadequate shoe cleaning (quantitatively ranked) was first placed, followed by uncut threads in the first month and leather defects after processing with dryer in the 3rd month.

Spot observations and the analysis of the results of a survey performed in the company allowed the development of the Ishikawa diagram, showing the causes of occurrence of the most frequent defect (fig. 4). The opinions of 20 workers directly from station No. 2, on the causes of the occurrence of nonconformities identified quality control footwear products were as follows: 37% is accounted for carelessness or irresponsibility workers, 22% - use of unsuitable materials, 19% - working method implemented, 11% - the machine used, 7% - incorrect measurements and 7% - work environment.

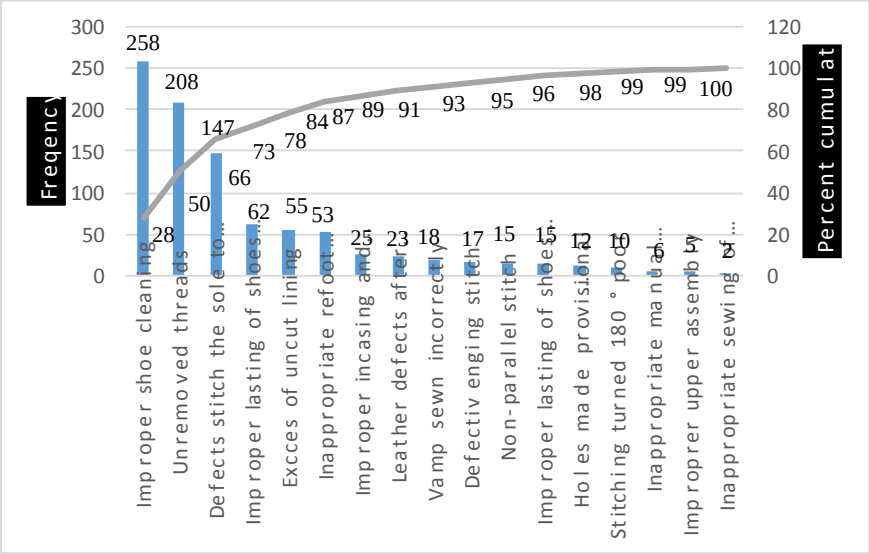

Figure 1. Pareto diagram – Ist month

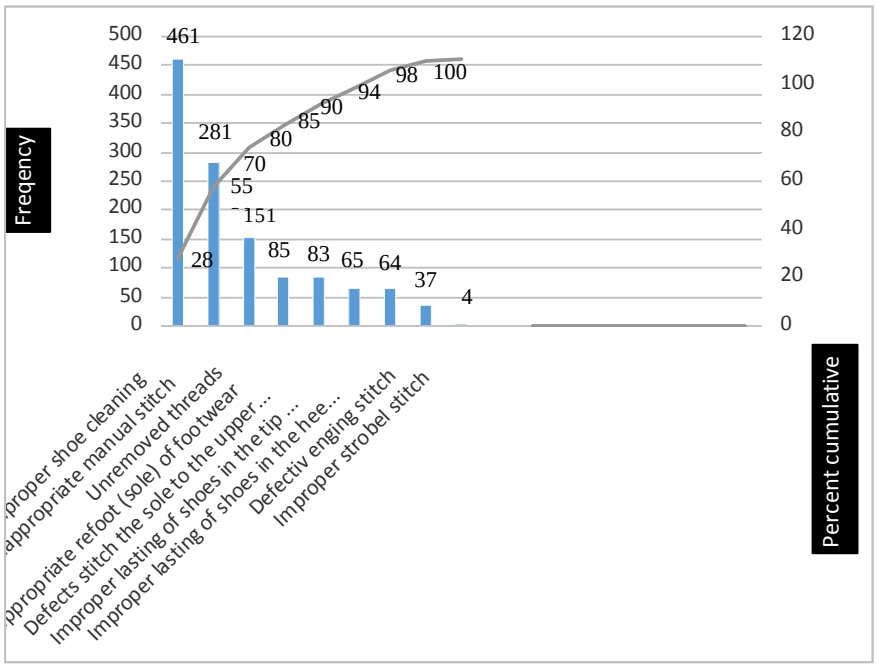

Figure 2. Pareto diagram II[nd] month

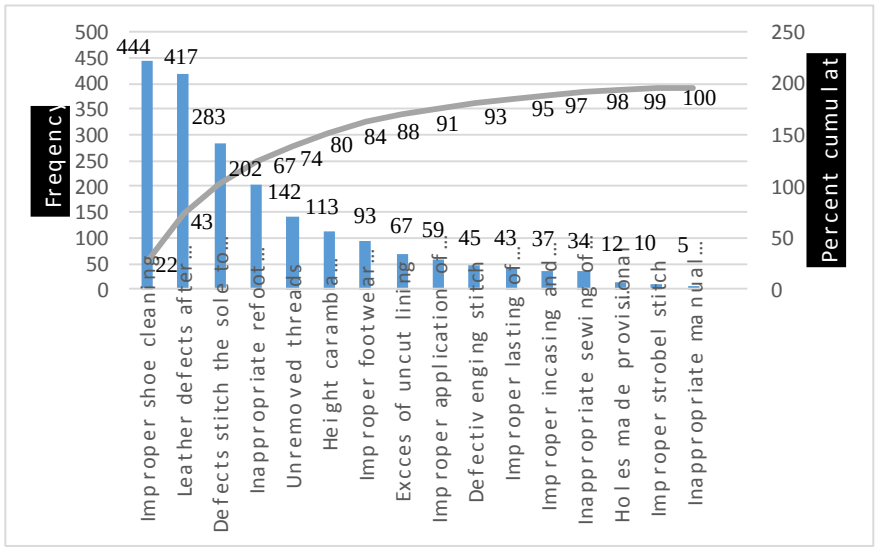

Figure 3. Pareto diagram III[rd] month

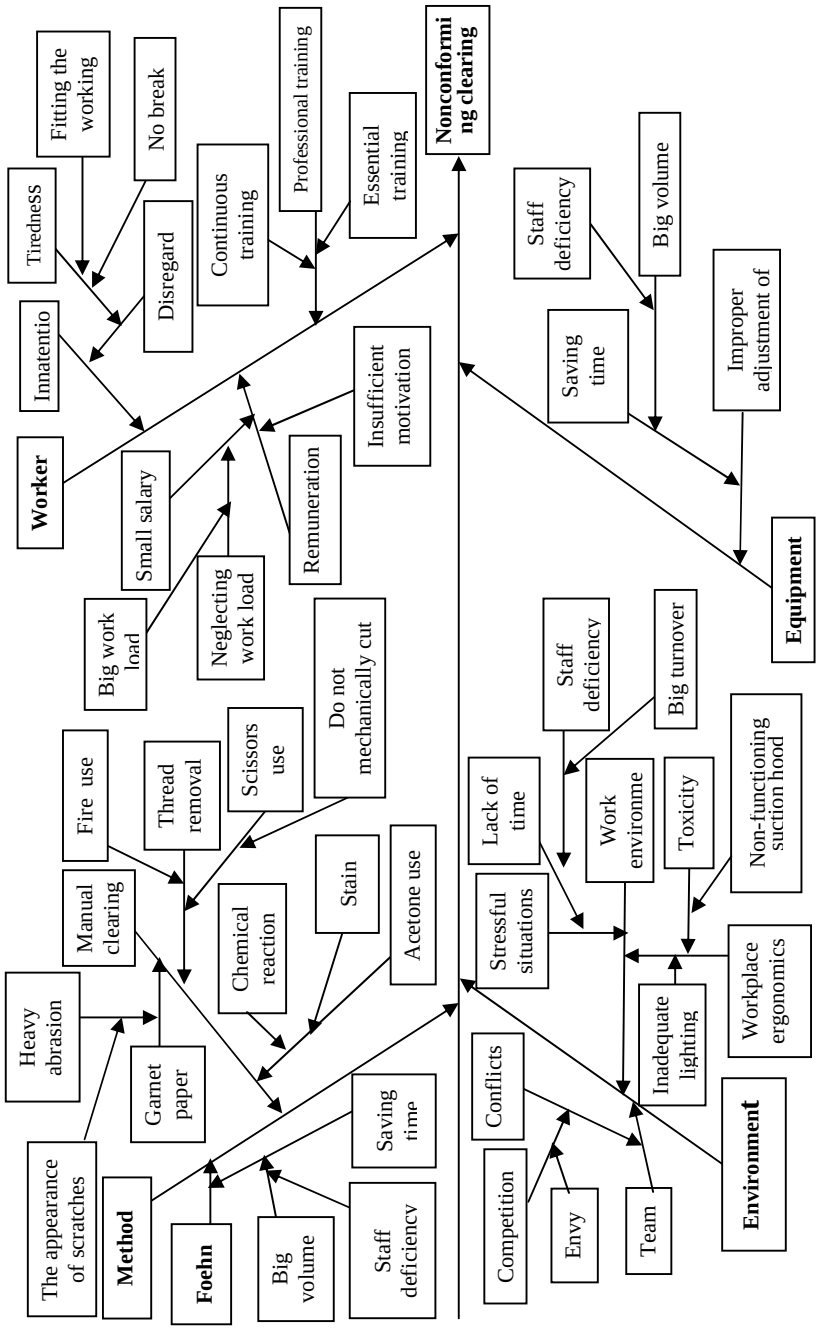

Figure 4. Ishikawa diagram for nonconforming clearing defect

It is noted that since 2011 S. A. "Zorile" implemented a motivating remuneration of quality controllers. It provides a structured remuneration of two components: the constant part that is 45% of salary and the floating part of 55%. The principle of granting the floating part is as follows: in the absence of comments on the product quality and finding that the volume rebut is less than 0,5% from floating part than it is given in full; if the rebut is 0,5 to 1,0% then the floating part is reduced by 25%; if the rebut is 1,1% to 1,5% then the floating part is reduced by 50% and in case the rebut is more than 1,5% then it is not granted. It was also planned work rotation for quality controllers, but due to their large fluctuation, the procedure is performed sluggishly.

RECOMMENDATIONS FOR QUALITY IMPROVEMENT

Based on the actual situation of the footwear quality in the enterprise "Zorile" it is recommended improve the quality of processes and products through the implementation of actions aimed to remove the underlying causes identified and relate to:

- professional training of workers provided by the employment of graduates and the organization of continuous training (Ciobanu, 2009; Kobayashi, 2001);
- staff motivation by increasing their remuneration accounted from salary supplements for the quality and ensuring the professional growth, organizing the activities in such a way that the employee be able to meet the standard of work produced by staff to improve recognition of the company in terms of continuous improvement quality;
- revision of the rules of time;
- organizing activities to prevent occurrence of nonconformities by continuous ensuring with materials needed for technological operations;
- implementation of new technologies that would require organic substances, total or partial mechanization;
- providing a favorable work environment, proper lighting, ventilation, workplace policy, workplace convenience.

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EU POLICY FOR SUSTAINABLE CONSUMPTION AND PRODUCTION – ECOLABEL FOR FOOTWEAR

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The EU Ecolabel is a voluntary scheme that forms part of overall EU policy to encourage more sustainable consumption and production. The award of an EU Ecolabel to a product is denoted by use of the logo „Flower". This paper provides a brief overview about EU Ecolabels with special emphasis on EU Ecolabel for footwear product group, criteria for a first assessment only and a short description of the global and European leather footwear industry.

Keywords: Ecolabel, footwear, sustainable development, sustainable consumption

INTRODUCTION

Sustainability means the ability of meeting the needs of the present generation without compromising the ability of future generations to meet their own needs. Hence sustainability is a holistic concept, which takes into account social and environment issues as well as problems that are specific to a region.

The EU Ecolabel is a voluntary ISO Type I environmental label, i.e. independent of the manufacturer and producer. Initially established in 1992, its aim is to: i) decrease the environmental impacts of products throughout their lifecycle; ii) promote the resource efficiency of industrial production; enable consumers to make informed decisions based on a product's environmental performance.

The award of an EU Ecolabel to a product is denoted by use of the logo „Flower".

The Ecolabel scheme is an important component of the EU's Sustainable Consumption and Production Action Plan (European Commission, On the Sustainable Consumption and Production and Sustainable Industrial Policy Action Plan, 2008) adopted by the Commission on 16 July 2008, complementing the Ecodesign Directive (Directive 2009/125/EC) by providing a best practice benchmark and integrating with the Green Public Procurement (GPP) agenda (Green Public Procurement).

However, the scheme has had difficulty in terms of market penetration and, following a review, the scheme was revised in 2009/10 and a new Regulation (EU Regulation 66/2010 on the EU Ecolabel) was published.

EU Footwear Labeling Directive

Directive 94/11/EC, also called EU Footwear Labeling Directive, is specifically related to the European market on the approximation of the laws, regulations and administrative provisions of the Member States relating to the labeling of materials used in the main components of footwear for sale to the consumer (http://www.ibisworld.com/industry). For the purposes of the Directive, 'footwear' shall mean all articles with applied soles designed to protect or cover the foot, including parts marketed separately as referred to in Annex I of the Directive, and recalled on Figure 1. Respective labels must contain information related to the main footwear component parts, such as: the upper, the lining and insole sock, and the outer-sole. Materials must be labeled in one of four ways: leather; coated leather; natural, synthetic and non-woven

textile; and all other materials. The labeling shall provide information on the material covering at least 80% of the surface areas or 80% of the volume of the outer-sole. The information must be conveyed by means of approved pictograms or textual information, as defined by the Directive.

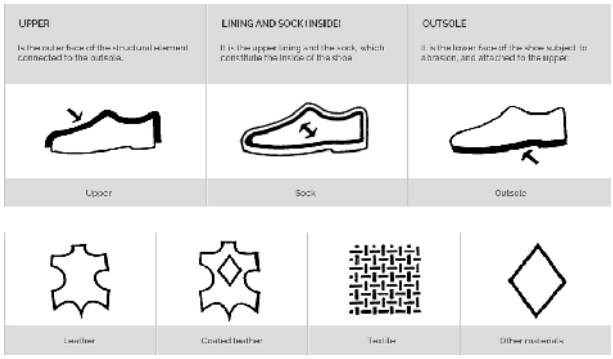

Figure 1. Footwear Pictograms in line with the Directive 94/11/EC

European and non-European Ecolabels

The main European Ecolabel schemes that address both footwear and/or leather containing product group(s) are shown in the Table 1.

Table 1. European Ecolabels

No	Ecolabel name	Logo	Scope
1	EU Ecolabel (Europe)		Footwear
2	Nordic Swan (Denmark)		Textiles, Skins and Leather
3	Blue Angel (Germany)		Footwear
4	Environmentally Friendly Products Ecolabel (Czech Republic)		Footwear
5	Distintiu de garantia de qualitat ambiental (Catalunya)		Leather

Global Footwear Market

Industry revenue for the Global Footwear Manufacturing has increased 2.2% in 2012 to total USD 122.9 billion, up from USD 107.4 billion in 2011: this represents an annual growth of 2.7% over the last 5 years (Eurostat). According to APPICAPS estimates world-wide production of footwear reached 21 billion pairs in 2011. When referring to the quantity of shoes produced, about 87% of the manufacturing takes place in Asia, mainly China (60.5%), followed by India (10.4%), Vietnam (3.8%), Pakistan (1.4%), and Bangladesh (1.3%). South America accounts for 5% of global production, 3.8% of which comes from Brazil. The European footwear production accounts for

approx. 3% of the world total, followed by the North America (2%). Africa shows a slight increase in the production (currently 3%) with respect to previous years. The only European country included on the top-ten list is Italy, with an overall share of 1% of world production. Figure 2 shows the production distribution of the top-ten list countries.

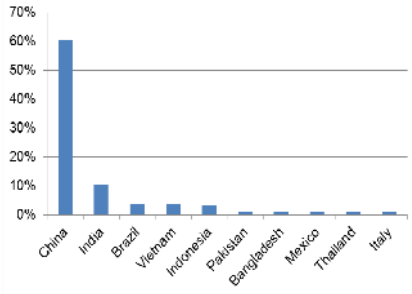

Figure 2. Top 10 of 2011 world footwear producers (volume)

In 2011 Asia was also the biggest consumer of footwear volume accounting, for 47% of world total, followed by Europe (21%), North America (17%), South America (8%), Africa (6%), and Oceania (1%). China accounts for 15.9% of global footwear apparent consumption (in volume), followed by the United States (12.9%), and India (12.7%). As is evident on the Figure 3, apparent consumption of footwear in Germany is the highest in Europe, representing 2.5% of global consumption.

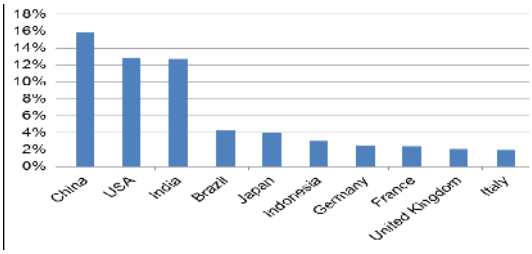

Figure 3. Top 10 of 2011 world footwear consumers (volume)

Footwear is an active product on the international markets, being one of the most widely traded and universally used commodities in the world. The level of international trade has risen steadily from 63.5% of industry revenue in 2006 at $75.2 billion, to $83.7 billion in 2010: this represents an annual average rise of 3.3% (Eurostat). The intra-European value of footwear trade corresponds to 35% of world total value, and 83% of overall European export. This is followed by Asian exports to North America and Europe, which represent respectively 19% and 17% of the world total. Intra-Asian exports, at 13%, are also very significant. On the other hand, European exports to Asia and North America represent only 3% and 2% respectively, of the world total. At 2% of world total, Asian exports to Africa is the only other flow exceeding 1% (APPICAPS). Exports from China have increased as the major international footwear companies have outsourced their production to take advantage of the lower labour and production costs. Asia dwarfs all other continents as a footwear exporter, with 84% of the world total volume. Europe is a distant second, with 11% of global export share. Europe leads the

ranking of world importers. However, after reaching a maximum of 44% in 2008, its share of the world total volume has been declining for the last three years to 40%. At the other end of the table, Africa's imports have been growing steadily over the last decade, currently representing approximately 7% of the world footwear trade volume.

European Footwear Market

Southern Europe, especially the Mediterranean region, is the main European footwear manufacturing area. Italy, Spain, Portugal and Romania together represent approximately 76% of the overall European production value, and 72% of production volume in 2011 (APPICAPS). The European footwear industry dominates production of high quality products in the medium to elevated price category. The average European production price has increased from 21.39 EUR in 2007 to 25.65 EUR in 2012. Because it has the highest share of the European market value (48%) and volume (34%), Italy leads the EU27 in manufacturing medium to highly-priced shoes. Romania, Bulgaria, Hungary, Slovakia have also recorded a decrease in production. Despite benefiting from increased market demand due to the market extension after the EU entrance, they competed poorly against Asian suppliers, many of whom have both lower cost bases and are technologically well developed (Eurostat).

Table 2. Top-12 of European producers (EUR millions) (APPICAPS)

	2007	2008	2009	2010	2011	Production share of 2011	Growth 2007-2011
EU27	13 838	12 898	11 218	11 772	12 951	100%	-6%
Italy	6 364	6 196	5 304	5 426	6 262	48%	-2%
Spain	1 836	1 707	1 454	1 476	1 563	12%	-15%
Portugal	1 137	1 117	1 094	1 204	1 270	10%	12%
Romania	1 017	877	716	756	793	6%	-22%
Germany	603	602	547	595	643	5%	7%
France	807	257	429	412	421	3%	-48%
Poland	351	354	269	298	307	2%	-13%
Slovakia	276	217	178	206	254	2%	-8%
Austria	187	209	224	252	248	2%	33%
UK	209	212	140	219	207	2%	-1%
Hungary	133	133	127	133	158	1%	19%
Finland	124	126	103	129	134	1%	8%
Others	793	892	633	665	690	5%	-13%

European footwear production experienced an overall decrease of 22% volume and 6% value within the last 5 years, particularly in Italy, Spain, and Portugal, due to the economic recession and intense competition within the footwear industry. Notwithstanding the global footwear market redistribution, the top European producers have not changed much since 2002.

The last decade consumers' preferences oriented to "ecological leathers" (Adiguzel Zengin *et al.*, 2012; Mutlu *et al.*, 2014; Deselnicu, V. *et al.*, 2014; Crudu *et al.*, 2014; Deselnicu, D.C. *et al.*, 2014).

HOW TO APPLY FOR THE ECO-LABEL

In order to apply for the Eco-label, the submitting organisation must send a technical dossier which contains all the details of how the product meets the new criteria to an

Eco-labelling competent body. There is one in each EU member state, which will assess the dossier against the criteria and decide if the product meets the requirements. The Table 3 presents criteria which have to fulfill a product for awarding EU Ecolabel.

Table 3. Check-list (for a first assessment only)

Life Cycle Step	Criterion	Expectations
Materials (packaging)	Use of recycled material	• Cardboard boxes: 100% recycled material • Plastic bags: at least 75% recycled material or biodegradable or compostable
Manufacturing (processes and chemicals)	Limitation of water pollution	Treatment of tannery waste water: • <250mg COD/L of water discharged if released directly into fresh water. • In line with minimum community requirements according to Council Directive 91/271/EEC if released into municipal waste water treatment plant/facility • Cr (III) < 1mg/L
Manufacturing (processes and chemicals)	Reduction of air pollution	• Volatile Organic Compounds (VOC): VOC < or equal to 20 to 25g/pair (according to type of footwear)
Manufacturing (processes and chemicals)	Reduction of water consumption	Limits to water consumption for the tanning of hide and skin: • Hides < 35m3/t • Skins < 55m3/t
Manufacturing (processes and chemicals)	Energy consumption	Information shall be declared
Manufacturing (processes and chemicals)	Exclusion of the use of substances harmful for health and the environment	• Pentachlorophenol, tetrachlorophenol and certain azo dyes excluded • C10-13 chloralkanes excluded from leather, rubber or textile components • Certain N-Nitrosamines excluded from rubber • No dyes meeting the criteria for classification as carcinogenic, mutagenic, toxic to reproduction, hazardous/dangerous to the following R-phrases: R40, R43, R45, R50, R51, R52, R53, R60, R61, R62, R63 or R68 (or any combination) shall be used • APE and PFOS shall not be used • Phthalates classified with the phrases: R60, R61, R62, R50, R51, R52, R53, R50/53, R51/53, R52/53 in accordance with Directive 67/548/EEC • Only biocidal products products containing biocidal active substances included in Annex IA of the Directive 98/8/EC shall be allowed for use • DNOP, DINP, DIDP are not permitted in the product
Use	Performance and durability	• Occupational and safety footwear must carry the EC mark (Dir. 89/686/EEC). • Other footwear must be tested for the following parameters: uppers flex resistance, uppersole adhesion, uppers tear strength, outsoles flex resistance, outsoles abrasion resistance, outsoles tear strength, colour fastness of the inside of the footwear
Use	Advice to consumers	• If the shoes have been treated to improve their water resistance, no further treatment required • Invitation to repair and recycle the product when possible

End of life	Limitation of toxic and other residues in the shoes	• For shoes made of leather, there shall be no Cr(VI) in the final product • As, Cd and Pb shall not be detected in the final product. • Formaldehyde: - in textile: not detectable - in leather: < or equal to 150ppm • No electric or electronic components

CONCLUSIONS

The EU Ecolabel is a voluntary scheme, which means that producers, importers and retailers can choose to apply for the label for their products. The scheme is designed to encourage businesses to market products and services that are kinder to the environment, as well as providing guidance for people seeking safer and more sustainable consumption.

The EU Ecolabel logo on Footwear tells you: i) Limited water pollution during production; ii) A reduction of emissions of volatile organic compounds during production; iii) The exclusion of substances harmful for the environment and health; iv) Limited residues of metals and formaldehyde in the final product; v) The use of recycled packaging; vi) The careful control of different aspects of durability.

Excluded or limited substances (non-exhaustive list): i) Exclusion of certain azo dyes; ii) Exclusion of PVC (except recycled PVC for outsoles); iii) No arsenic, cadmium and lead in the final product; iv) Limited use of formaldehyde and hexavalent chromium.

Acknowledgements

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NEW APPROACH RELATED TO THE EMERGING RISKS GENERATED IN THE OCCUPATIONAL ENVIRONMENT IN THE PROCESS INDUSTRIES

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Working environments are continuosly changing under the influence of new technologies and of shifting economic, social and demografic conditions. In this context, the Community strategy on health and safety at work called on the European Agency for Safety and Health at Work to set up an European Risk Observatory to anticipate emerging risks (any risks that are both new and increasing) in the world of work, in order to ensure high levels of safety and health at work The emerging risks were identified by means of the Delphi method. (that is based on an iteration process in which the results of the previous rounds are fed back to the experts for new evaluation). The experts invited to participate in this survey covered 27 European country and the USA. The „top" emerging risks agreed by experts in the process industries are nanoparticles, diesel exhaust, epoxy resins and isocyanates, vibrations, thermal discomfort, new technologies (complex human-machine interfaces, automation), repetitive work, new/ precarious forms of employment contract, job insecurity, work intensification and outsourcing. European Risk Observatory focus on emerging risks in order to ensure, in the future, a high level of safety and health at work.

Keywords: emerging risks, industry.

INTRODUCTION

Because the world of work is constantly changing, multiple exposures is increasing and each year approximately 170.000 workers die in the EU-27 of the consequence of their work, according to estimates from the International Labour Office. As many as 160.000 fatalities can be attributed to work-related diseases (Ossian, 2009). The implementation of the REACH Regulation should encourage the industry to develop safer work environment as well as generate information on the risks in the occupational environment and the means of managing these risks and hence contributing to the improvement of workers' protection. The Community strategy on health and safety at work 2002-2006 called on the European Agency for Safety and Health at Work to set up a risk observatory and to „anticipate new and emerging risks" in order to tackle the continuosly changing of the occupational environment and the new risks and challenges it brings (http://hwi.osha.europa.eu). Between 2002 and 2006, the Agency took the first step towards establishing an European Risk Observatory. Four expert forecasts have been carried out through questionnaire-based survey following the Delphi method on emerging risks related to occupational safety and health on physical, biological, chemical and psychosocial risks. In total, 520 experts from 27 countries and one international organisation were invited to participate in the survey. The results of this expert survey on emerging risks should be seen as a basis for discussions to set priorities to manage these new risks in the occupational environment.

METHODOLOGY

Concept of Emerging Risk

An emerging risk can be defined as any risk that is both new and increasing (http://www.inspectmun.ro/Ziua%20SSM%20index%202010/Ziua%20SSM.html).

By **new** is meant that:

- the risk was previously unknown and is caused by new processes, new technologies, new types of workplace, or social or organisational change; or

- a long-standing issue is newly considered as a risk due to a change in social or public perceptions; or
- new scientific knowledge allows a long-standing issue to be identified as a risk.

The risk **is increasing** if:

- the number of hazards leading to the risk is growing; or
- the likelihood of exposure to the hazard leading to the risk is increasing (exposure level and/or the number of people exposed); or
- the effect of the hazard on workers' health is getting worse (seriousness of health effects and/or the number of people affected).

Factors Generating Emerging Risks

New and emerging risks for workers and employers arise from:

- the development of new substances or materials with modified composition, such as epoxy resins with enhanced properties;
- the development of new technologies such as nanotechnologies and conversion technologies;
- continuously changing workplaces and work practices (new forms of employment contracts or non–traditional employment practices as outsourcing, temporary work, part-time work, flexible work);
- changing work processes: work intensification associated with shift in production organisation modes towards automation, shift in work organisation towards greater autonomy and more worker responsability, greater individualisation of human resource management and change in work evaluation and control mechanisms.

Method to Identify the Emerging Risks: **Delphi Method**

The Delphi method is a methodology used widely to create foresight information on topics for which only uncertain or incomplete knowledge is available. This method is based on an iteration process with three survey rounds in which the results of the previous rounds are fed back and submitted again to the experts for new evaluation. Delphi process implemented for the expert forecast on emerging risk:

Expert identification

▼

SURVEY ROUND 1

(emerging risks were identified using questionnaires with open-ended questions regarding risks of the next 10 years)

▼

creation of a list of emerging risks

▼

SURVEY ROUND 2

(validation of issues identified in round 1) The participants rated each item on a five-point Likert scale (strongly agreed as emerging, agreed as emerging, status undecided, agreed as non-emerging, strongly disagree as emerging)

▼

prioritised list of emerging risks (complemented by new added risks) drawn up based on the mean values (MV) of the item rating and the standard deviation (SD)

▼

SURVEY ROUND 3

(final consultation on prioritised list of emerging risks using the same five-point Likert scale used in the second round)

▼

EXPERT FORECAST

For each risk, the MV of the ratings and the SD were calculated. While the mean values help to prioritise the risks, the standard deviations reflect the level of consensus on one item among the respondents.The following areas have been defined for the interpretation of the MV, based on the definition of the five-point Likert scale used in the survey, and in order to have a reasonable balance of items between the different areas:

- the risk is *strongly agreed to be emerging* if MV > 4;
- if 3.25 < MV ≤ 4 means that the item is considered to be an *emerging risk*;
- when 2.75 ≤ MV ≤ 3.25 the status of a risk is regarded as *undecided*;
- when 2 ≤ MV < 2.75 the risk is regarded as *not emerging*;
- if MV<2 the risk is *strongly disagree as emerging.*

EMERGING RISKS IDENTIFIED BY UE EXPERTS IN THE PROCESS INDUSTRIES

Emerging Chemical Risks

The expert forecast identified eight risks **strongly agreed as emerging (MV > 4),** namely: nanoparticles and ultrafine particles; the risks resulting from the poor control of chemical risks in small and medium enterprises (SMEs); outsourced activities performed by subcontracted workers with poor knowledge of chemical risks; the increasing use of epoxy resins; the exposure to dangerous substances in the treatment of domestic, clinical and industrial waste; dermal exposure leading to skin diseases; diesel exhaust; isocyanates.

Short comments on the strongly agreed as emerging chemical risks in the process industries.

Nanoparticles (NPs) (MV=4.60)

Nanoparticles (NPs) (MV=4.60) is the main *strongly agreed as emergent risk*. Key applications of nanotechnology in the process industries include the chemical industry (manufacturing of the paints, pigments and other covering materials), textile industry (manufacturing both intelligence military and civil clothes) and construction material industry (cement manufacturing). *The risk management* regarding the NP exposure is not satisfactory due to the fact that there is insufficient knowledge and data concerning nanoparticle characterisation, detection, measurement, toxicology and fate in humans and the lack of easy-to-use, portable devices for measurement of nanoparticles in the air and therefore lack of exposure information. The main preventive measures include conventional ventilation, engineering control and filtration approaches. Collective and personal protective equipment should be evaluated and improved for reducing workplace exposures to NPs (DEFRA, 2005; NIOSH, 2005).

Allergenic and Sensitizing Agents: Epoxy Resins and Isocyanates. Dermal Exposure

The prioritised list of the strongly agreed to be emerging risks is presented in Table 1.

Table 1. The prioritised list of the strongly agreed as emerging risks of the allergenic and sensitizing agents

Allergenic and sensitizing agents	MV	SD
Epoxy resins (e.g. manufacturing in chemical industry)	4.14	0.743
Dermal exposure leading to skin diseases.	4.11	1.027
Isocyanates leading to allergic reactions: exposure occurs not only at the production stage (the chemical industry) but also during further processing (e.g. thermal or chemical degradation of polyurethane, grinding and welding of products containing polyurethane)	4.02	1.067

Skin diseases are caused both of sensitizing agents (Cr, Ni, Co, epoxy resins, natural rubber protein, pains) and irritative effect agents (cleaners).

The main protective measures include *to avoid contact* with skin, whenever possible, *replace* harmful epoxies by alternative epoxy systems with reduced risk of sensitization, provide *ventilation* to prevent airborne dermatitis, *wear protective clothing*, particularly effective gloves (e.g. fluorinated rubber gloves), skin protective spray, *courses* of occupational safety and health, establishing of occupational exposure limits for all ingredients of plastics.

Diesel Exhaust (MV=4.02)

The International Agency for Research on Cancer, (IARC) clasified diesel exhaust as „probable cancerigen to humans" and the European Agency for Safety and Health at Work agreed this risk to be strongly emerging. Diesel exhaust is made up of a complex mixture of thousands of gases, vapours and fine particles; the major components are carbon dioxide, carbon monoxide, nitrogen dioxide, nitric oxide, particulate matter and sulphur dioxide (Kittelson, 1998). All workers in the process industries which operate diesel engines are exposed to diesel exhaust. *Risk management and the protective measures* include the use of modern, low emission engines; low sulphur fuel; appropriate exhaust after-treatment devices such as filters and oxidation catalysts; ventilation; closed, environmentally-conditioned cabs; diesel engines should be appropriately operated and maintained.

Emerging Physical Risks

The strongly emerging physical risks identified in the process industries are vibration, thermal discomfort and complexity of new technologies and human-machine interfaces.

Risks Related to Vibration

The risks of vibration both to the handarm and to the whole-body systems have gained more attention with the European Directive 2002/44/EC. They are also perceived as emerging as the use of transportation systems and of industrial technologies grows and the working population exposed increases. The physical risks identified in the forecast reflect a growing concern for multi-factorial issues, e.g. combined exposure to vibration, awkward postures and muscular work (see Table 2).

Table 2. Prioritised list of the risks (*strongly emerging*) related to vibration in occupational environment

Risks related to vibration	MV	SD
Combined exposure to vibration and awkward postures	4.56	0,629.
Combined exposure to vibration and muscular work	4.38	0.619

Other risks are related to combined exposure to vibration and poor ergonomic design (e.g. poor seat support for the lumbar spine), combined exposure to vibration and unfavourable environmental factors (e.g. low temperature, exhaust emissions), combined exposure to vibration and dangerous compounds.

Occupational exposure circumstances in the process industries include the work to concrete mixers (construction material industry), to mechanic looms (textile industry), to forge, pneumatic hammers and to press.

The main *protective measures* are related to technical measures (e.g. the installation of damping elements) and organisational measures (e.g. proper maintenance of equipments, improvement of the work programme a.o.).

Thermal Discomfort (MV = 4.40)

These type of risks were identified in the construction material industry (manufacturing of cement, lime, brick, ceramic materials, terra cotta a.o.), oil distillation, glass manufacturing (hot microclimate). The food industry use especially the cold microclimate (activities of food freezing). Special protective clothes causing thermal stress represents today a problem insufficiently tackled.

Complexity of New Technologies and Human-Machine Interfaces (MV = 4.35)

If the design of the interface does not take into consideration the cognitive processes involved when operating such a system, the mental and emotional demands on the operator is higher. Hence a potential increase in the incidence of stress, human errors and accidents. *The risk management* consists of the determination of a maximum number of function units that an operator can handle without his mental workload, so decreasing the risk of accidents, the ergonomic design of the joystick, its compatibility with the machine response and the positioning of the functions on the device.

Emerging Psychosocial Risks

The list of 10 most important emerging psychosocial risks identified in European Agency for Safety and Health at Work' survey is the following (http://riskobservatory.osha.europa.eu):

- precarious contracts in the context of unstable labour market (MV=4.56, SD=0.51);
- increased workers' vulnerability in the context of globalisation (MV=4.38, SD=0.72);
- new forms of employment contracts (temporary-work, part-time, telework, mobile-workers, day-hire, on-call) (MV=4.25, SD=0.68);
- feeling of job insecurity (MV=4.25, SD=0.77);
- ageing workforce (MV=4.19, SD=0.54);
- long working hours (MV=4.13, SD=0.62);
- work intensification (MV=4.07, SD=1.03);
- lean production and outsourcing (MV=4.05, SD=0.68);
- high emotional demands at work (MV=4.00, SD=0.52);
- poor work – life balance (MV=4.00, SD=0.73).

One can see that all the identified risks are *strongly emerging*. Not only the industrial sector is characterised by this type of risks, but also all sectors of economic activity (health, education, wholesale and retail trade, public administration, immovable and bussines activities and other service sectors).

The risk management and *preventive measures* are related to start prevention programmes focusing on communication on health and safety or training and instruction on general and job-specific safety and health practices. With regard to *shift work*, night work should be reduced as much as possible; a semi-rapid to rapid rotation with two to four similar shifts in a row is preferable to slow rotation. A later start of the morning shift is also preferable. The resting period between two or more night shifts and the day shifts should be at least 56 hours (two complete nights). The duration of shifts should not exceed 10 to 12 hours. To prevent the job insecurity, the company management has to inform employees in good time about the planned changes, even if this information might be painful, because a realistic information helps employees to adapt to the situation. The company management has also to use the services of outplacement agencies, which through their job advisors, can help employeer choose a new job. Also, a better work design can result in the *reduction of work intensity*. *Age management* is related to an ergonomic workplace design, an age-appropriate job design, fostering healthy work

processes, reducing time pressure, support the intergenerational treansfer of know-how in companies. *Violence and bullying management* includes avoiding deficiencies of the design of the job (e.g. adequacy of workers'workload, demands and control, elimination of conflict); maintaining good quality leadership and management systems (e.g. recognition of conflicts and handling them adequately, managing information well); and good management of discrepancies, complaints and conflicts (Sterinman, 2006).

The employees should not constitute a collection of individuals working nearby one another, but a group where solidarity has evolved and where feelings of trust connect the individuals. Over the course of time, this leads to group-specific structures of mutual help and cooperation, helping the company.

CONCLUSIONS

New work situations existing in present (the introduction of new technologies, substances and work processes, changes in the structure of the workforce and the labour market and new forms of employment and work organization) bring new risks and challenges for workers and employers. In this context, the European Agency for Safety and Heath set up an European Risk Observatory to identify emerging risks related to occupational safety and health (OSH), using expert forecast. The expert forecast on emerging OSH risks were reached through questionnaire-based survey following the Delphi method.

This work shows, first time in our country, the strongly emerging risks identified by the experts in the process industries, namely, chemical risks (nanoparticles, allergenic and sensitizing agents: epoxy resins and isocyanates, diesel exhaust), physical risks (vibration, thermal discomfort and complexity of new technologies and human-machine interfaces) and psychosocial risks (precarious work contracts, new forms of employment contracts, feeling of job insecurity, long working hours, work intensification, high emotional demands at work a.o.).

The work emphasizes the main preventive measures and means of managing these risks, which can ensure in the future high levels of safety and health at work.

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THE IMPORTANCE OF TURKISH LEATHER SECTOR IN EUROPEAN UNION MARKET FOR RAW AND FINISHED LEATHER

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Because the effects of globalization and intense competition are deeply felt nowadays, the businesses which want to maintain their existence, expand into foreign markets and increase their participation in the international activities. The internationalization process and the assessment of the factors affecting this process have great importance for the businesses with this thought. In 2013, the world economy generally showed a yield below the average. Throughout the year, the growth rate of developing countries has been a slowdown while the economies of developed countries were slowly recovering. European Union (EU) consisting of 28 member countries met in order to keep peace and to stand economic and social improvement has become a big power. Trade realized with the EU countries is very important for the leather sector which is one of the leading sectors of Turkish Economy and also has a very important share in the economy. When considering that the intensity of foreign trade with EU countries is based on Customs Integration, it should be noted that the EU norms will be the reference for the sector exporters. When we have a look at the foreign trade of Turkish leather sector in terms of the export and also import values, it can be easily seen that the trade with EU countries is very extensive. The EU countries with the features of higher income per person and being open to consumption, is the market where is held 33.9 % of our total leather exports. It is a very important market for Turkish leather sector. However, in such a large market, a number of criteria that need to be considered should not be ignored. In this study, the EU share of Turkish leather sector in recent years is investigated. However, Turkey – EU relations in terms of Turkish leather sector are examined, export and import data of the sector are also considered by years and listed among EU member countries. In the last part of the study, The problems experienced with EU on foreign trade and the criteria which should be taken into consideration, are stated.

Keywords: European Union, leather and leather products, export-import.

INTRODUCTION

Economic developments in Turkey and the world continue at a dizzying speed. Today, with the increasing world trade volume and more severe competition, the efforts of the companies have rapidly increased in order to rise their market share. To survive in this competitive environment is bound to be successful in the international area. The industrialized and newly industrialized countries, which aim to get rid of this situation with a minimal loss, have begun to give more importance to their economic security. In the progress of globalization in the international trade, it is observed that the obstacles such as the restrictions of goods and quantitative have decreased but the regional integrations have strengthened. The positions of the countries have changed by placing in global organizations like “World Trade Organization” or participating in regional entities like “the European Union” and “Customs Union”. In this context, the EU is appeared as the most important economic integration for European countries (DPT, 2013).

The decision of the Customs Union taken by Turkey-EU Association Council has been the most important development affecting the entire economy after the liberalization of Turkey’s economy in the 1980s. The Customs Union which has led to several changes on the legislation of Turkey trade, competition and policies, has created both new opportunities for the Turkish economy and also the factors that are required to effort.

Within the dynamic and static effects, Customs Union has become inevitable for the future of the Turkish economy in the period after the Customs Integration which was built on the 1st January 1996 with the EU (Uyar, 2000). Then, Turkey, in December 1999, was accepted to the European Union as a candidate country.

The countries that are not members of the European Union have become nervous at first but then over time they have begun to take advantage of the Union as a tool in their economic and social policies in order to develop their countries. In this progress, while were leaving the "protective- closed economy" perspective and leading to the "open-competitive economy" perspective, the EU countries were having new policies on the issues such as production, marketing, product and technology developing in order to increase their competition capacity (Kutlu, 1998).

Undoubtedly, the effects of all these developments were observed on Turkish Leather Industry. According to the figures obtaining from Turkey Exporters Assembly Records, as seen in Table 1 below, Turkey's overall export in the period of January-December 2013 remained the same when compared to the previous year and was realized as 151.7 billion dollars. In the same period, Turkish leather and leather products exports rose to 1 billion 901 million Dollars with an increase of 16.4%. Thus, Turkish leather sector's share in Turkey's total exports became 1.3% with the realized export as 1 billion 901 million Dollars in the period of January-December 2013. This rate stood at level 1.1% in 2012 (ITKIP, 2014).

Table 1. The Share of Leather and Leather Products Within Turkey's Total Export

Unit 1000 $	Jan.-Dec. 2012	Jan.-Dec.2013	12/13 Change %
Turkey's Total Export	151.695.192	151.707.002	0.0
Leather and Leather Products Export	1.633.988	1.901.404	16.4
The Share of Leather and Leather Products Within Turkey's Total Export (%)	1.1	1.3	

THE IMPORTANCE OF THE EU MARKET FOR TURKEY

Since the 1980s, with the "export-based growth" perspective, Turkey has directed to foreign trade and Turkey's export products have shifted from agricultural products to industrial products. Furthermore, Turkey's export has been increased by the foreign trade policies applied until the 1990s. However, the developments in Turkey and the world economy were reflected in foreign trade and the performance shown during the 1980s failed in the 1990s. Thus, the export ratio unfortunately remained at the very back in the 2000s (Ener, 2003).

Among the economic integration movements, The EU integration has the greatest impact in terms of Turkey. With the Customs Integration which was built on the 1st January 1996 with the European Union, Turkey has obliged to consider European norms in deciding not only on foreign trade, but also economic, law and even political issues. The steps taken towards to both regional and global integration directly affect Turkey. In particular, the World Trade Organization (WTO-WTO) which has begun to play an

important role in globalization closely follows Turkey's agenda. Besides, taking an active role in this organization has a great importance for our country (Nebioglu, 1997).

Turkey carries out the strategic studies and policies in order to enhance export, ensure the balance of export-import and also to get a larger share from the EU market. However, the desired stability could not be achieved in Turkey because of the economic and political developments lived with the EU which is placed in the global market. When analyzed Table 2, the importance of the EU is clearly seen in terms of export and import values. The increase of export and import should be carefully evaluated in the period after the Customs Union (DTM, 2003).

Table 2. The Share of The EU Countries Within Turkey's Foreign Trade

	Export	Import	Export	Import	Foreign Trade Balance	Exp. / Imp.
	EU(27) / Turkey(%)	(Million $)	EU(27)/ Turkey (%)	(Million $)	(Million $)	(%)
1999	58,0	15,424	55,4	22,530	-7,106	68,5
2000	56,4	15,664	52,3	28,527	-12,862	54,9
2001	56,0	17,546	47,9	19,823	-2,287	88,5
2002	56,6	20,415	49,8	25,689	-5,274	79,5
2003	58,0	27,394	50,7	35,140	-7,746	78,0
2004	57,9	36,581	49,3	48,096	-11,515	76,1
2005	56,3	41,365	45,1	52,696	-11,331	78,5
2006	56,0	47,935	42,5	59,387	-11,452	80,7
2007	56,3	60,399	40,2	68,395	-7,996	88,3
2008	48,0	63,390	36,8	74,408	-11,017	85,2
2009	46,0	47,013	40,1	56,509	-9,496	83,2
2010	46,3	52,685	38,9	72,180	-19,494	73,0
2011	46,2	62,347	37,8	91,128	-28,781	68,4
2012	38,8	59,241	37,0	87,446	-28,205	67,7
2013	41,5	63,026	36,1	92,446	-29,420	68,2

Because the effects of globalization and intense competition are deeply felt nowadays, the businesses which want to maintain their existence, expand into foreign markets and increase their participation in the international activities. The internationalization process and the assessment of the factors affecting this process have great importance for the businesses with this thought (Ozdag, 2003). In 2013, the world economy generally showed a yield below the average. Throughout the year, the growth rate of developing countries has been a slowdown while the economies of developed countries were slowly recovering. In this regard, the world economy especially the EU has struggled with the recession and unemployment problems of developed countries. Despite all the efforts of Turkey in order to strengthen the economy in recent years, the problems have been basically continuing for the economies of developing countries especially in the EU. The share of EU countries within Turkey's foreign trade is presented in Table 3.

TURKISH LEATHER SECTOR IN THE EUROPEAN UNION MARKET

Leather and leather products exports was realized as 1,9 billion dollars by increasing 16.4% in 2013. If we evaluate this figure in terms of Turkey, our export

realized with the EU countries, which is the largest market of us for raw and finished leather, rose to 644, 4 billion dollars with an increase of 18% in the period of January-December 2013. As seen in Table 3 and Table 4, Italy, which has a share 6.9 % of the export among the EU countries that is a total of 131.6 million dollars. That is followed by Germany having a share 6% of the export which refers to 114.6 million dollars. On the other hand, France, which has a share 3.8 % of the export, and England are the largest buyers following the others. During this period, the export of leather and leather products has increased by 3.6% in Italy, 8.2% in Germany whereas the export has increased by 14.3 % in the UK . Only France has fallen by 0.7% (ITKIP, 2014).

Being so close to Turkey, having higher living standards than us, our best quality production and timely delivery of orders are the underlying factors why the EU countries have a large share in our foreign trade. In addition, because of environmental pollution, increased production and labor costs in industry and such reasons occurred in the leather sectors of the developed countries, the beginning of the leather sector transition into the less developed and developing countries are the other effects for Turkey to become an important leather market in the EU countries. However, despite being so close and producing best quality goods, it is observed that only the export realized with Germany among country groups has been remarkable (DTM, 2013). However, with our advantages mentioned above, it is possible to realize high export to the other EU countries. Moreover, through trade delegations and market research, the presentation of our products and opening stores and show rooms in certain points of these countries may be useful in order to improve the trade (DTP, 2013).

Table 3. Turkey's Export Records of Leather and Leather Products Within Countries

	2012 Dec. 1000 $	2013 Dec. 1000 $	Change (%)	Share (%)	2012 Jan.-Dec 1000 $	2013 Jan-Dec. 1000 $	Change %	Share %
EU Countries	42.806	54.160	26.5	30.2	545.998	644.421	18.0	33,9
Former USSR Countries Total	73.963	79.245	7.1	44.1	567.262	622.439	9.7	32,7
Middle East Countries Total	15.245	16.880	10.7	9.4	174.134	225.900	29.7	11.9
Asia Total	14.074	14.107	0.2	7.9	133.390	181.223	35.9	9.5
Turkish Republics Total	6.020	4.650	-22.8	2.6	79.371	78.663	-0.9	4.1
African Countries Total	4.984	4.885	-2.0	2.7	61.708	65.188	5.6	3.4
United States Total	2.854	3.461	21.3	1.9	38.827	42.199	8.7	2.2
Other European Countries Total	2.499	1.280	-48.8	0.7	25.302	31.338	23.9	1.6
Free Zone	755	613	-18.8	0.3	5.558	6.966	25.3	0.4

Total Leather and Leather Products Export Records Total	162.995	179.531	10.1	100.0	1.633.988	1.901.404	16.4	100.0

Table 4. The Leather and Leather Products Export to The EU Countries

Unit: 1000 $	2012 Jan.-Dec. 1000 $	TOTAL LEATHER SHARE %	2013 Jan.- Dec. 1000 $	TOTAL LEATHER SHARE %	2012/2013 CHANGE %
GERMANY	105.945	6.5	114.633	6.0	8.2
ITALY	127.083	7.8	131.602	6.9	3.6
LITHUANIA	3.620	0.2	74.860	3.9	1968.1
FRANCE	72.590	4.4	72.102	3.8	-0.7
ENGLAND	63.027	3.9	72.070	3.8	14.3
BULGARIA	21.087	1.3	22.354	1.2	6.0
SPAIN	23.307	1.4	21.033	1.1	-9.8
NETHERLANDS	19.057	1.2	18.726	1.0	-1.7
ROMANIA	13.676	0.8	18.273	1.0	33.6
AUSTRIA	19.236	1.2	18.211	1.0	-5.3
DENMARK	13.452	0.8	12.229	0.6	-9.1
BELGIAN	11.753	0.7	11.673	0.6	-0.7
SWEDEN	7.398	0.5	10.592	0.6	43.2
GREECE	10.168	0.6	10.078	0.5	-0.9
ESTONIA	5.332	0.3	7.891	0.4	48.0
POLAND	9.626	0.6	7.784	0.4	-19.1
CZECH REPUBLIC	4.461	0.3	4.198	0.2	-5.9
SLOVAKIA	2.346	0.1	3.418	0.2	45.7
FİNLAND	3.269	0.2	3.262	0.2	-0.2
PORTUGAL	1.782	0.1	2.533	0.1	42.1
HUNGARY	2.624	0.2	2.415	0.1	-8.0
CROATIA	673	0.0	1.746	0.1	159.3
LATVIA	3.184	0.2	852	0.0	-73.2
SLOVENIA	219	0.0	767	0.0	249.6
IRELAND	535	0.0	627	0.0	17.3
EU(27) TOTAL	545.998	33.4	644.421	33.9	18.0
Turkey leather and leather Products Export	1.633.988	100.0	1.901.404	100.0	16.4

The reasons of the increases and decreases related to the EU countries are stated as the return to Euros and the hidden inflation lived and consequently the consumption and market recession observed in Germany. With the image of fashion and brand Turkish leather and leather goods can prevent this market recession without compromising the quality. The increases in Spain and Italy are based on the orders directed by the best known brands of these countries to Turkey, relying on the product quality. Goods are always purchased just for the quality in the EU countries and primarily preferred

according to their origin and brands. In terms of the quality, Chinese goods are unlikely to compete with Turkish products in the EU market (Yonsel, 2011).

CONCLUSION

The EU market has always been an important market for Turkey. And the trade realized with the EU is indispensable for the leather sector which is one of the leading sectors of Turkish economy. Turkey, which is located close to the EU market, uses its geographical position as an advantage for Turkey's exports and imports. Despite radical changes since the Customs Union and the competitive pressures of the EU countries Turkey have been successful to resist them. Besides, it can be said that the Turkish leather industry owns a competitive, dynamic and flexible structure and is directed by a sophisticated understanding of entrepreneurship. Because it creates environmentally harmful waste, leather industry is kept ecologically under strict control. The EU countries pay much more attention to such criteria due to the environmental health.

European Union (EU) consisting of 28 member countries met in order to keep peace and to stand economic and social improvement has become a big power. Trade realized with the EU countries is very important for the leather sector which is one of the leading sectors of Turkish Economy and also has a very important share in the economy. When considering that the intensity of foreign trade with EU countries is based on Customs Integration, it should be noted that the EU norms will be the reference for the sector exporters.

The Turkish leather industry directing to the production with the clean technology will have a stronger chance to compete in the EU market in the future. When considered the density of Turkish leather foreign trade with the EU countries, it should be noted that the EU standards are the references for Turkish exporters. The competitiveness of the industry has been developed through the modernization investments realized by leather sector for the quality. Furthermore, the importance which is given to the quality concept by the companies in leather industry has increased.

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A THEORETICAL INSIGHT INTO THE BUSINESS PROCESSES FRAMEWORK

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The recent years have brought a plenty of researches and knowledge in the field of business process management as an effect to the interest on understanding and managing the enterprise functioning. Due to the growing importance of digital era and IT advance, enterprises striving to achieve competitive advantage are required to elaborate a common understanding of working environment by configuring their business processes. The paper aims to explore the core concepts embedded in the two well established frameworks for business processes: BABOK Guide (Business Analysis Body of Knowledge) and ISO 9001:2008 standard. The research methodology was consisted of a literature review on today's enterprise processes architecture that helps understand and organize knowledge about business processes models, followed by a comparative analysis of the frameworks chosen, from different views. The results capture the key differences and similarities between the frameworks and highlight the limitation of the BABOK Guide compared to ISO 9001:2008, with respect to business processes framework. However, regardless of the architecture, the business processes framework enables the development of the coordination mechanism of the processes relationships, diminishing the variance of input and output values with significant improvements on the predictability of process behavior. Finally, the authors share their view on how business processes framework is becoming a cutting-edge vehicle toward achieving the enterprise excellence, gaining thus the competitive advantages.

Keywords: business process management, business analysis, competitiveness.

INTRODUCTION

The business process management is a continuously growing body of knowledge since it helps to create a common understanding of enterprise functioning, representing the first step in automating the working environment. Regardless of the industry, the key challenge of any enterprise consists of defining the business processes framework in accordance with the given set of constrains including regulations, time, budget, and so on, towards meeting the stakeholders' needs and expectations, in a cost-effective manner. In this context, the scientific community has been striving to integrate the business processes framework with the developments of technological advance in an attempt to create an effective vehicle for gaining competitive advantage on business market.

With a high pace of growth, the process oriented enterprises are requiring an increased expertise in business process management since they are interested in adopting and leveraging different business processes frameworks. As consequence, business process management approach has become a powerful tool for any enterprise striving at gaining competitive advantage in these ever changing market requirements, (Mathiesen *et al.*, 2011).

In addition, business processes are seen as the capability of an enterprise to execute its strategy, based on a continuous approach to improve work processes in a bounded timeframe. Interestingly, the focus is on viewing business processes as a well-defined and properly managed corporate asset that requires specialized skills with respect to different roles of process owner, process analyst, and process architect, (Olding and Rosser, 2007; Bandara *et al.*, 2009).

In response to this growing need for a deeper understanding of the business processes, the research methodology has commenced with studying the most important models that proposed different views on business process frameworks. Within these circumstances, the scope of the comparative analysis was limited to the Business Analysis Body of Knowledge Guide (BABOK) and ISO 9001:2008 frameworks since they have gained remarkable attention, being the widely used models which allow the alignment of business goals to business strategy through the processes glue.

METHODOLOGY

There are a significant number of studies underway to formalize the various aspects of managing business processes. These efforts represent a maturation of the process movements of the 1990s that include Business Reengineering, Business Process Innovation, Six Sigma and Total Quality Management. Business Process Reengineering has proposed changing or entirely reconceptualization of processes by thinking in terms of comprehensive processes instead of changing in an incremental manner, (Hammer and Champy, 1993). Total Quality Management has established the processes required for managing the quality such as quality planning, quality control and quality improvement whereas Six Sigma has emphasized a mixed of process analysis and statistical quality control techniques to continuous process improvement in organization, (Pyzdek and Keller, 2010).

The evolution of IT has moved the focus on process modelling based on enterprise resources planning (ERP), work flow management, process modeling and simulating applications. These modelling techniques aim to assure the optimal convergence between the resources and the strategic direction needed to offer added value to the customers (Harmon, 2010).

As a result, the enterprises are typically interested in acquiring and using structured methods, methodologies and frameworks to better manage their business processes. According to scholars, a wide variety of business processes frameworks and models came under the broad umbrella of Business Process Management. The Association of Business Process Management Professionals, has been defined the Business Process Management (BPM) as a disciplined approach aims to identify, design, execute, document, measure, monitor, and control both automated and non-automated business processes to achieve consistent, targeted results aligned with an organization's strategic goals (ABPMP, 2009).

Another key underpinning that addresses the challenges of business processes frameworks is the widely accepted standard Business Analysis Body of Knowledge (BABOK Guide) developed by the International Institute of Business Analysis (IIBA), first published in 2006 and extensively revised and improved in 2009, IIBA (2009). By reflecting the current generally accepted practices with respect to the roles of process owner, process analyst or process architect, the BABOK Guide has proposed a systematic vehicle for creating, monitoring and sharing new knowledge in business processes area. It has defined seven knowledge areas focused exclusively on the business need and adding business value, as follows:

1. Business Analysis Planning and Monitoring: describes the processes required to complete the business effort.
2. Elicitation: describes the processes required to identify and understand the stakeholders' needs and concerns.

3. Requirements Management and Communication: describes the processes required to manage issues, conflicts and changes to the business solution scope.
4. Enterprise Analysis: describes the processes required to identify, refine and clarify the business need, and define a solution scope.
5. Requirements Analysis: describes the processes required to prioritize and progressively elaborate business solution requirements.
6. Solution Assessment and Validation: describes the processes required to assess, identify gaps and shortcomings in solution, and determine necessary workarounds of changes to the solution.
7. Underlying Competencies: describes the behaviors, knowledge, and other characteristics in support to an effective performance of business analysis.

In addition, the ISO 9001:2008 is also an outstanding standard that addresses the challenges of business processes frameworks, drawing valuable insights from the APQC's Process Classification Framework, APQC – PCF (2012). The PCF framework was developed by the American Productivity and Quality Center and enables to make objective comparison of organizational performance within and among organizations through an out-of-box thinking and to search for insights not typically found within intra-industry paradigms. Since its inception in 1992, the Process Classification Framework has been enhanced with updates inputs from a large number of industries and has created a common language by outlining all of the practiced processes in fourteen industries such as automotive, banking, consumer electronics, consumer products, telecommunication, etc. That's way, the business processes framework proposed by APQC represents a cross-industry high level business processes classification that organizes enterprise's processes into two groups with a twelve enterprise-level categories, as follows:

The operating processes group, aiming at setting, creating and fulfilling the stakeholders demand, is tightly connected to the enterprise value chain and comprises five operating processes: 1.0. Develop Vision and Strategy; 2.0. Develop and Manage Products and Services; 3.0. Market and Sell Products and Services; 4.0. Deliver Products and Services; 5.0. Manage Customer Service, APQC – PCF (2012).

The management processes group is focused on ensuring a coherent functioning of the enterprise by setting the goals and by enabling to achieve these goals based on providing capable resources: 6.0. Develop and Manage Human Capital; 7.0. Manage Information Technology; 8.0 Manage Financial Resources; 9.0. Acquire, Construct, and Manage Assets; 10.0. Manage Enterprise Risk, Compliance, and Resiliency; 11.0. Manage External Relationships; 12.0. Develop and Manage Business Capabilities, APQC – PCF (2012).

As reasoned earlier, the ISO 9001:2008 standard has taken advantage of the APQC's Process Classification Framework and has configured an interestingly processes framework by grouping business processes into four categories: Mission Management, Demand Creation, Demand Fulfillment, and Resources Management (Hoyle, 2009). Moreover, the ISO 9001:2008 standard has been rearranged and expended the business processes elements proving thus a context to the process management concept as a base to satisfy the stakeholders' demands using the available resources and constraints. The core concept of process management is referring to the well-known PDCA cycle (Plan-Do-Check-Act) develop by Deming and enhanced as DMAIC cycle (Define, Measure, Analyze, Improve, and Control) by Six Sigma in an attempt to make every employee responsible for process quality (Harmon, 2010).

As consequence, the business processes frameworks proposed by BABOK Guide and ISO 9001:2008 were selected for the critical analysis as they support the requirements of a wide range of stakeholders, being globally accepted and having high applicability in a wide range of industries.

RESULTS AND FINDINGS

As a result of the conceptual research in the scientific literature, the comparative analysis of the business processes frameworks proposed by the BABOK Guide and ISO 9001:2008 reveals the key similarities and differences, as presented in the table 1.

Table 1.The BABOK and ISO 9001:2008 frameworks – comparative analysis

Key similarities	BABOK Guide	ISO 9001:2008
Aim	Enterprise coordination	Enterprise coordination
Management process	Planning, executing, monitoring and controlling (PDCA cycle)	Planning, executing, monitoring and controlling (PDCA cycle)
Constraints	Limited resources	Limited resources
Implementation	Human resources	Human resources
Key differences	**BABOK Guide**	**ISO 9001:2008**
Structure	Knowledge areas that interconnect management and business processes	Five requirements classes with specific clauses and a subset of requirements
Terminology	Different terms used for describing the processes based on the appropriate knowledge area	Macro processes for describing the management process Micro or support processes for accomplishing the customer requirements
Analyzed entity	Internal customer	Internal and external customers
Key elements	Focus on business processes	Focused on management processes
Process components	Inputs, techniques, outputs	Process requirements
Responsibility	Business analyst	Management team

The underlying concept for the similarities between business processes frameworks is consisted of designing the coordination mechanism that enables a thorough understanding of business in terms of processes and interactions. This serves as a basis for defining the structure, policies and operations of any enterprise with positive impact on satisfying the stakeholders' needs in a sustainable way.

In regard to the coordination purpose of the enterprise functioning, each of the frameworks takes advantage of the management processes represented by the well-established Plan-Do-Check-Act cycle defined by Stewart, improved by Deming and promote by ISO 9000 family of standard (Hoyle, 2009).

The BABOK Guide and ISO 9001:2008 frameworks for the business processes are both dealing with the resources constraints such as materials, equipment, time, budget, and human resources knowledge and expertise, in defining the cost-effective solution for satisfying the business need or stakeholders demand.

Worthy to be mention are the concepts that capture the key differences between the BABOK Guide and ISO 9001:2008 frameworks. The structure of the content for the Business Analysis Body ok Knowledge encompasses the seven knowledge areas that define what should be done to understand the enterprise: business analysis planning and

monitoring, stakeholders requirements elicitation, requirements management and communication, enterprise analysis, requirements analysis, solution assessment and validation, underlying competences, IIBA (2009). Each knowledge area has defined the required processes to ensure the expected outcomes with the exception of underlying competences that provides only a description of behaviors, characteristics and personal qualities needed to support the practices of business analyzing.

As opposed to this, the ISO 9001:2008 consists of five classes of requirements on quality management and system development, management responsibility, resource management, product realization, and measurement, analysis and improvement as a means of achieving sustained success in a complex and changing market. Each class comprises a subset of requirements triggering the definition and execution of specific processes, (Hoyle, 2009).

As far as the terminology, the comparative analysis of process thinking gets out a roughly process configuration for the BABOK Guide compared to ISO 9001:2008 that proposes a highly structured process architecture composed of macro or main processes from the enterprise value chain and micro or support processes needed for satisfying the requirements of the internal customer. That's way, the BABOK Guide do not clearly structure the management and the support processes, being in fact grouped and getting different notations depending on the knowledge area in question. Anyway, the BABOK Guide explicitly defines the business processes with a strong focus on inputs, outputs, and expected techniques.

An interesting aspect is referring to the accountability for improving the enterprise performance. The BABOK points out the responsibility of business analyst in understanding the structure, policies and operations to define solutions that enable the achievement of enterprise goals whereas the ISO 9001:2008 accounts for the management responsibility in building an working environment focused on employees commitment towards accomplishing the performance goals.

CONCLUSIONS

Undoubtedly, the business processes configuration proposed by Business Analysis Body ok Knowledge has brought valuable insights to the business process management area and business practitioners but it has significant shortcomings arisen from its very narrow approach of business system. With a strong focus on capturing the processes architecture that enables developing only the internal optimal solution to satisfy the business need, the BABOK Guide proposes a suitable business processes model towards improving the internal performance of any enterprise.

The journey for enterprise's competitiveness has also brought to light a more complete business processes architecture captured by the ISO 9001:2008 standard characterized by a broader and deeper understanding of the enterprise functioning as a system. Through a cross-industry process viewpoint, the ISO 9001:2008 configures two specific processes in accordance to the output stakeholders: business management processes for satisfying external stakeholders' requirements and support processes aiming at satisfying the internal customers.

Finally, the enterprises striving to satisfy their stakeholders in an attempt to raise the competitiveness on the market can either go through a process of trial and error selected from a wide management body of knowledge, or take advantage of one or more management frameworks available that combine proven concepts and principles needed to develop the enterprise capability. That's way, the conceptual analysis of business

process frameworks highlighting their strengths and weaknesses may give a clearer perspective on the subjected area, serving as a basis for raising the ambition level of practice for enterprises aspiring to gain high competitiveness.

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ANALYSIS OF ERRORS IN THE MANUFACTURING USING DESIGN FOR SIX SIGMA. (DFSS)

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This paper uses the results of research in the field of automotive, manufacturing line process optimization. Achieving quality components with documentation, in line with the conditions of a large series and mass production, generates the need for an optimization process at a global level (logistics, manufacturing, assembly, sales), depending on the components 'magic triangle' quality costs and time. Simultaneously, an improvement (downwards) the error rate towards achieving manufacturing - assembly lines of robust products generates an improvement in the net value of production, an increase in the competitiveness of the company by increasing production capacity, labor productivity and delivery terms. DFSS is considered a way to improve the training process of product components, by diminishing the number of defects. It aims to improve the management of specific aspects of manufacture, reducing manufacturing risk by avoiding the use of bad or defective components, from the design stage of the process. DFSS was used to analyze the structure of functions of electronic parking brake fitted to the car (Electronic Parking Brake - EPB); model was used for analysis V or "cascade" each come with the design, to avoid errors, and especially to the structuring tasks, functions of each component in the system structure. DFSS implementation stages followed DICOV circle (Definition, Identification, Characterization, Optimization, Validation).

Keywords: functions of product, continuous improvement components, magic triangle, the car's electronic parking brake (EPB)

INTRODUCTION

Due to the significant economic potential for the prevention and reduction of production scrap in recent years, an increasing number of preventive quality management techniques have been used; the actual report addresses the Six Sigma method as part of the scientific research program.

Only those who can react in a short time to the customer changes in the life cycle of the product and who can can also show a higher quality of the product supplied, in parallel with the reduction of errors in production (achieving zero-defects), may invest on long term in crucial resources for product development.

The increasingly larger competition from the automotive market has caused many companies to seek a sustainable concept, through which company processes may be optimized in accordance with quality criteria.

Quality, cost and time are known in many areas as the "magic triangle" of product development, considering that these are key elements that ensure sustainable development in the industry.

In the automotive industry and particularly in the relationship with suppliers these factors are in the spotlight.

A superficial analysis highlights the fact that manufacturing of products at the quality level required to meet the requirements of beneficiaries with lower costs, generates a discordance between the required quality or requested quality, the time consumed for repair and the additional costs of bringing the product to parameters.

However, it can be shown that an improvement of the error rate leads to an improvement in the net value of the output. A strategy of "zero defects", increases the

competitivity of a company by the increasing production capacity, increasing employment and the decrement of manufacturing duration, ensuring compliance with/reducing of the delivery times.

ERROR ANALYSIS ON THE MANUFACTURING LINES

The goal of every product developer ist o detect in time production errors and also to establish the necessary measures to avoid such errors, so as to ensure the success of the finished product for the customer. About 70-80% of all faults in the structure of a product can be caused by deficiencies in planning, in which the greatest contribution lies in the research, development, and production-preparation sectors.

Discovering and fixing errors usually occurs in the late phase of production or even when the product arrives to the client. This means that the very late discovery of errors in the development product process generates higher costs to eliminate these errors. This phenomenon can be viewed by using the so-called magic triangle (Figure 1).

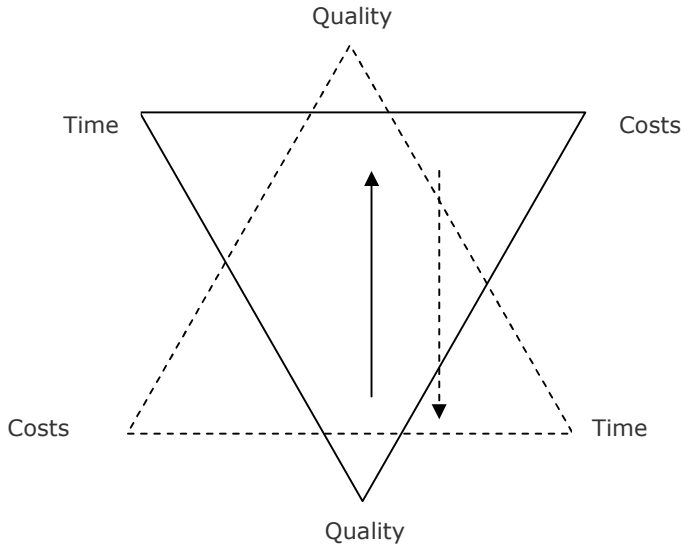

Figure 1 Triangle cost - quality - time (magic triangle)

From the figure it is observed that, if for a product there are high levels of quality required even in the early stages of manufacturing, any deviation from the required quality will be removed with low cost and in irrelevant time spans. However, if for a product low quality levels are required, deviations from quality will be corrected with additional consuming time and increased costs.

In practice, for preventing production faults along the lines of production, various methods can be applied. A comparison between the two methods highlights specific differences. Thereby:

The **Six-Sigma Method** used for the analysis of the basic method DMAIC (Define - Measure - Analyze - Improve - Control) to make the quality of existing processes measurable as well as of those resulting from their continuous improvement over time.

The DFSS Method uses the **DICOV** model (Define - Identify - Characterize - Optimize - Validate) to develop the product, to make it safer, more robust, beginning with the design and manufacturing phases. The differences between the objectives of the two methods is given by the following characteristics (Table 1).

Table 1. Objectives of Six Sigma and DFSS

No.	SIX SIGMA	DESIGN FOR SIX SIGMA
1.	It focuses on existing processes	It focuses on product development
2.	Optimizing production in order to create new value	Optimization of design, with strong emphasis on continuous innovation
3.	Company-specific requirements and customer requirements.	Future requirements at the company, taking into account the diversity of customer requirements
4.	Avoid the additional costs of deviations from the planned processes.	Avoid the additional costs of possible deviations on the entire manufacturing cycle of the product

The DFSS Method aims:

- A complete understanding of the products requirements, imposed requirements determined by the system in which it is integrated.
- Identification of areas with early stage design problems, and addressing solutions to ensure robust structure for the designed product.
- The products interface must to be clearly defined from the design stage.

For correlating product requirements with the requirements of production line capacity is frequently used in the V model known as model in cascade.

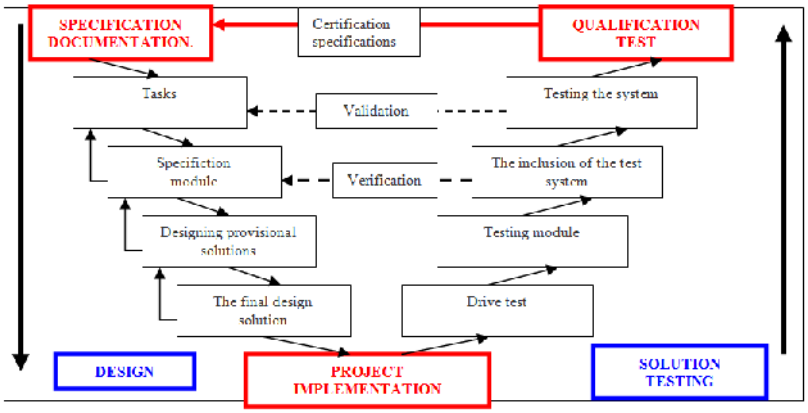

Figure 2. The Model in cascade

The Cascade Model

From Figure 2 it is seen that the Cascade Model has the advantage of a direct correlation between design activities, both at the system level and at the level of modules or specifications, related to such documentations specification purposes. At the same time each solution, before being made permanent, is tested in terms of the

parameters that must be provided. In designing the final solution, one starts from the product specifications required by documentation, and continues with the selection of modules according to the tasks resulted for each module; then continues with the choice of interim final solutions which, by combining functions, generates a final solution. So the design process follows a TOP – DOWN approach, namely from the product to the components, using optimization solutions that ensure objectives and ensure implementation possibilities in the manufacturing components line, modules and systems. After the implementing of the final solution, verification tests of the new elements introduced in the project are performed, validating the newly created module with respect to the design requirements, and after the qualification test pecifications required by the product documentation are certified. The staged testing process follos a BOTTOM-UP approach, in parallel with the top-down approach. Only after this test, one may pass to large serial production or to mass production under the condititions required by the documentation specifications.

This method has at least two advantages:

- it reduces implementation time of new solutions in the manufacturing,
- it eliminates possible errors that may occur after implementing the process solution through verification testing, validation taking place alongside with the design process.

Each of the activities of the cascade model are analyzed using the DICOV method (Definition, Identification, Characterization, Optimization, Validation); by using the method of analysis it is intended to ensure the optimal solution for the development of that activity so that further work can be carried out in the most favorable conditions, taking account of the requirements of the specifications in the documentation.

CASE STUDY - DFSS APPLICATION FOR THE ELECTRONIC PARKING BRAKE (EPB)

Electrically operated brakes of the type “duo servo” are used in combination with electronic parking brakes (EPB) braking systems. Parts of the brakes structure are safety pieces. Because of this, registration, listing and filing each operation of the production process and highlight errors / rejects is necessary. The error can be avoided by resuming the activity as specified in the budget documentation; the spoilage requires replacement of the defective part and its traceability analysis until the test. Documentation must exist to prove that during the production period the audit has been conducted and that the nominal parameters, provided in the documentation have been met.

EPB assembly line comprises assembly of the new posts, placed in a continuous stream; from each running one specific operation, as shown in Figure 3.

Operation # 1 is a manual operation combined with a robotic operation, which ensures logistic processes at the job 1.

Operation # 2 ensures printing of the code and batch of the finished manufacturing product.

Operation # 3 mechanical component assembly 2.

Operation # 4 mechanical component assembly 3 and lubrication.

Operation # 5 installing protection systems.

Operation # 6 installing electrical and electronic assemblies.

Operation # 7 # 8 End of Line(EOL) control and measurement of system parameters.

Operation # 9 visual check, packaging and storage.

The production quantity consists of 12,000 pieces/month, in a system consisting of two shifts of eight hours each; tact follows the line T=3.3 min/piece. This follows the implementation of the project, which at its time structured optimal solutions in terms of the modules selected and from the point of view of the specifications.

Under these conditions at the test operation EOL, during a work week a number 8 ... 10 exchanged defective products were noted. Applying DFSS operations # 7 # 8 and analyzing the causes which have generally found defects classified as "critical failures" that do not belong to management solutions, but are due to the poor structure of the components that enter into the structure of braking.

Given the annual production of about 150,000 units EPB/year, preliminary statistics have a value of 500 pcs. rejected items/year. Considering the correlation that exists between DPMO (Defects Per Million Opportunities) and Six Sigma function, it follows that for a production of one million units, the company is on a position close to the lower limit of 4σ, with about 3333 units produced which are considered critical flaws in terms of product quality.

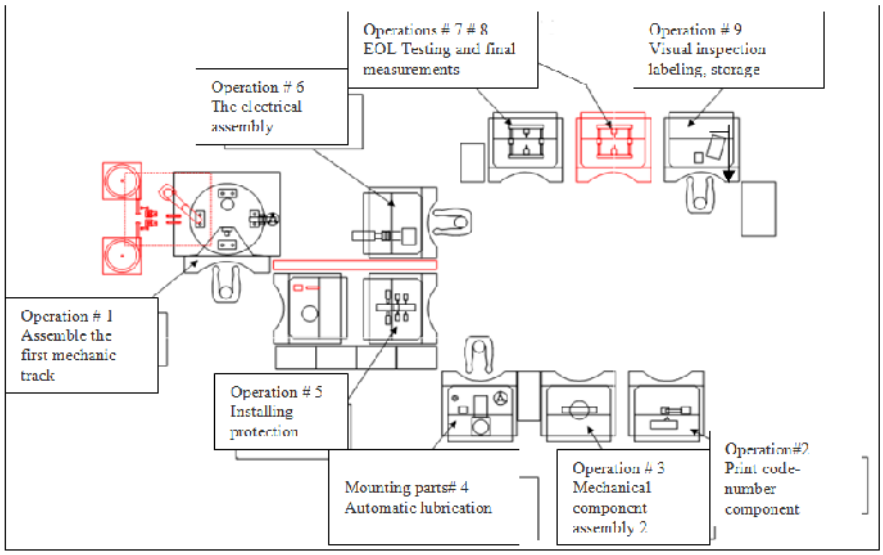

Figure 3 Assembly line components parking brake (EPB)

Given the connections between function Six-Sigma, DPMO and costs for improving product quality, costs of quality can be statistically determined (Table 2):

Tabel 2 Costs related to quality production

Level σ	DPMO	Costs related to quality
2	308 537 (Companies can not compete on Marketplace)	No sense issue of quality costs
3	66 807	25 – 40 % of turnover
4	62 10 (medium)	15 – 25 % of turnover
5	233	5 – 15 % of turnover
6	3,4 (very good)	> 5% of turnover

CONCLUSION

1. Using DfSS in the design process allows removing errors from the design phase, and at the same time optimizing single or multi-criteria solutions adopted by the design team of a product. The project team can focus on the product development and at the same time costs incurred by improving quality, both in design and manufacturing-cycle of products can be reduced.

2. The lower DPMO is, the lower costs to improve the quality get, and as a result economic efficiency of the company's work is better.

3. Knowing where the company stands in relation to the Six Sigma, allows setting out strategies on medium or even long term. So in the case of hardware EPB companies, after applying DfSS for mounting activities, the strategy applied took into account some specific issues such as:

- Fundamental change in business thinking of the company's management team. While in a classical situation one tries to eliminate the problems, by implementing DfSS in the organization, one tries to avoid them. The classical management perspective focuses on the product, while the DfSS perspective is oriented towards quality of the manufacturing process.
- Since EPB is a product that contributes to human security, applying DfSS is a process that provides a vision regarding the anchored quality in the company's business model, as in all important processes regarding the relationship with the customer. The road to implementing DFSS becomes thereby a process of continuous improvement.
- The use of DFSS has completely changed the "genetic code" of the company, since by reducing the variation limits of its manufacturing process, an error is evaluated differently and the improvement programs are different, more transparent, and more flexible.

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ASPECTS OF RISK MANAGEMENT AT COMPANY LEVEL

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Companies belonging to the former communist states, when accessing a European or global market, a risk factor collides. It appears unexpectedly where you are not expecting! If the management team does not have information about the possibility of risk, at any time, in any form, it can not take effective measures to eliminate the risk. Consequently, the additional costs resulting from the planned reduction benefit. Programs developed by the companies highlighted, particularly in the last decade, the need for protection against business risk. These programs will include elements of risk management; they can be considered risk management tools, as in applications, the value resulting from the process must exceed the costs of it. The increasing complexity of business, changes in market and financial uncertainties in some markets (such as energy market, grain market, commodity market, the IT market) requires an increased awareness of the risk factor in time, and thus increase the need and importance of the Chief-Risk-Officers in the company. It is presence throughout the program creates the premises highlighting, synthesizing and undertaking risk to optimize the trials themselves. Overall analysis of how financial risk arises, the company points out that now must shift from an approach to the economy that seeks certainty and eliminating risks to accept uncertainty and risk taking as a contempt of uncertainty and that philosophy to stimulate progress.

Keywords: risk, Chief Risk Officiers, business risk.

INTRODUCTION

When accessing a market, any company is required to solve some problems caused by the emergence of risk and uncertainty. In the paper entitled "Limits of uncertainty", Giarini and Walter R. Stahel show that "any system that is meant to obtain a result in the future, operates by definition in a situation of uncertainty, even if the different situations are characterized by different risk degrees". If the management team does not have real and especially useful information about the possibility of risk, you may experience it at any time and of any form, or if the team does not recognize the risks manifestation possibilities, the team cannot take effective measures to eliminate the risk. As a result, at the end of that markets activity, additional costs that lead to the decrease of the expected benefit or even to entering the risk of bankruptcy of the company can occur.

Considering that between companies and banks appears an ever more active collaboration, the pressure of financial markets is increasingly felt in the sales markets where the main actors are the companies.

In Romania private property becomes the core element of market economy, free price formation in the market price and competition are elements that define more frequently the situations of uncertainty and risk in the market. They are amplified by the fact that external phenomenon of globalization becomes more intense, and that humans are increasingly less protected of borders. Peter F. Drucker shows, in a paper entitled "Post-Capitalist Society", that "... productivity and innovation will be crucial in creating wealth. As a result, situations of risk and uncertainty will gain new characteristic features, shades and new ways of asserting and therefore new strategies for risk prevention will be sought ".

IDENTIFIED RISKS IN THE COMPANYS' ACTIVITIES

Risks to the conduct of corporate activities may be included in the term "GLOBAL RISK". An overview of the overall risk structure highlights two risk groups:

1. The natural risk.
2. The country risk.

1. The natural risk is either naturally linked to the company's business profile, or to the ownership.

2. The country risk can develop as a bankruptcy risk or as a decision risk.

The decision risk may cover several areas: technical or technological risk, investment risk, innovative risk, social-economic risk, financial risk, liquidity risk, commercial risk, operational risk, risk related to human factor.

Decision-making risk measurement at existing structure level in a company, is usually coming down to a person or a group of people specialized in this field; known as Chief-Risk-Officers in the literature.

Interdependence of information and decisions-making system being permissible, risk measures are taken through conjugate analyzing of the two systems; at each of these two systems two states are recognized: centralized or decentralized for the decision-making system and formal and informal for the information system. With these components you can define three classes of decision-making risk: large, medium and small.

The decision-making risk is of particular significance because it covers not only business segments but also complete development strategies. Making a decision requires competence and responsibility, especially if the employed resources (time important resource) are irreversible.

The decision-uncertainty relationship is of special significance due to the fact that a number of factors generated by natural environmental conditions can not be predicted; so, the decision maker is forced to decide under uncertainty; fact that generates two alternatives:

A. – the decision-maker can use the best information available and his/hers own experience and judgment to:

a) identify and assign subjective probabilities of nature conditions, or

b) assess the consequences of the results for each strategy available in every state of nature;

B. – if the level of uncertainty is so high that the decision maker prefers not to issue assumptions on the probability of state of nature, he may neglect these probabilities, or consider them equal.

Strategies which are required to maintain and develop market access of a company should consider measuring and assessing risk at any time of the activity.

At company level there are several ways to act for preserving the competitive advantages and to minimize, this way, the risk emergence; we mention here:

1. Involvement of the whole system of values that the company has to diminish chance of developing risk in the business.
2. Applying the continuous improvement process to ensure the sustainability of the company's products in the expanding market segments.
3. Elimination of premises that determine sectoral risk: hazard of potential new clients on the market, the emergence of substitute products, negotiating with clients, and/or negotiating with suppliers.

The risks that can develop at a company's level can be generated by four factors operating within the company. At the same time, competition within the company is influenced by a number of issues arising from external suppliers, customers, level of competition (Figure 1).

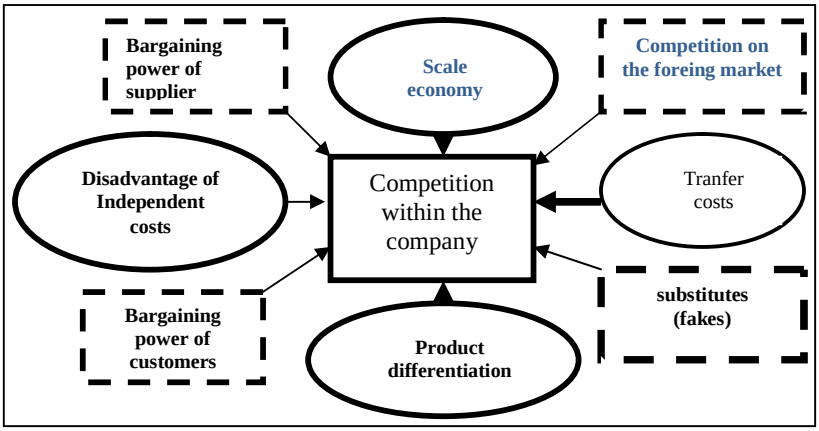

Figure 1. Components of the competitive environment in a company

In order to have an overview of the risk manifestation, external forces that put pressure on the company must be analysed, these are also known as "company entry barriers": scale economies (embodied of the amount of capital required to run a particular activity type), product differentiation and transfer costs incurred by it (direct access to distribution of products or through specially trained intermediaries), the negative effect of the independent production costs (research costs, design costs). Each of these elements generates a particular type of risk, which is reflected in the overall risk. The Chief-Risk-Officer's role is to identify these types of risks and to manage the process in such a way that the overall risk does not affect the overall economic efficiency of the company.

The following risk categories were identified at company level (Figure 2):

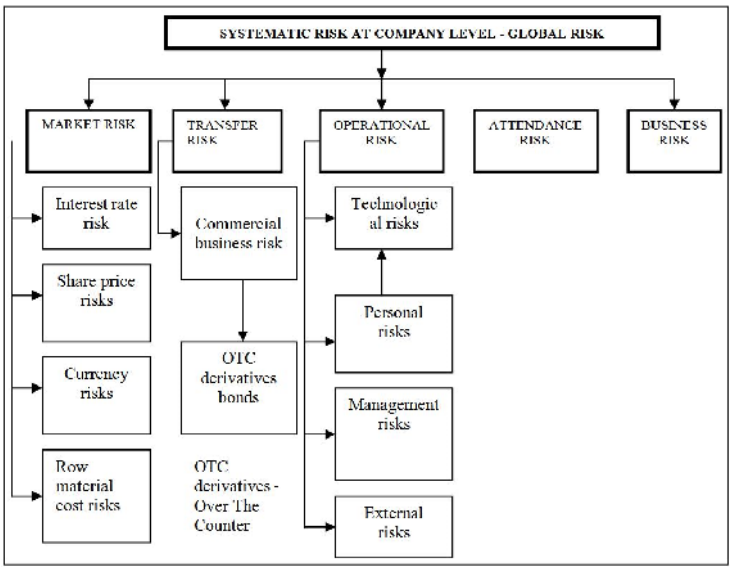

Figure 2. Categories of risk that may develop in the company

Methods of risk control at the level of companies, regardless of their structure or purpose, generally follow the same type of algorithm, which has the following steps:

1. **Identifying the risks**: Identifying and classifying risks according to their origin.

2.**Quantification, analysis and risk assessment**: Risk assessment based on quantitative and qualitative factors which generate them.

3. **Risk limitation:** Risk classification processing systems to limit quantifiable risks.

4. **Risk activities management:** Development of a strategy to avoid risks or if this is not possible, to minimize their effects

5. **Monitoring risk**: Control risk limits, the global and unquantifiable risk profile analysis.

6. **Risk reporting**: Establishing a reporting system regarding risk information.

THE CHIEF RISK OFFICER'S (CRO) ROLE IN THE COMPANY

After working as a manager for credit risk, market risk and liquidity for capital risk, James Lam introduced in 1993 the term "Chief Risk Officer".

The CRO is responsible for how the development of the overall business risk is intended to contribute to good corporate governance Not only the identification, measurement, risk analysis and risk reporting, are the CRO's responsibility; among his/hers skills there are risk control activities and opportunities. The CRO takes decisions based on the overview of business risks and opportunities for the development of company objectives; in these activities the CRO cooperates with other managers in the company.

The specific functions of the CRO depend on the size and complexity of the company. Among the most important attributions we include:

- Development of a framework for highlighting the risk; implementation and control of risk management at company level.
- Development of corporate risk policy, including the establishment of risk tolerance and risk tendency.
- Informing the management structure of significant risks events.
- Ensuring compliance with regulatory risk requirements by identifying and monitoring significant risks.
- Ensuring business continuity in time through centralization of risk assessment, through planning, capital allocation, and risk transfer to the opportunities arising during the course of business.
- Development of alternative risk strategies, or strategies that make use of lower risks.
- Explanation about the company's management strategy risk understandable to investors.
- Education and professional training of the company's staff about the management policy risk and its structure.

The question is: **How widespread is the role of the Chief Risk Officer in the company?**

Important companies in the financial, energy and security fields established the CRO function, whose role increases because of the growing risk and increasing regulatory requirements.

A study, conducted in 2003 in the United States, analyzed the factors that determine the need for CRO. The basis for the study was the data from 26 U.S. companies that have published in the press, between 1997 and 2001 a job ad for the position of CRO. Survey findings reveal that at least one of the major companies in each sector, has in its

organizational chart a CRO position, or collective which serves as a CRO; more than 58% of specialized financial services companies interviewed have in their organizational a CRO position; 41% of the energy companies have a CRO position provided in their organizational chart; most of them are companies with a significant capital loan (external), in this case the CRO's role is to reduce the risks associated with variable costs (Risk-Shifting-Problem).

In 2003, Switzerland KPMG conducted a survey on "How many companies have already established a CRO?" A number of 50 Swiss companies in the medium and large company sector were studied. The survey revealed that 60% of the interviewed companies provided in their organizational chart CRO position; each of these companies has over 2,000 employees; most companies operate in the financial sector.

A survey from Ernst & Young showed in 2003 that only 25% of U.S. insurance companies have had a CRO.

In 2004 "The II Research Foundation" investigates the presence of CRO in 175 large companies structured as it follows: 68% U.S., 10% in Canada and 5% in the UK, 17% in other countries. Companies, with high income (approximately $ 1.3 billion/year) particularly in the industrial field, finance, education institutions were interviewed. Study results showed that 33% of companies have already implemented CRO, 27% redistributed the CRO functions to other managers in the company's divisions, 22% of the company's general manager took over the CRO's full duties, at 18% of the companies the CRO's tasks have been taken over by the company's management council.

Deloitte conducted in 2010 a survey on "Global Risk Management". 131 large international banks and financial institutions in America, Asia and Europe with a turnover of over $ 17 trillion / year were interviewed.

The survey revealed that 73% of the banks and institutions have already introduced the CRO position in the organization. This represents an increase compared with the measured data in 2004, of 81%, and from 2002 of 65%. Over 8% of the respondents plan to establish the CRO position in the company; 19% of respondents did not intend to introduce CRO, its tasks are distributed to other vectors (reporting by executive management or access to company management - senior management).

According to a forecast made by Forrester Research Inc. in Cambridge in 2007, all the big companies in the financial and energy sector will have the CRO function 75% integrated. Figure 3 presents the CRO dynamic during the last eight years, in global banks, financial institutions and insurance institutions. From the figure it is observed that banks have introduced CRO to control the risks specific to financial flows. Insurance institutions responded with a lower trend to this objective(they started with 20%, and then stabilized at around 40%).

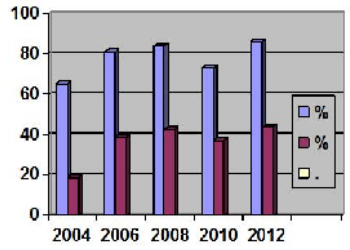

Figure 3. CRO dynamics during 2004-2012 in banks, financial institutions and insurance companies

CONCLUSION

Risk management at a company is a new concept, which is developing a highly dynamic trend. Risk management programs are among the most important tools of management if they are well applied, additional benefits outweigh the costs of implementation..

Implementation of a risk management program does not automatically mean just establishing a CRO, but the presence of a CRO is a sign of the existence of a risk management program in the company.

The beginning of the CRO era was in the financial sector. The CRO position was established precisely in this industry. In other sectors, such as energy and pharmaceutical industry the introduction of the CRO quickly gained in importance. The increasing complexity of business, changes in market or other financial uncertainties of the energy market require an increased awareness of the risk occurring and thus the need and importance of the CRO function in the company is growing.

Both now and in the future the CRO function will be connected, on one hand, to fulfilling the continuous growth of the legal and governed by specific legislation in various areas of business requirements, and on the other hand, to the dynamic risks of competition on the market. The continuous emergence of new risks such as the risk of over-regulation, globalization and expanding business strategy must be identified and monitored in time. The overview of risks and significant knowledge of the relationships within the company is an important prerequisite for the success of the company's activities in any field.

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ADAPTIVE MULTI-AGENT CONTROL OF LEATHER MANUFACTURING PROCESSES BY USING SMITH PREDICTOR

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The conventional control systems used in leather manufacturing proven their inefficiency due to their centralized architecture, being a critical point of failure that impose operational bottleneck. Multi-agent systems (MAS) represent a viable alternative for making a system agile, providing flexibility and modular development of the control system. A MAS represents a more natural way in dealing with complex distributed problems due to their characteristics autonomy, social ability, mobility, modular development. Agents part of the MAS are distributed geographically and communicate one with another via a network. Each node of the network is represented by an agent that represents a manufacturing resource. Communication between agents generates delays that can affect functionality of the system. This paper presents an adaptive multi-agent control system used on leather manufacturing processes which is based on Smith predictor. The proposed adaptive control strategy based on Smith predictor aims to eliminate the negative effects generated by communication delays between agents on production system performance. Communication delays are modeled as pure delay elements. The proposed control strategy has an estimator for the communication delays and these estimates are used to adjust the controller, specifically to adapt its parameters to the new values of communication delays. Simulation results are presented and they demonstrate the performance improvement when the proposed control strategy is used.

Keywords: leather industry, agent architecture, smith predictor, multi-agent systems, leather manufacturing.

INTRODUCTION

Conventional automation systems based on staled centralized control model proven to be an inefficient solution for dealing with the complexity and the diversity of the leather manufacturing operations (Guta and Dumitrache; Guta *et al.*, 2013; Hanchevici and Guta, 2012). These concentrate all control functions within a single central controller that is rigid, not provide agility, flexibility, being a critical point of failure that impose operational bottleneck.

The problem of effective tannery automation has numerous variable such as raw material diversity, discontinuity of the technological process, time-variance and non-linear behavior of the manufacturing operations, lack of mathematical models that accurately express the reality. The traditional solutions did not offer expected results to the above mentioned problem. In this situation adoption of multi-agent system (MAS) solution that extends the functionality of an existing automation system represents a viable solution (Guta and Dumitrache).

Guta *et al.* (2013) emphasize the superiority of the MAS technology for leather industry in comparison with conventional solution. Its superiority is mainly in terms of agility, flexibility, modular development, fault tolerance, robustness.

MAS technology is used for solving complex problems that are difficult or even impossible to solve by a centralized system (Wooldridge, 2009; Paolucci and Sacile, 2004). A MAS is composed of a society of autonomous entities (software agents) distributed geographically that represents the manufacturing entities (mainly resources). These agents are sociable, have a partial knowledge or view of the manufacturing

system, interact one with another sharing knowledge and delegating task. Thus the result of agent interaction is an emergent behavior that is similar to that of humans, proper for solving complex problem (Guta, 2014; Monostori *et al.*, 2006). The agents part of a MAS are distributed across the whole tannery and communicate one with another via an industrial network or even wireless. During their communication acts through message exchange, communication delays can occur.

The paper addresses the issues generated by the variant communication delays which can occur in MAS used for the control of the leather manufacturing processes.

PROPOSED CONTROL STRATEGY

In this paper is proposed a multi-agent control strategy for linear SISO (single input – single output) systems which have variant communication delays. This control strategy is based on Smith predictor. The communication delays between the agents are modeled as pure delay elements. The control strategy used for fixed communication delays is presented in Figure 1.

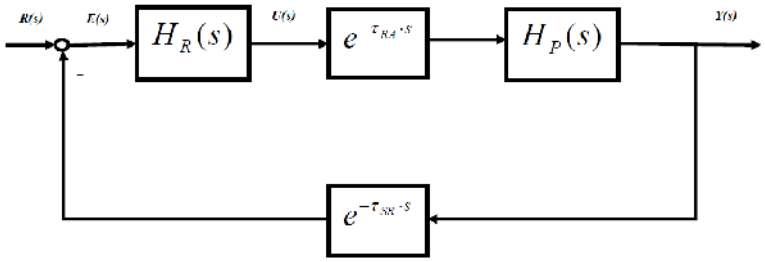

Figure 1. The control strategy used for fixed communication delays (Hanchevici, 2013)

where:

- R(s) represents the set-point for the closed loop system;
- E(s) is the error of the closed loop system;
- U(s) represents the control computed by the control agent;
- Y(s) denotes the controlled parameter;
- $H_R(s)$ is the mathematical model of the control agent;
- $H_P(s)$ represents the mathematical model of the plant;
- $e^{-\tau \cdot S}$ represents the mathematical model of the communication delays. $e^{-\tau_{RA}\cdot S}$ is the mathematical model of the communication delay between the control and actuation agents, and $e^{-\tau_{SR}\cdot S}$ is the mathematical model of the communication delay between the sensing and control agents.

The control strategy presented in Figure 1 is used for the case when the communication delays between agents are fixed. If the communication delays are variant, then is used the proposed control strategy presented in Figure 3.

In Figure 2 is described how it is used the Smith predictor. The controller H_R* is designed for the case when the communication delays are placed outside the control loop.

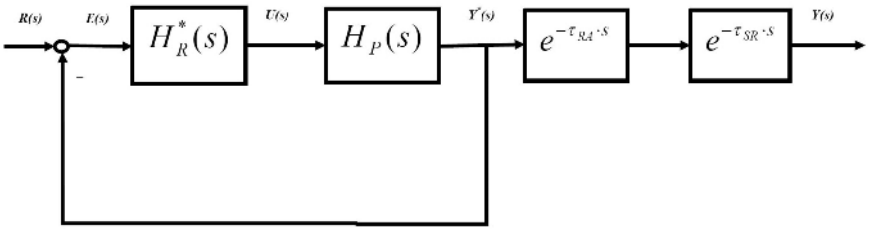

Figure 2. The control strategy which has the fixed communication delays placed outside the loop (Hanchevici, 2013)

The dependency between them is expressed by (1):

$$H_R(s)=\frac{H_R^*(s)\cdot e^{-\tau_{SR}\cdot s}}{1+H_R^*(s)\cdot H_P(s)\cdot\left(1-e^{-\tau_{RA}\cdot s}\cdot e^{-2\cdot\tau_{SR}\cdot s}\right)} \quad (1)$$

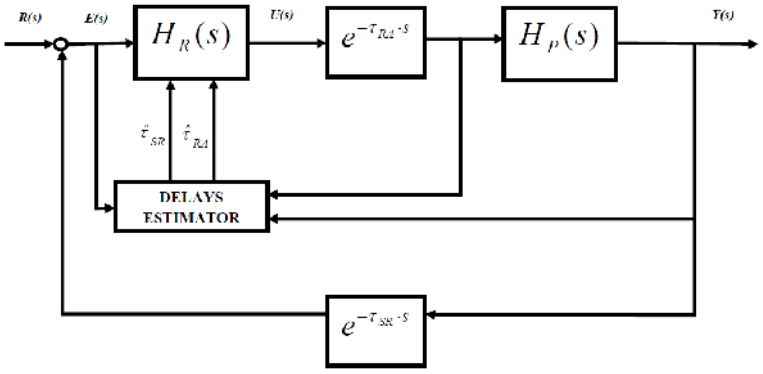

Figure 3. The control strategy proposed for variant communication delays

In the proposed control strategy is used an estimator for the communication delays. During every sampling time, the DELAYS ESTIMATOR is used to estimate the current values for the communication delays and these values are used to tune the control agent. The tuning represents changing the values of the controller's parameters according to the current values of the communication delays.

CASE STUDY

For this study the control of one parameter was considered, namely the pH value. This SISO (Single Input Single Output) system is non-linear and it can be approximated by the rational s-transfer function (2):

$$H_P(s) = \frac{K_P}{T_P \cdot s + 1} = \frac{1}{10 \cdot s + 1} \quad (2)$$

It is designed a control agent considering fixed communication delays, and it is tested in different cases when the communication delays are variant.

In Figure 4 is presented the influence of variant communication delays over the control, and in Figure 5 is presented the influence of the same delays over the output of the closed-loop multi-agent system.

By analyzing these responses, we can see that the performances of the closed-loop multi-agent system are worse in terms of overshoot. This mismatch is generated by the fact that when the variant communication delays appear, the controller designed for the case when the communication delays are fixed is not able to perform as requested and the performances of the closed-loop multi-agent system are getting worse.

In order to prevent the decrease of performances of the closed-loop multi-agent system, we have adopted the control strategy presented in Figure 3.

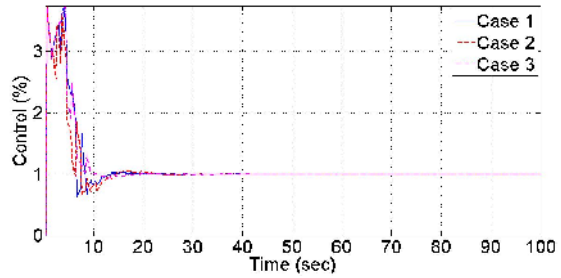

Figure 4. Simulated control analysis when is used the same controller and the communication delays are variant

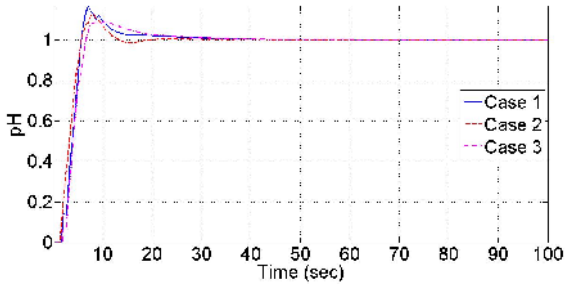

Figure 5. Simulated output analysis when is used the same controller and the communication delays are variant

In this study have been considered the following cases (Table 1):

- Non-adaptive represents the performances of the closed-loop multi-agent system obtained when the communication delays were variant between 0 and 2 seconds, and the controller was designed considering fixed communication delays;

- Adaptive represents the performances of the closed-loop multi-agent system obtained when the communication delays were variant between 0 and 2 seconds, and is used the proposed control strategy.

Table 1. Simulation study analysis

		Overshoot (%)
Non-adaptive	Case1: τ_{max}=2.0 sec	16.8
	Case2: τ_{max}=2.0 sec	12.9
	Case3: τ_{max}=2.0 sec	9.1
	Case4: τ_{max}=2.0 sec	19.9
	Case5: τ_{max}=2.0 sec	19.2
Adaptive	Case1: τ_{max}=2.0 sec	0.0
	Case2: τ_{max}=2.0 sec	0.0
	Case3: τ_{max}=2.0 sec	0.0
	Case4: τ_{max}=2.0 sec	0.0
	Case5: τ_{max}=2.0 sec	0.0

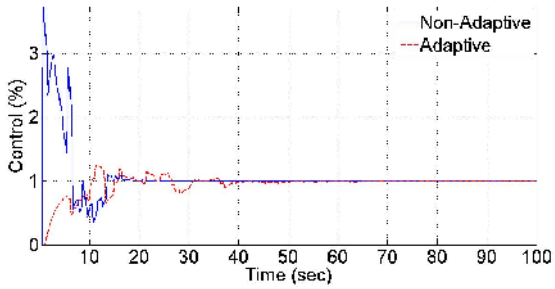

Figure 6. Simulated controls both situations with variant communication delays (case S5)

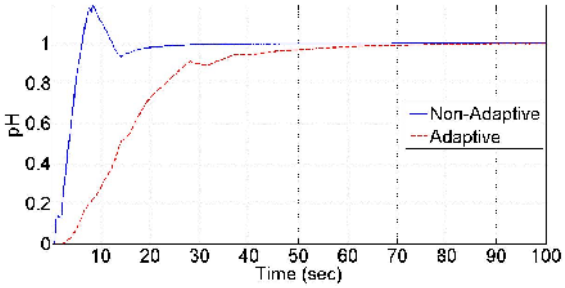

Figure 7. Simulated responses for pH value control in both situations with variant communication delays (case S5)

In Figure 6 are presented the controls, and in Figure 7 are presented the outputs of the closed-loop multi-agent system for two cases with variant communication delays. In

the first case (Non-Adaptive) is used the control strategy described in Figure 1, and in the second case (Adaptive) is used our proposed control strategy (Case 5).

By analyzing the responses presented in Figures 4, 5, 6, and 7 we can see that the performances, in terms of overshoot, of the closed-loop multi-agent system are improved when is used our proposed control strategy.

CONCLUSIONS

The paper pointed out the advantages of MAS technology in leather manufacturing in comparison with conventional control solutions. A thing that stands out is the fact that during the communication between agents, delays occur that affects the functionality of the system. The paper presented a solution that mitigates the negative effects caused by variant communication delays. An adaptive multi-agent control strategy was proposed for non-linear single input single output systems affected by variant communication delays. The proposed control strategy uses an estimator for the communication delays, and these values are used to tune the control agent according to (1).

By analyzing the results, the performances of the closed-loop multi-agent system are improved when is used the adaptive control strategy proposed in this paper.

In future work the proposed control strategy will be evaluated on a real industrial process.

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ENERGY EFFICIENCY THROUGH MULTI-AGENTS ADAPTIVE MICROMANAGEMENT

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Finding successful ways to reduce the energy utilization in commercial and residential buildings is of paramount importance in lowering CO_2 emissions and achieving the Kyoto Protocol commitments on climate change. Indoors energy consumption account for roughly 40% in US and EU. Building exploitation are linked to about 36% of the total CO2 footprint. The main approach to ameliorate this situation is to enhanced energy efficiency, decrease the overall consumption and switch to renewable, carbon-free energy sources. In the field of energy efficiency, enhancement of load control and adaptive demand response at every point of consumption are part of the solution. This paper presents the problematic and limits of energy consumption savings while accommodating human comfort propensity. Further we present the simulation results for a grid of independent, autonomous, collaborative agents that continuously monitor human activity in a closed environment and override the user configured comfort preferences towards a default optimum performance/cost whenever the changes do not affect the user experience. In order to better highlight the importance of local micromanagement and to obtain the best approximated average performance, the chosen simulation environment was a 250 rooms hotelier resort and targeted the heating/cooling annual energy cost with human behavior stochastic considerations.

Keywords: multi-agents, energy efficiency, intelligent buildings.

INTRODUCTION

A building's energy consumption depends on numerous factors like structure, insulation, climate, in-terrain positioning, residents, usage patterns, HVAC (heating, ventilation and air conditioning) performance, etc. These factors can be treated as systemic features or exploitation events.

Simulating a building's usage history over a significant period can test usage strategies and indicate improvement options. Real-time monitoring in large buildings is expensive, the case-study timeframe cannot be compressed and the events cannot be exactly rerun. The study results are strongly linked to the observed building and the improvement recommendations cannot be ported elsewhere. Modeling and simulating user activities in virtual environments is cost effective, fast, flexible and permits use-case scenarios testing for better systemic, organizational and procedural design (Fujimoto, 2001).

Multi-agent systems (MAS) architectures avoid the "single point of failure" structural vulnerability encountered in SCADA design, provide support for interconnectivity and interoperability of legacy systems in heterogeneous assemblies and mitigate network service interruption. MAS characteristics determine an inherent better reliability, robustness, extensibility, maintainability, responsiveness and flexibility of the developed solution.

The agents can either be explicitly organized in particular task groups or configured to auto-organize and cooperate towards a preconfigured set of goals (Vasyutynskyy *et al.*, 2007; Rutishauser, 2002). The tasks can be related to Smart Grid operations and

optimization, energy consumption with HVAC and lighting, user comfort, security and disaster management, etc.

While from a theoretical point of view the space positioning of some system elements (sensors/actuators) do not deny the conceptual systemic unity and the agent as set of roles distributed across multiple nodes is admissible (Ruairí & Keane, 2007), we consider an agent to be a dynamically configurable, compact unit capable of autonomous and collaborative behavior.

Energy efficiency strategies implemented at building level are impaired by individual originated demands and preferences. While most approaches treat the building's HVAC demand as a whole and propose strategies at building level, the objective of this paper is to demonstrate the importance of micromanagement at user and individual action level. Further, we prove the existence of an important savings margin by overriding a user's preferences whenever his comfort or experiences are not affected.

THEORETICAL BACKGROUND

The overall heat loss aggregates surface losses by conduction and radiation through windows, wall, doors etc., by ventilation and by infiltration. The superficial heat loss varies linear with ΔT and determines a constant cost per time unit to maintain a desired interior temperature for a given outside condition, with **U** = overall heat loss coefficient and ΔT = outside/inside temperature difference:

$$\textbf{Heat loss}\ (W) = \boldsymbol{A}\ (m^2) * \mathbf{U}(W/m^2K) * \Delta \boldsymbol{T}\ (K) \quad (1)$$

In order to estimate the annual energy cost with space heating or cooling we used the degree-days approach (Martinaitis *et al.*, 2010; Bhatia, 2013). This method was first developed in agricultural research and was used to observe the effect of atmospheric temperature dynamics on crops. The concept was imported into building energy consumption analysis as a link between weather changes and energy consumption and it allows to review a building's current energy requirements vs. its past performance.

Heating or cooling degree-days are computed as a daily exterior-interior temperature difference, the interior temperature being considered a reference temperature, usually 65ºF ≅ 18 ℃. The method assumes that any outside temperature below the reference temperature will trigger indoor heating in various degrees. An analogue approach anticipates the cooling demand.

$$HDD = \frac{\sum_{i=1}^{24}(T_{H,base} - T_o)}{24} \text{ for } T_{H\ base} > T_o \quad (2)$$
$$CDD = \frac{\sum_{i=1}^{24}(T_o - T_{C\_base})}{24} \text{ for } T_o > T_{C\ base}$$

Some models considered an hourly basis in sampling, averaging the sum of positive hourly differences, introduced as cooling degree hours (CDH) (Krese *et al.*, 2012). If detailed weather dynamics information is not provided, estimative calculation methods for degree-days have been proposed (Hitchin, 1984; Spano *et al.*, 2002). While the HDH/ CDH method produce better results than HDD/CDD, its predictive usefulness in real human activity scenarios is still reduced because one-hour sampling intervals cannot accurately support human activity modeling.

Both degree-days and degree hours formulas use one single indoor temperature reference although the human comfort zone is a variable interval that depends on climate, age, gender, culture, habits etc. We consider more accurate to use two indoor temperature references, a lower "need heating" reference and a higher "need cooling" one. Further, we used weather data for every minute in order to summate and compare temperature differences over an entire year in two different usage scenarios: with and without multi-agents HVAC micromanagement. Given the above considerations of heating/cooling cost being linear with ΔT , the less cumulative temperature differences have to be sustained over a year, the lesser energy will be required, being expected thus a lower exploitation cost.

SCENARIO SETUP

We considered an administrative task group of collaborative agents for each closed space that could have an independent HVAC configuration inside a building. The purpose of each task group was to reduce HVAC energy consumption whenever possible, within a series of constraints. Such HVAC independent spaces can include one or more linked rooms and have the capability to insulate the inside temperature from the rest of the building. We will name in the following such organizational space units “apartments”, regardless of their private, public, commercial or corporate destination.

The considered constraints were:

- Prior to use an apartment, a user must announce in advance with at least one hour and book the apartment for a determined period of time.
- A free (non-booked) apartment will function in “stand-by mode”, maintaining a cold (**Standby-Chill**) or hot (**Standby-Hot**) temperature until booked.
- By default, the apartment is set to function in optimum comfort temperature mode, if the user does not activate personal preferences.
- The agents override the user’s preferences and fallback to the default regime whenever the user’s experience is not affected (user is away or sleeps). The user’s preferences are reinstated immediately after the overriding condition cease to exist.

SIMULATION FRAMEWORK

For commercial buildings, ISO standard 7730 prescribes generic temperatures of 20-24°C in winter and 23-26°C in summer, with exceptions according to room destination. According to World Health Organization (WHO), a temperature of 21°C in the living room and 18°C in other occupied rooms represent “an adequate standard of warmth". In practice, the base temperature is recommendable to be established from case to case (Day *et al.*, 2003).

The presented agent model and scenario constraints were implemented using the SHIELD simulation framework. The framework is oriented to emulate complex collaborative interactions of heterogeneous agents in Intelligent Buildings (Neagoe, 2014).

Our simulation took the standard reference temperature as 18°C, the optimum heating comfort 21°C, optimum cooling comfort 22°C. These values were considered conservatively and prudent, to avoid exaggerating the comfort interval and force better

results. The **Standby Chill** and **Standby Hot** temperatures were set at 16°C and respectively 26°C. The simulation considered real weather temperatures recorded in 2013. Processing the injected Max/Min monthly values series, the simulation generates a plausible atmospheric variation (continuous weather scenario) for 525600 minutes (one year) or loads a previous generated pattern.

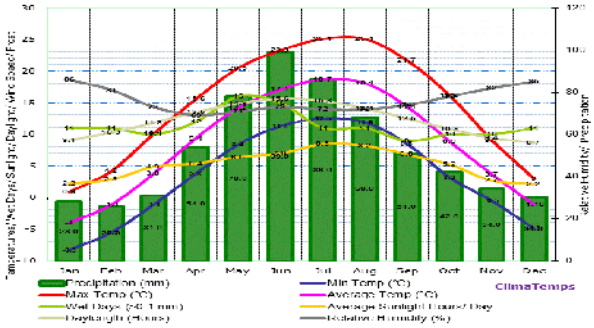

Figure 2. Real weather pattern for Brasov, Romania, 2013

The simulated environment emulated a hotel with 250 apartments with HVAC features managed for each apartment by a group of collaborative agents. The generated weather continuity chart highlights that buildings in Brasov deal mostly with heating demand (reality), cooling being a non-issue:

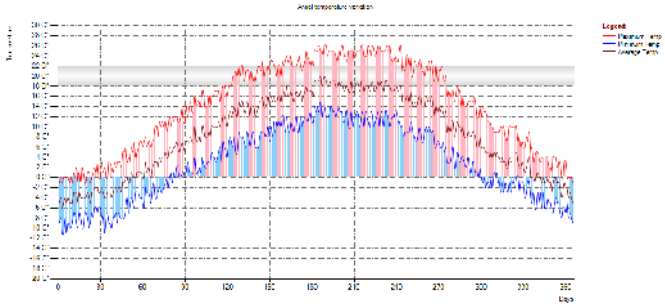

Figure 3. Annual heating and cooling demand, Brasov, Romania, 2013

Since most energy consumption occurs in the cold days with heating, the hotel scenario considered a plausible facility load history, giving a special attention to the possibility that unrealistic usage patterns injected as scenario input could artificially alter the result. The considered occupancy pattern as a (days in month, occupancy percent) series:

Monthly Facility Load = { IAN { 31, **95** }, FEB { 28, **85** }, MAR { 31, **65** }, APR { 30, **40** }, MAI { 31, **45** }, IUN { 30, **55** }, IUL { 31, **65** }, AUG { 31, **75** }, SEP { 30, **70** }, OCT { 31**, 65** }, NOV { 30, **75** }, DEC { 31, **100** } }; // {DAYS IN MONTH, HOTEL LOADING PERCENTAGE}

The occupancy pattern proved to be of major importance in the simulation outcome. The differences in the final result ranged from the lowest energy savings score (6,7%)

for a constant 100% occupancy to the highest (33, 94) for 0% occupancy. The interpretation is straightforward, the 0% occupancy fully "benefiting" from the **Standby-Chill/ Standby-Hot** HVAC regime while at 100% occupancy no standby regime was registered.

Further, for each apartment was generated the daily occupancy history and one of three user types was assigned to use the space. The three user types were: minimalistic user (***R1***), normal user (***R2***) and intensive user (***R3***), randomly generated with 20%-60%-20% probabilities. The ***R1*** type is mostly absent, does not change much or at all the standard HVAC setup and has no extreme preferences(demands 19-22°C in winter and 24-27°C in summer), the ***R2*** normal user is moderately absent and has moderate temperature preferences(21-24°C , 22-25°C) and ***R3*** intensive user that almost never leaves the room and has the costliest "demands" (23-26°C, 20-23°C). All user types "sleep" one single time per 24 hours, at night, for 6 to 10 hours(random).

For each user type, an user event sequence is created for every day he occupies the apartment. He leaves, returns, configures HVAC preferences or not, sleeps. All events have a "cool-down time" while they cannot happen again and do not describe impossible or abnormal user behavior (one cannot be away and asleep at the same time, will not change HVAC preferences 10 times per hour, etc.). The user actions are not altered by exterior influences like price incentives or constraints (Mohsenian-Rad *et al.*, 2010; Ozturk and Kumar, 2013; Ramchurn *et al.*, 2011). To permit an accurate comparison, the simulation stores the user action sequence for each day, for each apartment, in order to run the "unmanaged"/"micromanaged" scenarios in a row.

RESULTS

Using the degree method to compute the heating and cooling effort each minute produces large figures for each day, for the 250 simulated apartments reaching an 10^7 order. The following synthetic chart represents the "**C**lassic **H**eating/**C**ooling **D**egrees Needed" - **CHDN/CCDN** series, computed using two interior comfort reference temperatures Comfort Heat Needed = 21℃ and Comfort Cold Needed = 22℃ and the "**M**icromanaged **H**eating/**C**ooling **D**egrees **C**omfort" **MHDSC/MCDSC** series, where the local agents override user preferences with optimum values while the user is away/sleeps and fallback to **Standby Chill/Hot** mode while free of contract:

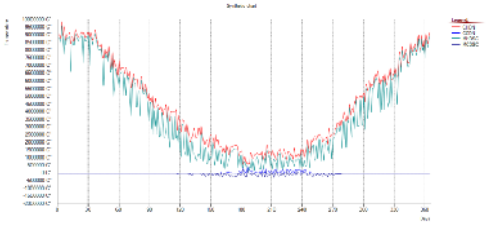

Figure 4. Comparative unmanaged vs. agent micromanagement HVAC effort

The two compared methods used the same annual weather pattern, occupancy series and generated user events series for all simulated apartments. Repeated simulation re-runs with the same input data (monthly min/max temperature series and monthly occupancy series) constantly produced result between roughly 14% and 15%. The above simulation run indicated a 14.421 % gain in energy efficiency.

CONCLUSION

The degree-days (hour/minutes) concept permits the analysis of energy management outcome regardless of a buildings physical characteristics or HVAC installations performance. These factors being the same for each case, the outcome difference comes from usage policy alone. Intelligent agent HVAC real-time micromanagement shows an important savings margin to a building's energy bill. For the simulated case-study presented in this paper the performance gain was equivalent with 35 apartments out of 250 running with no HVAC costs for an entire year. The fact that the simulation used conservative figures indicates a higher performance possible in extreme case scenarios (ex. natural disaster or calamity), when the comfort interval could be automatically adjusted to accommodate the transient conditions. The intelligent micromanagement technique can be applied to any building, residential or commercial, and can be improved with further automatic triggered events like automatic windows and reflective curtains operations, user incentives, humidity control etc.

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COMPETITIVENESS MANAGEMENT OF LEATHER COMPANIES: A CLUSTER APPROACH

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Manager's efforts aimed at developing efficient organizational forms of business, including clusters. Specified economic phenomenon can be identified as the structure of the vertically and horizontally linked by economic agents (companies, research and educational institutions, government agencies) in a particular industry and allied sectors. Their essential feature is the ability to complement and enhance the competitiveness of each other and the region as a whole. In this case principal in the identification of clusters is their ability to generate positive synergies from coordinated behavior and internal communications. Within the pale of the cluster mainly such forms of synergy find a manifestation, as synergies of scale, labor, sales, investment management, environment and operational synergies. Assessment of Ukraine leather companies regarding opportunities for cluster synergy cooperation was carried out by us in the following segments: manufacturing; logistics; sales; marketing; research and experimental development; exchange of information; communication with consumers. It is shown that the formation of cluster networks provides increasing competitiveness of companies by reducing logistics costs and marketing, accelerate innovation and by stimulating the exchange of knowledge and skills.

Keywords: competitiveness, cluster, positive synergies.

INTRODUCTION

Increased competition in the markets as a result of globalization of economic processes, accelerated upgrading of the technologies leads to the need to strengthen the competitiveness of leather companies. Areas in which these companies operate in many countries are experiencing stagnant production. However, the companies preserve the technological, human and scientific potential for successful development. Managerial efforts are aimed at developing efficient organizational forms of business, in particular, clusters. Specified economic phenomenon can be identified as the structure of the vertically and horizontally linked economic agents (companies, research and educational institutions, government agencies) in a particular industry and related sectors. Such companies must be located close to each other. Their essential feature is the ability to complement and enhance the competitiveness of each other and the regions as a whole.

Cluster study was initiated by Harvard Business School Professor Michael Porter (Porter, 1990). He found causes of competitiveness in individual sectors of the country on the basis of four indicators - "The diamond model". Before M. Porter, spatial agglomerations that are distinguished by increased competitiveness, were described by A. Marshall in his works (Marshal, 1961). Modern researchers identify spatial clustering as a kind of network form of organization (Enright, 2000; Sölvell *et al.*, 2003; Malmberg and Maskell, 2002; Powell and Brantley, 1992; Powell and Smith-Doerr, 1994; Perrow, 1993).

The main features of the cluster are preservation of competition between the companies; voluntary cooperation in certain areas in order to achieve common goals; geographical proximity of members; associations of companies that represent the main

production as well as related industries and servicing infrastructure. Fundamental in identifying clusters is their ability to generate positive synergies based on coordinated behaviour and internal links. The authors share the opinion that the cluster is just an association of companies that provides a positive synergistic effect reflected in the explicit and implicit financial effects. For the synergistic effect of cluster system to be maximal, it is necessary to optimally combine the elements that it includes. In addition, the volume of the synergistic effect will be significantly affected by the quality of the cluster system elements and the efficiency of their interaction. In each particular cluster, the occurrence of a synergistic effect depends on a combination of factors, among which the most significant are the number of members, number and qualifications of the staff involved, availability of resources, availability of areas of economic interest coincidence, quality of management, availability of the capital flows and information, government support (Eggertsson, 1990; Ansoff, 1999; Itami and Roehl, 1991).

Due to the aforesaid, **the aim of our study** is to develop the methods for evaluation of the relative synergistic effect and formation of scheme of optimal relationships between the cluster companies to ensure the maximum synergistic effect.

MATERIALS AND METHODS

As a result of study a scheme for optimal synergy cluster relationships for six companies of leather cluster in Kyiv was built. The methods by which the calculations were done is a sequence of the following steps: 1. identification of synergy factors that can be quantified; 2. calculation of matrix of synergy ratios between the two companies by certain factors; 3. calculation of generalized synergistic effect of cooperation between the companies by all synergy factors taking into account ratios of their significance; 4. establishment of ranks of various cooperation options; 5. construction of scheme for optimal synergy relationships between the companies in the cluster.

Most often, in practice, there are four types of synergism: synergism of sales, operational, investment and management synergism (Bushueva, 2002). Some researchers have also added to this list the synergy of innovation and "synergy of conglomerate" (Maljuk, 2009). It should be noted that in each particular cluster, depending on the stage of its development, degree of integration of the members, sources of the synergistic effect will vary. For the leather industry companies, the main sources of synergies in cluster structure can be such areas as manufacturing; logistics; sales; marketing; research, design and experimental development; exchange of information; communication with consumers. The occurrence of a synergistic effect due to the presence of common interests of companies in such areas, which are based on the use of the same or similar technologies, equipment, logistics channels, relations with the same suppliers and consumer segments, usage of the shared infrastructure, system of dealers, repair services, as well as the design and usage of scientific research results. Schematically, sources of synergy in the interaction of companies in the cluster are shown in Figure 1.

The diagram shows a few possible areas of coincidence of production and marketing interests of enterprises that can generate synergies. Much more of such synergy factors can be identified in practice. In addition, it should be noted that they are not static and may vary depending on the life cycle of companies and the cluster as a whole, as well as changes in market conditions. In the cluster structure, the companies' management faces

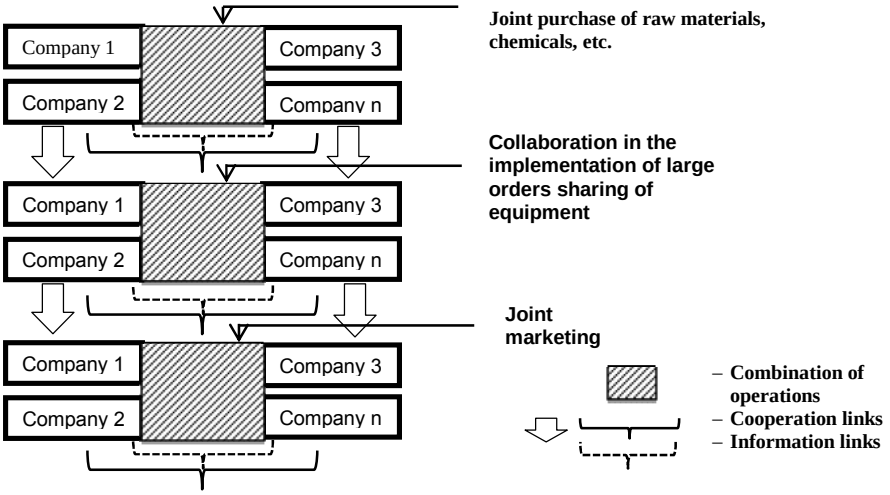


Figure 1. Sources of synergy in the interaction of the cluster companies

the task of researching and identifying the most complete list of sources of synergies generation. The more thoroughly they will be investigated; the better will be the evaluation of the total synergistic effect of the cluster. Based on our analysis of the possible sources of synergies generation in a cluster of leather industry companies in Kyiv, the following synergy factors have been distinguished: cooperation in repair servicing of the equipment (X_1); joint purchase of raw materials (X_2); joint promotional events, organization of exhibitions, etc. (X_3); cooperation in research and development (X_4); sharing of infrastructure facilities (X_5). All listed factors can be quantified and currently is the most significant for the companies' cooperation in the specified cluster. For their evaluation we proposed to use the following indicators:

1. Relative share of types of equipment that coincide in usage by the companies under study in the general list of equipment. The indicator is used to calculate the relative synergistic effect from the organization of joint repair service.

2. Relative proportion of raw materials that coincide in usage by the companies under study in their general list. The indicator is used to calculate the relative synergistic effect from the organization of joint purchase of raw materials, chemicals, etc.

3. Relative proportion of identical goods produced by the companies under study in their general list. The indicator is used to calculate the relative synergistic effect from the organization of joint promotions, joint participation in trade shows, collection and analysis of market information.

4. Relative proportion of products that coincide by production time and technology of the companies under study in their general list. The indicator is used to calculate the relative synergistic effect from the organization of joint research and development, project documentation, researches, etc.

5. The relative share of infrastructure facilities, which coincide in use by the companies in their general list. The indicator is used to calculate the relative synergistic effect from the organization of joint transport, storage etc. support.

RESULTS AND DISCUSSION

The proposed list of indicators can be changed and enhanced according to practical needs. After the synergy parameters for each of the above types of cooperation, the total synergistic effect between the companies under study in the cluster shall be determined. For each company its synergistic attractiveness is measured as the sum of two groups of effects: as a synergy generator for partner companies and as a synergy receiver from them (Ansoff, 1989). Determination of relative evaluation of synergistic effect generated in the cluster has been conducted for each of the five distinguished synergy factors (Table 1). For ease of handling, all calculated indicators were multiplied by 10. Five separate matrices by these factors were formed in total. This article gives only one matrix, but the final calculations are presented in Table 2. The corresponding lines of the Table 1 contain elements a_{ij}, which values are calculated by the above formulas of synergy ratios with pair interaction between i-th and j-th companies. Generalized evaluation of synergy generated by the pair interaction of the cluster enterprises is calculated as the sum of the synergistic effect ratios by of all synergy factors (Table 2).

Table 1. Formation of relative synergy evaluations by factor (X_1 - joint repair service of equipment)

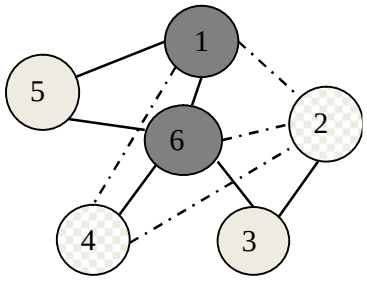
Synergy generator companies	Synergy receiver companies						Average value of generated synergistic effect
	C 1	C 2	C 3	C 4	C 5	C 6	
C 1	–	2.5	1.2	3.8	4.2	5	2.9
C 2	2.5	–	3	4.1	1.6	1.2	2.1
C 3	1.2	3	–	2.1	2.2	4.8	2.1
C 4	3.8	4.1	2.1	–	0.8	1.3	2.0
C 5	4.2	1.6	2.2	0.8	–	0.2	1.5
C 6	5	1.2	4.8	1.3	0.2	–	2.1

Columns 2, 4, 6, 8, 10 of Table 2 correspond to the synergy ratios by factors under study (X_1-X_5). Column 2 of Table 2 is filled with elements from the matrix Table 1 corresponding to the pairs of companies under study. Columns 4, 6, 8, 10 are filled in the same manner. To increase the accuracy of calculations, evaluation of total synergistic effect (C_Σ) of interaction between companies in the cluster was carried in view of the significance coefficients (kx) of the synergy factor under study (X_1-X_5). Columns 3, 5, 7, 9, 11 of Table 2 were calculated as the product of the synergistic effect ratio from possible cooperation and synergy factor significance coefficient: $C_\Sigma = \sum C_{i,j} * kx$. Synergy factor significance coefficient was determined on the basis of expert assessment. The six leading experts of leather companies under study in Kyiv have been selected as experts. It should be noted that the presented calculations are based on the analysis of cooperation between pairs of companies. However, this technique makes it possible similarly to algorithm presented above to evaluate the relative synergistic effect of the interaction of three or more members. Based on the calculation of the generalized synergistic effect, we have formed rating of various options for the companies' cooperation. Evaluation of priority was based on the following scale: if $C_\Sigma \geq 2$ – combination of companies is optimal; if $2 > C_\Sigma > 1{,}5$ – interaction option is quite

efficient; if $C_{\Sigma} \leq 1,5$ – combination of companies will have insignificant synergistic effect.

Table 2. Generalized evaluation of synergistic effect by synergy factors under study

Combinations of companies	Total value of synergistic effect by synergy factors										$C_{\Sigma}=\sum_{ij} * k_x$	Rank
	X_1 (k_{x1}=0,2)		X_2 (k_{x2}=0,23)		X_3 (k_{x3}=0,31)		X_4 (k_{x4}=0,15)		X_5 (k_{x5}=0,11)			
	C_{ij}	0.2 *C_{ij}	C_{ij}	0.23 *C_{ij}	C_{ij}	0.31 *C_{ij}	C_{ij}	0.15 *C_{ij}	C_{ij}	0.11 *C_{ij}		
1-2	2.5	0.5	1.3	0.3	1.8	0.56	1.7	0.26	1.1	0.12	1.7	8
1-3	1.2	0.24	0.9	0.21	0.8	0.25	1.1	0.17	3	0.33	1.2	13
1-4	3.8	0.76	0	0	0.9	0.28	1.3	0.2	5	0.55	1.8	7
1-5	4.2	0.84	1.4	0.32	1.2	0.37	0.3	0.05	4	0.44	2.0	6
1-6	5	1	2	0.46	0	0	1.4	0.21	8	0.88	2.6	3
2-3	3	0.6	1.3	0.3	4.6	1.43	1.2	0.18	3.1	0.34	2.8	2
2-4	4.1	0.82	1.5	0.35	0	0	2.4	0.36	1.6	0.18	1.7	9
2-5	1.6	0.32	0	0	1.9	0.59	0	0	0	0	0.9	15
2-6	1.2	0.24	0.2	0.05	2.8	0.87	2	0.3	1.9	0.21	1.7	10
3-4	2.1	0.42	0	0	1.2	0.37	2.2	0.33	0	0	1.1	14
3-5	2.2	0.44	1	0.23	1.8	0.56	1.7	0.26	0	0	1.5	11
3-6	4.8	0.96	5	1.15	0	0	0	0	2.3	0.25	2.4	5
4-5	0.8	0.16	4.2	0.97	0	0	0	0	2.9	0.32	1.5	12
4-6	1.3	0.26	3.8	0.87	3.1	0.97	3.1	0.47	4	0.44	3	1
5-6	0.2	0.04	3.7	0.85	2.4	0.74	3	0.45	3.9	0.43	2.5	4



Combination of the companies that is optimal according to the synergistic effect:
[4-6] – 3 (generalized synergistic effect)
[2-3] –2,8 (generalized synergistic effect)
[1-6] – 2,6 (generalized synergistic effect)
[5-6] – 2,5 (generalized synergistic effect)
[3-6] – 2,4 (generalized synergistic effect)
[1-5] – 2,0 (generalized synergistic effect)

Figure 2. Scheme of clustering interaction between the companies that is optimal according to the generalized synergistic effect

Based on the analysis of Table 2, the scheme of clustering interaction between the companies was built. It is optimal according to the generalized synergistic effect Fig. 2. Scheme of cooperative interaction between the companies under study in the leather cluster enables to conclude that cluster-forming in our case are the company's №.6 and №1. Company №2, №4 have more branched but less powerful in terms of synergy relationships as compared to companies №3, № 5. The scheme helps to identify and

direct management efforts to support those areas of cooperative interactions which may potentially generate the greatest total synergistic effect.

It should be noted that, in practice, the implementation of the identified potential synergies will depend on many factors. The most significant among them is the willingness of companies' management to organize co-operation with the partners. Moreover, the possibility of obtainment of a synergistic effect in the cluster will depend on the business environment, radius of trust between economic entities, features of companies that are defined by the asymmetry of their economic development, organizational culture, etc. Generation of synergistic effect in the cluster may also decrease because of the complexity of coordinating the activities of formally independent companies, and because of the lower stability of mutual relations in the cluster structure compared to hierarchical one.

CONCLUSIONS

The study conducted resulted in the development of a method for evaluation of synergy ratios between leather companies in the cluster of Kyiv City, which enabled to build the scheme of cluster interaction of the companies that is optimal in terms of synergistic effect. It was established which of the investigated companies are cluster-forming, priority of options for cooperation with the various partners was determined. Moreover, the most significant areas for coincidence of interests in the manufacturing, sales, marketing and innovation in terms of synergy were defined. The presented method can be used both in newly formed clusters and in the existing structures to evaluate and reformat partner relationships to maximize the synergistic effect.

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THE EVOLUTION OF PRODUCTIVITY FACTORS IN THE ROMANIAN MANUFACTURING: AN ANALYSIS OF THE TEXTILE, CLOTHING AND LEATHER SECTOR

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The three pillars of productivity are new technologies, capital and labour. Observation of data of the economy shows that positive developments in some sectors are based excessively on labour and capital accumulated and less on new investments and capitalization of R & D results. Using the correlation method aims to identify the factors which ensured industrial output growth during 2000-2012. The application takes place in the textile, clothing and leather.

Keywords: productivity, indicators, textile

PRODUCTIVITY FACTORS

According to BusinessDictionary.com productivity is „a measure of the efficiency of a person, machine, factory, system, etc., in converting inputs into useful outputs". As a general approach, „productivity is computed by dividing average output per period by the total costs incurred or resources (capital, energy, material, personnel) consumed in that period. Productivity is a critical determinant of cost efficiency".

Krugman (1997) described the role of productivity in words that have remained famous and are often cited: „Productivity isn't everything, but in the long run it is almost everything. A country's ability to improve its standard of living over time depends almost entirely on its ability to raise its output per worker."

There are several known ways of measuring productivity, namely:

Single factor: labour productivity, capital productivity (with entry type: production value, gross added value)

Multi-factorial: in relation to capital and labour costs

Multi-factorial: KLEMS (L- labour costs, K - capital (partial) costs, M - material costs, S - services (parts) costs, E - technological utilities costs)

Total Factor Productivity (TFP) is a variable which represents the total production effects caused by other inputs that are traditionally measured (labour and capital). Total Factor Productivity (TFP) can be taken as a long-term assessment of technological changes of an economy or technological dynamism.

In different approaches linking inflows to outflows of capital and labour, it is introduced the total factor of productivity, the most common formulas being formula Cobb-Douglas and Solow formula.

Cobb-Douglas formula is:

$$Y = A \times K^{\alpha} \times L^{\beta} \quad (1)$$

Where Y - total output; A - expression of total factor productivity, K - capital inflows, L - labour inputs, α and β the sizes that take into account the contributions of L and K

Solow formula is:

$$Y(t) = [K(t)]^{e}[A(t)L(t)]^{1-\alpha} \quad (2)$$

where Y(t) the total production associated to the time interval t; K(t) capital employed; L(t) the number of people at work; A(t) multifactor of productivity, technology; α measure that takes into account the contributions of L and K.

There are many factors influencing the relationship between the output and the input, but in most cases, three main pillars of productivity growth are considered in the overall economic approach, namely:

- Technology - new technology used directly in the production;
- Capital - contributions that support production but are not directly involved in technology renewal;
- Workforce - education and training (integration capability, use of resource, motivation).

Without aiming to make an estimation of the quantities included in the previous formulas, we believe it is useful to analyse how the increase of production value in some economic sectors is correlated with data sets that contribute in different approaches to the determination of indicators of productivity.

DEVELOPMENTS OF ECONOMIC INDICATORS IN TEXTILES, CLOTHING AND LEATHER SECTORS

For analysis we targeted a group of economic activities that can be aggregated, consisting of three industries, namely: Manufacture of textiles (NACE Division 13), Manufacture of wearing apparel (NACE Division 14), Tanning and manufacture of leather, manufacture of luggage, handbags, saddlery and harness, dressing and dyeing of fur (NACE Division 15).

We tried to emphasize the factors that have influenced the value of production and whether the contribution of capital and labour can be evidenced in the form of some correlations with production.

The period considered for analysis is marked by two major events: restructuring of industrial activities in the post privatization and EU accession, which brought major structural developments regarding performance indicators. Significant indicators for the financial statements of companies are presented in the following chart.

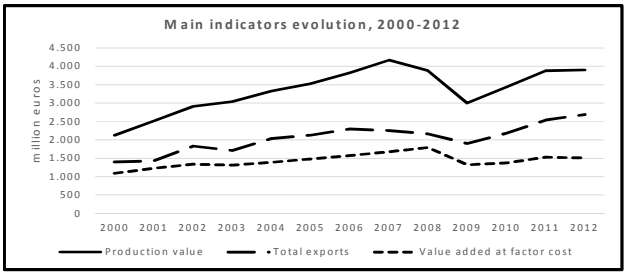

Figure 1. Main indicators evolution of textile, clothing and leather manufacturing in Romania (2000-2012) – Data source: INS

Although the crisis led to a considerable drop in the production value, this was rather a consequence of declining domestic orders, while export maintained an overall positive growth trend. Gross added value remained at a very low level and does not reflect the positive developments in the growth of production and exports.

The causes do not seem to be related to the volume of orders but rather to the efficiency of manufacturing processes of enterprises in Romania. Compared to the situation from France and Italy, the added value per employee for businesses in Romania had a hard to tolerate level, being 10-12 times lower. The values are shown in the following figure.

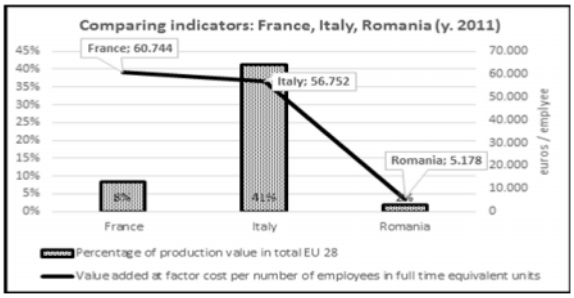

Figure 2. Comparison of Romania expressed labour productivity versus EU countries (y. 2011)

The results of increased productivity, including better use of resources, machinery and labour create not only more and cheaper consumption goods, but also more resources for wage increases. Both effects contribute to increasing the living standard, major goal of state policy.

Poor performance in terms of productivity, expressed as added value to cost factor per employee, does not support efforts to increase the quality of life, nor ensure the accumulation of resources for increasing competitiveness in manufacturing of textiles, clothing and leather. The products in this segment contribute directly to economic welfare but do not provide adequate wages increases.

RESEARCH HYPOTHESES

Starting from the two formulas of the productivity factors, the research aims if the assumed factors contributed to the growth of the mentioned economic sector.

The preliminary observation of economic indicators and the situation in the segment analysed during 2000-2012, suggest that to some extent, the increase in production value is a consequence of export orders and that there is no major concern in increasing the efficiency of production processes through investment and R&D.

It also may suggest that the increase in gross added value per employee is a consequence of the restructuring plan, expected in the post privatization period, rather than as an effect of the investment and the application of new technologies.

Since it is expected that the behaviour of enterprises will be different by size category, there were analysed both total businesses and series for large and medium-sized enterprises.

CORRELATION FACTORS

The correlation analysis between series of indicators will be done using Microsoft Excel 2013 Correlation function in the Data Analysis pack.

Data sets are shown in the following tables (from 1 to 3). Data was taken from publications of the National Statistics Institute available both online (www.insse.ro) and in print.

Table 1. Indicators of textiles, clothing and leather manufacturing from Romania (2002-2012) - total enterprise, billions lei

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Producția exercițiului	4,24	6,54	9,10	11,42	13,48	12,79	13,47	13,91	14,29	12,71	14,48	16,44	17,36
Număr mediu de salariați	488.211	520.888	530.081	537.032	515.370	465.286	386.033	374.779	316.162	255.583	239.887	253.023	250.401
Exporturi directe	2,80	3,72	5,73	6,48	8,25	7,71	8,10	7,52	7,96	8,05	9,18	10,76	11,98
Valoarea adăugată brută la costul factorilor	2,17	3,20	4,19	4,94	5,63	5,37	5,54	5,59	6,60	5,59	5,79	6,47	6,73
Investiții realizate	0,36	0,55	0,80	1,08	0,63	0,80	1,03	0,90	0,94	0,52	0,55	0,65	0,59
Mijloace fixe	1,59	2,38	3,22	5,24	6,90	6,94	7,63	10,50	8,73	7,89	8,11	7,88	8,13
Cheltuieli totale din activitatea de cercetare-dezvoltare - milioane lei	2,87	1,84	2,08	4,08	3,27	4,13	2,36	5,02	6,90	1,90	3,43	1,41	8,84

Table 2. Indicators of textiles, clothing and leather manufacturing from Romania (2002-2012) – enterprises with more than 250 employees, billions lei

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Producția exercițiului	2,52	3,85	4,87	5,89	6,84	5,63	6,01	6,02	5,82	4,89	5,60	6,65	7,73
Număr mediu de salariați	300.705	301.568	291.145	287.795	256.758	220.559	187.602	156.156	124.205	96.678	89.390	94.304	95.845
Exporturi directe	1,58	2,39	3,64	4,11	5,04	4,15	4,58	4,01	3,95	3,86	4,61	5,60	6,79
Valoarea adăugată brută la costul factorilor	1,41	1,94	2,34	2,75	3,07	2,73	2,65	2,64	2,74	2,30	2,36	2,74	2,92
Investiții realizate	0,19	0,28	0,56	0,36	0,69	0,32	0,33	0,28	0,29	0,15	0,15	0,20	0,24

Table 3. Indicators of textiles, clothing and leather manufacturing from Romania (2002-2012) – enterprises with 50 to 249 employees, billions lei

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Producția exercițiului	1,05	1,33	2,74	3,72	4,51	4,69	4,74	5,11	5,80	5,19	5,73	6,52	6,39
Număr mediu de salariați	136.277	163.293	180.156	183.657	184.617	171.906	162.790	142.336	122.443	99.957	95.696	104.505	98.879
Exporturi directe	0,51	1,17	1,55	2,00	2,56	2,95	2,71	2,88	3,52	3,53	3,72	4,39	4,38
Valoarea adăugată brută la costul factorilor	0,54	0,94	1,28	1,66	1,87	1,79	2,01	2,17	2,48	2,16	2,29	2,53	2,53
Investiții realizate	0,12	0,23	0,94	0,49	0,44	0,28	0,31	0,31	1,54	0,17	0,20	0,24	0,21

The correlation coefficient is a measure of the extent to which two measurement variables "vary together." The value of any correlation coefficient must be between -1 and +1 inclusive. The correlation analysis tool can be used to examine each pair of measurement variables to determine whether the two measurement variables tend to move together — that is, whether large values of one variable tend to be associated with large values of the other (positive correlation), whether small values of one variable tend to be associated with large values of the other (negative correlation), or whether values of both variables tend to be unrelated (correlation near „zero"). The following guidelines on strength of relationship are often useful (Source: https://explorable.com/statistical-correlation).

Table 4. Guidelines for interpretation of the correlation coefficient

Positive correlation		Negative correlation	
Coefficient value	Strength of relationship	Coefficient value	Strength of relationship
1.0 to 0.5	Strong	-1.0 to -0.5	Strong
0.3 to 0.5	Moderate	-0.5 to -0.3	Moderate
0.1 to 0.3	Weak	-0.3 to -0.1	Weak
-0.1 to 0.1	None or very weak	-0.1 to 0.1	None or very weak

Measurements generated the results shown below in Figures 4 and 5. According to the level of correlation coefficients of the indicators with the production value (Figure 4), the exports, added value to factor cost and total value of fixed assets record a strong

positive correlation. R&D expenditures and investments recorded a moderate or low correlation and the number of employees a strong negative correlation. These results are recorded, with small differences, for medium and large enterprises as well.

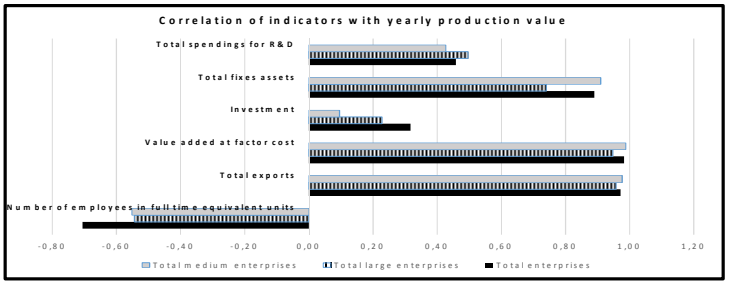

Figure 3. Correlation of indicators with yearly production value

The medium and low correlation of R&D spending and investment do not appear to be factors that contributed to the increase in production value. Not even inclusive analysis of these types of expenses offset by one or two years would still not have brought a higher level correlation. Despite the low precision of the linear mathematical model, one may find the idea that R&D and investments were intended more to solving organizational problems, including environmental costs, restructuring measures of administrative functions or support quality assurance. The negative correlation to the movement of the number of employees is explained by job losses in the post privatization period which has brought a reduction almost by half of the jobs in the economy segment analysed.

The correlation with added value per employee shows similar results, with a greater differentiation between the large and the medium enterprises (Figure 5).

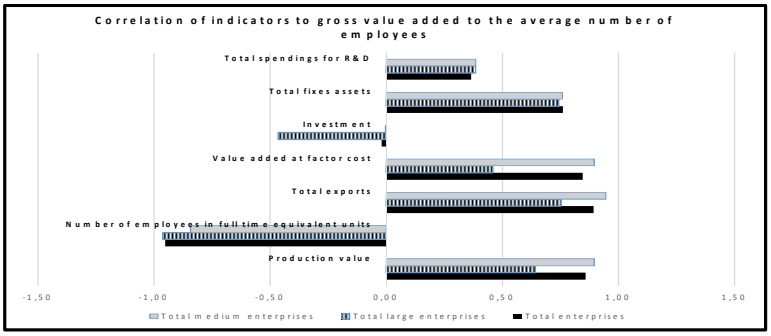

Figure 4. Correlation of indicators to gross value added to the average number of employees

For correlations with added value per employee, the results are marked by the reduction of staff based on post-privatization restructuring criteria.

To interpret the actual results of correlation is important to observe the position of the total industry economic segment put into analysis. The segment analysed accounts 15.3% of the enterprises from the total manufacturing industry, 21.7% from the

employees, achieving only 6.7% of production value, 10.2% of the exports and 11,2% of the added value to factor costs.

In terms of outlook, the economy segment analysed targets an increase in production value increasing with higher rates than the industry, especially in textile and leather manufacturing segments, as detailed in Figure 7.

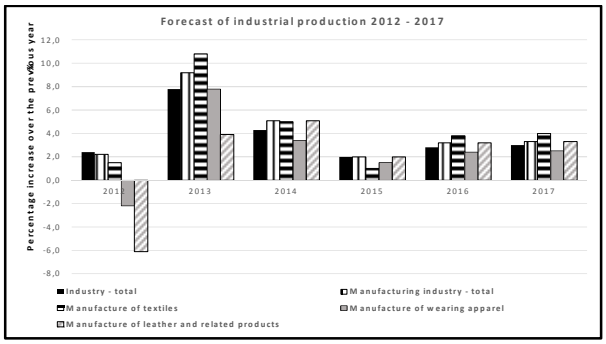

Figure 5. Forecast of industrial production 2012 - 2017 (Source: CNP)

CONCLUSION

In the period 2000-2012, the growth of production value of textile, clothing and leather items had as correlation element the growth of exports, fixed assets and gross added value to factor cost. The period was marked by a massive drop by almost 50% in employment during the same period.

The correlation to R&D expenditure and investment was moderate or low, which could lead to the conclusion that these efforts have not consistently supported increased production value and added value per employee. R&D expenses represented 0.05% of the production value in 2012, while new investments accounted for only 3.41%.

Situation of analysed sector is atypical if related to existing models. Productivity growth is rather a result of increased utilization of labour and the introduction of new technologies is greatly reduced.

Valorisation results of R & D entities in Romania can be a solution to the economic efficiency of the sector, in particular by promoting smart materials, individual protective equipment and advanced finishing technologies.

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QUALITY ASSESSMENT OF LEATHER PRODUCTS USING THE METHOD OF ABSOLUTE VALUE PARAMETERS

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In the last two decades, the development of leather goods industry, rom the point of view of sustainability, was characterized by emphasizing the two groups of the relevant parameters: (1) Development of production volumes, from the point of view of the assortment, of product quality, the areas of sales, but also in terms of quantities produced; (2) Development of new technologies, capable of providing, in real-time, and in the quantities required by the market, products at a quality level according to customer requirements. As a result of the above, in the quality evaluation of skin products, it is necessary to develop a generally quality index, to consider themselves not only the quality of the finished product, but also aspects of quality product manufacturing process parameters finished. From the set of these parameters, some can be quantified numerically, others do not. This paper aims to develop a methodology for calculating the Synthetic Quality Index (SQI) for leather products, taking into account the effects of manufacturing parameters on the quality of the finished product. All these values will be reported in nominal quality requirements accepted.

Keywords: synthetic quality index, quality characteristics, coefficient of importance.

INTRODUCTION

With the diverse assortment of products, manufacturing processes involve a growing range of components, which by their individual contribution affects the quality of the finished product; furthermore, by combining these components, complex effects result, which, in their turn, have a relevant effect on the final quality of the product. The analysis of product quality is even more important as production volumes become higher. This raises the need to address a method of evaluating the quality of products, taking into account a large number of quality features, which in turn can give a more complete picture of the overall quality of the product. In other words, product quality resulting from the manufacturing process should be as close (or identical) as prescribed in the product documentation, in conjunction with the customer's requirements.

The set of indicators used in assessing the quality of a product contributes to assessing the usefulness of the product on the market. Because by combining selective indicators technical performance of the product are taken into account, the social effects generated by a certain quality of product delivered to the market; all this correlate with the wishes of customers and users of the product choice.

The system of indicators covering quality of a product highlights three distinct levels, namely: level 1 - corresponding to analytical indicators, level 2 - corresponding to synthetic indicators and level 3 - corresponding to complexity indicators.

An embodiment of how you can use the indicator system is the leather industry; this happens because the raw hides production is projected to increase further, especially where export restrictions persist; export deliveries of Romanian leather industry and leather goods exceeded half a milliard euros after the first quarter of 2013, thus prefiguring a new development of the field both in processing raw hides and in related

processes. In this area, the used indicator system takes into account both technological aspects and specific issues related to the leather industry market.

Technological aspects take into account the fact that sustainability was ensured by investments in order to maintain highly skilled jobs in traditional sectors, which allowed increased competitiveness of domestic producers.

Meanwhile, skin and leather products have a high profile in public perception; consumers increasingly want to know where and how to produce the products they buy. Some studies point out that they are among the first products to which there has been recorded a demand for more responsibility from social and environmental point of view. It seems that users are more acutely aware of social and environmental problems due to harmful potential of some chemicals used in leather processing.

The above considerations "forced" the leather industry to find valid answers to a range of issues such as product traceability, transparency and guarantee, performance related to the environment, social common responsibility producer-user.

A possible answer is to develop a system of synthetic indicators, which allows product placement in terms of its intrinsic quality, but also takes into account the influence product use has, in terms of quality, on the social ambient environment.

In the specialized literature, there is presented a variety of methods to evaluate the quality of products that have the above considerations.

Of the many possibilities for evaluating, the paper presents the development of a system of indicators, using the absolute value of the parameter.

ABSOLUTE VALUE METHOD OF PARAMETERS

The general index of the quality has two types of parameters in its structure targeting quality:

- numerically measurable quality parameters and
- quality parameters that can not be numerically quantified

The calculation algorithm is:

1. The selection is made of the quality characteristics of the possible crowd that characterize product quality, involved technologies and the short and medium term effects requirements arising from use of the product. (X, Y, Z, ...)

2. For these features, prescribed values are adopted; they will be the reference values or nominal values (X_N, Y_N, $Z_{N,...}$). They express the values and minimum quality requirements imposed by the designer.

3. The actual characteristics measured or evaluated by tests(for parameters that can not be quantified numerically) are determined using methods and specific equipment; to test a lot of products the medium value of each quality characteristics, X_r , Y_r , Z_r are determined using the formula:

$$X_r = [\Sigma X_i]/m; \quad Y_r = [\Sigma Y_i]/n; \quad Z_r = = [\Sigma Z_i]/p \qquad (1)$$

where m, n, p – number of pieces in the lot; i-values of characteristics obtained from tests or measurements.

4. To operate with average indicators expressed in different units, ***the medium quality index*** (I_X ,I_Y ,I_Z …) is determined for each quality characteristic separately:

$$I_X = X_r / X_N ;\Uparrow \quad I_y = Y_r / Y_N ;\Uparrow \quad I_z = Z_r / Z_N \Uparrow; \qquad (2)$$

Because the values X_N ,Y_N ,Z_N represent normal values, the minimum quality requirements and indices above have above unit values (⇑).

5. Based on these indices the ***synthetic quality index*** (I_C) is determined*:*

$$I_C = (I_X + I_Y + I_Z + \ldots)/ m \qquad (3)$$

where m= number of considered quality characteristics.

6. To highlight the importance of the features in the synthetic's quality index structure, the coefficients of importance of Characteristics of Quality (K_I) are use; the formula is:

$$I^*_C = \frac{K_X .I_X + K_Y .I_Y + K_Z .I_Z + \ldots + K_N .I_N}{K_X + K_Y + K_Z + \ldots + K_N} \quad (4)$$

in which:

I^*_C= ***Synthetic quality index ordering the importance of the quality characteristics***.

K_x, K_y, K_z, K_n= coefficients of importance; if they are expressed by numbers, they may be chosen from 1 to 10, if they are expressed as a percentage, they are represented by the sum of the percentages = 100%($\Sigma K_I = 100$)

Sometimes, the user shares the purchased multitude of the product's characteristics into two groups: the group in which they are grouped i = n base important coefficients(K_B) and another group in which they are grouped j = m aid important or auxiliary coefficients (K_A); in this case ***the synthetic quality index (I^{**}_C)*** may be written as:

$$I^{**}_C = [K_B \Sigma I_{Bi} + K_A \Sigma I_{A\,j}] / (K_B .n + K_A .m) \quad (5)$$

Using quality characteristics degrees of importance (gi), expressed in percentages ***the synthetic quality index IC***** may be written:

$$I^{**}_C = gx.I_X + gy.I_Y + gz .I_Z + \ldots + gn.I_n \quad (6)$$

in which: $1 < gi > 0$ and $\Sigma gi = 1$

Since the weights of importance given to different quality characteristics not only depends on the absolute level of features, but also on their relative level to each other, to provide a correlation of these shares in relation to the calculation of the synthetic quality index for the synthetic quality index can be written:

$$I^{**}_C = (I_X)^{\gamma x} + (I_Y)^{\gamma y} + (I_Z)^{\gamma z} + \ldots + (I_N)^{\gamma n} \quad (7)$$

or the logarithm:

$$\lg.I^{**}_C = \gamma_X .\lg.I_X + \gamma_Y \lg I_Y + \gamma_Z \lg I_Z + \ldots + \gamma_N \lg I_N + \lg a \quad (8)$$

in which: a- the logarithm constant.

To calculate γ_X ....γ_N we use the economical operation function E.

Considering that the economics of exploitation (E) integrate the technical, functional, aesthetic, economic, including social aspects that the product's presence rolls onward, we can write to E:

$$E = \varphi(X, Y, Z, \ldots, W) \quad (9)$$

wherein φ-the envelope function determined by the user.

To highlight the influence of each quality characteristic on the behavior of the E, we proceed to grant a 1% change in values for each quality characteristic in part.

For the position of economy, with one changed feature, we can write:

$$E_X = [x(1 \pm 1/100), y, z, ..w]; \; E_Y = [x, y(1 \pm 1/100)..w]; \; E_Z = [x, y, z(1 \pm 1/100), ..w]; \; E_W = [x, y, z, .., w(1 \pm 100)] \quad (10)$$

The differences are calculated: $\Delta E_X = E - E_X$; $\Delta E_Y = E - E_Y$; $\Delta E_Z = E - E_Z$;…, $\Delta E_W = E - E_W$.

Then the weights of quality characteristics(γ_i) are calculated:

$\gamma_X = \Delta E_X / E$; $\gamma_Y = \Delta E_Y / E$; $\gamma_Z = \Delta E_Z / E$; …; $\gamma_W = \Delta E_W / E$

With the quality indices and weights of each quality characteristic the synthetic quality index (I^{**}_C) can be determined.

A scale for assessing the quality of products of the same class is prepared, with advance set limits: I_{C1}-I_{C2} –satisfying; I_{C2} –I_{C3}-good; I_{C3}-I_{C4}-very good; I_{C4}-I_{C5}-excellent. The calculated value of the synthetic index introduced in the appreciation scale allows relative assessment of the quality of the analyzed product, compared with other similar products.

CASE STUDY – THE LEATHER INDUSTRY

Production processes at a tannery can be grouped into four categories: storage of hides, skins soaking operations, operations that occur after tanning and finishing operations. Transforming the raw hides and skins into tanned ones is achieved through technological process in which chemical, biochemical, mechanical processes alternate. To assess the performed process quality, the following quality features were considered relevant:

- The amount of waste generated per product, expressed in Kg/ m^2 $\rightarrow$[X_N =2,12 kg/ m^2]
- The amount of solvent consumed product, expressed in g/ m^2 $\rightarrow$[Y_N =43,36 g/m^2]
- The incidence of environmental costs in the turnover of the company, expressed in %. [Z_N = 4,2%]

Accepted or standardized values are the ones above.

To assess the quality of manufacturing processes, the trade level, a total of five lots of skins was selected; the synthetic data necessary to determine the quality index (ICS) is shown in Table 1.

The rating scale of the synthetic index has the following values: 0 to 0.550 insufficient; 0.555 to 0.999 sufficient; 1.000 to 1.499 satisfactory; 1.500 to 1.999 fine; <2 very well.

Table 1. Medium quality clues table

No.	Batch size	The medium values of the characteristics			I_X	I_Y	I_Z	
		[X]	[Y]	[Z]				
	pieces/ lot	kg/ m^2	g/ m^2	%	–	–	–	
1	50	2,10	45,00	5,1	0,99	1,03	1,2	
2	70	1,92	49,08	5.8	0,90	1,13	1,3	
3	60	3,20	38,05	4,1	1,50	0,87	0,9	
4	50	2,80	48,5	4,3	1,33	1,11	1,02	
5	40	1,86	43,02	4,25	0,87	0,99	1,01	
The medium value of the quality indexes I^m_i					1,118	1,026	1,086	i= X, Y,Z

I_C^{**}= (1,118+1,026+ 1,086) / 3= 1,04;

If we consider the share of importance of quality characteristics in the leather processing - P_X = 0,42%; P_Y = 0,20%, P_Z = 0,38%, the synthetic quality index value, with the evaluation weight of importance I^{**}_{CP} is:

I^{**}_{CP} = 1,118.0,42+1,026. 0,20+1,086.0,38 =1,08

In both cases it is observed that from the point of view of the quality index the processing loads are situated in the the synthetic acceptable.

CONCLUSIONS

The quality of the finished leathers can be exposed by a number of numeric values on different references: technical (physical, mechanical and other properties), economical (cutting area, physical productivity, chemical, water or energy quantity used) and usability of the product. Besides all these and without the possibility to be measured but fundamentally important, it's added some esthetic and organoleptic properties (dye uniformity, finishing uniformity, conformity with the sample, physical natural defects or provoked during the processing, physical-mechanical tests , etc). One of the specs that can be targeted is the role of the best practices on the sustainability of transformation the raw hides in finished leathers, analyzing especially the impact of these approaches at the technological processes quality's level.

From the economical point of view, leather is a key material, generating welfare and jobs in a large variety of value chains in which is involved as main compound as: shoes industry, garment, leather goods, upholstery, automotive, aircrafts, medicine, etc. That's why to calculate the synthetic indicia of quality for each lot or batch becomes a very important and imminent job to do!

Follow the environment indicators and their inclusion into the quality analyses to the factories in our country (small or big!), can contribute to the sustainability of the specific processes from the leather industry. Their implementation in the vision, mission and strategy of the leather factories can lead to the testing of the possibilities of collaboration with the local communities as well as with the business communities in projects linked on environmental sustainability.

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ERRATA TO PROCEEDINGS OF THE 5TH INTERNATIONAL CONFERENCE ON ADVANCED MATERIALS AND SYSTEMS 2014

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Papers

Full paper entitled *Synthesis of Antimicrobial Materials with Regenerative Function by LBL Method*, authors Oana Cristina DUTA, Denisa FICAI, Anton FICAI, Ecaterina ANDRONESCU, Roxana TRUSCA, Madalina Georgiana ALBU, Marius RADULESCU, Grigore MIHAESCU, Lia Mara DITU is missing from **BIOMATERIALS Section**, inserted after Errata, at page 723.

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SYNTHESIS OF ANTIMICROBIAL MATERIALS WITH REGENERATIVE FUNCTION BY LBL METHOD

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In this study, a series of COLL(/HA)$_n$/ZnO/Ampi multifunctional materials was obtained by LBL method (consecutive immersions in oppositely charged solutions, each immersion in the oppositely charged solution/suspension being equivalent with one layer of HA). These systems have regenerative role because of the support (COL/HA composite materials with various content of HA), antiseptic and anti-infective due to the synergic presence of ZnO and ampicillin (Ampi). Starting from these premises, different systems were obtained and characterized: COL(/HA)$_n$ and COL(/HA)$_n$/ZnO, COL(/HA)$_n$/ZnO/ampicillin. The coexistence of both components (zinc oxide and ampicillin) is very important because ZnO exhibit long-term antimicrobial activity while ampicillin is rapidly delivered and confers a strong antimicrobial activity, short-term after administration. The final products were characterized by XRD, IR spectroscopy and SEM microscopy. The delivery rate was determined by UV-Vis while the antimicrobial activity was determined by Minimal inhibitory concentration (MIC) adapted from CLSI standard method. It can conclude that drug release and antimicrobial activity are dependent on the composition and consequently on the number of depositions.

Keywords: layer-by-layer process, controlled release, multifunctional materials

INTRODUCTION

Layer-by-layer method was intensively used for different applications since discovered in the yearly 1960s (Iler, 1966). By short, this technique consist in alternative dipping of the starting material in (poly)cationic and (poly)anionic solutions/suspensions. Usually, the deposition of these layers can be controlled from the point of view of deposited weight/thickness, morphology by controlling the deposition parameters (concentrations of the solutions, pH, temperature, ionic strength, etc) (Ficai *et al.*, 2009a; Ilie *et al.*, 2011). This method was successfully applied for obtaining sensors (Hasan *et al.*, 2006; Rajesh *et al.*, 2009); coated liposomes (Angelini *et al.*, 2008), highly structured drug delivery systems (Bhadra *et al.*, 2004; Chen *et al.*, 2009; Mu *et al.*, 2012), bone graft materials (Calvert *et al.*, 1998; Ficai *et al.*, 2009b; Ilie *et al.*, 2011; Peng *et al.*, 2009), tunable surface coatings for different medical devices or implants (Citterio *et al.*, 2008; Kunjukunju *et al.*, 2013), opto-electric materials (Promnimit *et al.*, 2008; Pronmimit *et al.*, 2007), batteries (Wang *et al.*, 2013), etc. Antimicrobial materials are very interesting materials for many medical applications, especially in surgical interventions. It is well known that infections can occur once with the surgical intervention (Haerdi-Landerer *et al.*, 2010; Pritchard *et al.*, 2013). These antibacterial grafts exhibit lower systemic toxicity comparing with the systemic administrated antibiotics especially because of the loco-regional release where the concentration of antimicrobial agent is high while in the rest of

body this concentration is low (Jain *et al.*, 2005). Layer by layer technique was also used for obtaining collagen/hydroxyapatite composite materials for bone grafting and repair. In our previous studies (Ficai *et al.*, 2009b; Ilie *et al.*, 2011) we studied the physico-chemical properties of the materials obtained by LbL. In the case of collagen (COLL)/hydroxyapatite (HA) composite materials, previous these studies shown that this technique allows a linear deposition of the mineral phase (HA) onto the organic layer (collagen). Up to 7 layers, the linearity of the deposition is very good, the amount of deposited HA being proportional with the concentration of the precursors (Ilie *et al.*, 2011). This paper is devoted to the preparation of new, multifunctional bone graft materials. The multifunctionality of the obtained systems are assured by the support material - regenerative properties induced by the COLL/HA composite materials and by ZnO and ampicilline – antibacterial properties.

MATERIALS AND METHODS

Materials

Collagen matrices were provided by the National Research Institute for Textile and Leather, Collagen Department, and were obtained from calf collagen gel (MW = 300kDa) followed by cross-linking with glutaraldehyde. $Ca(OH)_2$ (Chimopa), NaH_2PO_4, ampicillin (Sigma Aldrich) were used without further purification.

Preparation

The mineralization process was carried out directly on the collagen matrix (21 pieces of collagen matrices, each of ~1x1cm; together weighing 0.5g) by repeating immersion in $Ca(OH)_2$ suspension and NaH_2PO_4 solution. In a first step collagen matrices were immersed in 200mL $Ca(OH)_2$ suspension 0.05M for 15min followed by immersion in 100mL NaH_2PO_4 0.04M, for 10 min. Once this two consecutive immersions occur, the first layer of HA is deposited (Ficai *et al.*, 2009a; Ilie *et al.*, 2011). By further alternative dippings in $Ca(OH)_2$ suspension and NaH_2PO_4 solution new layers can be deposited (LbL1-LbL9). Once all the samples are mineralized, all these samples are re-suspended in 70mL ZnO suspension (14,3mg/ml) and stirred 20 minutes. During this step, ~ 0.01g ZnO nanoparticles are deposited on each composite matrix. Finally, 5 samples are immersed 70 mL ampicillin containing 0.5g ampicillin let 20min for absorption and than the wet samples are dried by freeze drying.

Characterization

The obtained samples were morphologically and structurally characterized by X-ray diffraction – XRD, Fourier transform infrared spectroscopy – FTIR and scanning electron microscopy – SEM. The evolution of the content of the deposited HA with the number of layers was monitored continuously by gravimetric analysis. The ampicillin release was also monitored by using a liquid chromatograph equipped with DAD detector. The antimicrobial efficiency of the samples was determined by Minimal Inhibitory Concentration (MIC) method, adapted from the CLSI standard method (Clinical and Laboratory Standards Institute, 2004). The IR spectra were performed using a Thermo iN10 MX FT-IR microscope. All the spectra were obtained in reflection mode, using a cooled imaging detector (MCT Array), and by co-adding 16 spectra at a spectral resolution of 8cm while the time of acquisition was 3 second/scan.

X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu-K radiation from a Cu X-ray tube (ran at 15mA and 30 kV) was used. The samples were scanned in the Bragg angle, 2θ range of 10 – 80, with a sampling interval of 0.02.

SEM images were recorded on a HITACHI S2600N electron microscope coupled with an EDS detector, on samples covered with a very thin silver layer.

The antimicrobial activity of COLL(/HA)$_n$/ZnO/Ampi multifunctional materials was tested against *Escherichia coli* ATCC **25922** ampicillin susceptible strain. Considering that the biopolymers fragments are containing 1 mg of ampicillin, each of them were immersed in 1 ml Müller Hinton broth (to reach a final concentration of 1mg/mL) and inoculated with 10 µl bacterial suspension of 0.5 Mc Farland standard density (1 , 5x10^8 CFU / ml) and incubated at 37°C. In order to establish the release rate of ampicillin, samples were taken from each working version, at different time after inoculation (immediately, after 20 minutes, 60 minutes, 4 hours, 8 hours, and 24 hours) and viable counts (colony forming units / ml) were established, thus allowing the assessment of bacterial culture development under the influence of delivered ampicillin.

RESULTS AND DISCUSSION

In order to study the influence of certain components (LbL deposition, ZnO, ampicillin) appropriate analyses were considered especially by comparison with the pure, well characterized, COLL/HA composite materials.

X-ray diffraction recorded for mineralized composites shown that HA is the main component of the deposited phase (Ficai et al., 2009a), especially at high level of mineralization (LbL7 or 9) but also different hydrated calcium phosphates are easily identified (the most important being brushite) as well as species containing zinc (not only ZnO) proving that during the preparation procedure ZnO is partially transformed (Figure 1). When 7 or 9 layers are deposited, the mineral phase mainly consist in HA [84-1998] and brushite [72-1240].

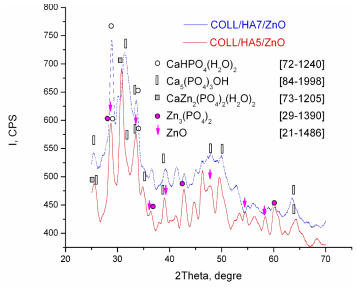

Figure 1. X-ray diffractogram recorded for material composite COLL/HA/ZnO

At low mineralization level (LbL1-LbL5), the presence of ZnO, $Zn_3(PO_4)_2$ and $CaZn_2(PO_4)_2$ $(H_2O)_2$. Ampicillin peaks can not be identified due to the small amount of ampicillin absorbed onto the COLL/HA$_n$/ZnO composite materials as well as due to the overlapping over the HA peaks. Based on the recorded diffractograms it can seen that the characteristic peaks of hydroxyapatite does not suffer significant shifts towards the positions of the pure HA.

COLL/HA_n/ZnO/Ampi multifunctional materials exhibit a special morphology most probably due to the presence of ZnO microplatelets. The morphology of these materials is strongly dependent on the content of ZnO. The samples COLL/HA_n/ZnO/Ampi, with n=1-5, the SEM images highlight the platelet form characteristic to ZnO while the samples with n=7 or 9 are similar with the pure COLL/HA (ZnO platelets are not visible). The explanation is relatively simple and takes into account the content of ZnO. Once with the increasing of the number of layers, the content of HA increase and the content of ZnO decreases and consequently, the ZnO platelets are less present. The microplatelets can reach 20-150nm (thickness) and up to 10x10 µm (width x length).

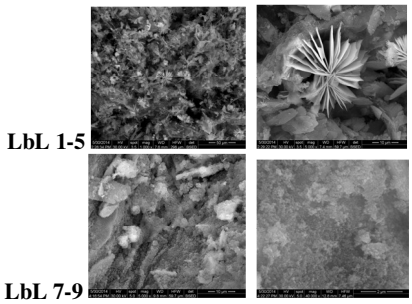

Figure 2. SEM images recorded on composite materials COL/HAn/ZnO/ampicillin

IR spectroscopy is very useful for the analysis of composite materials as well as for study the influence of zinc cation on the phosphate group. Once with the increases of the number of layers, the relative intensity of the collagen peaks decrease due to the increase content of HA (Figure 3). Unfortunately, the absorption of ampicillin from solution leads to low level of ampicillin which cannot be identified in the spectrum of COLL/HA_n/ZnO/Ampi. Even if ZnO is added from suspension (and was not directly precipitated into the COLL/HA_n matrices), the presence of ZnO leads to a strong phosphate band splitting, as result from the insert (Figure 3). This splitting of the peak form ~1030cm^{-1} is a consequence of the partial substitution of Ca^{2+} with Zn^{2+}, the FTIR data being in good agreement with the XRD data because in the case of COLL/HA_5/ZnO both analyses highlight the presence of brushite (band from ~943cm^{-1}) and three compounds containing phosphate (1030, 1065 and 1120cm^{-1}) can be identified.

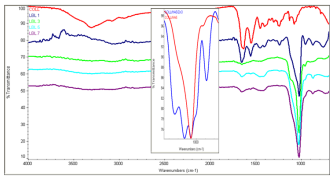

Figure 3. IR spectra of pure collagen and COLL/HA materials obtained by LbL; insert COLL/HA_5 and COLL/HA_5/ZnO (750 – 1350cm^{-1})

Ampicillin release was monitored by UV-Vis spectroscopy after separation. It can conclude that ampicillin release is strongly influenced by the degree of mineralization. Based on the release curves of the five monitored samples (Figure 4) it can be seen that the release rate decreases with increase of the content of mineral phase deposited most

likely due to the increased density of the material and also due to the increased interaction of HA with ampicillin.

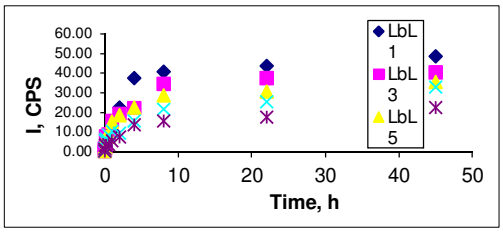

Figure 4. Release curves obtained for the systems based on inorganic compounds collagen matrix for each composition

The antimicrobial activity of COLL(/HA)$_n$/ZnO/Ampi multifunctional materials was performed toward *Escherichia coli* ATCC **25922** ampicillin susceptible strain, being evaluated the ampicillin release dynamics in bacterial growth broth medium. In most cases, the accumulation of ampicillin in the medium, to an active concentration (IC50), occurred after 8 hours of incubation, when the CFU/ml values has reached a very low level.

Bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours or more. In the intestinal tract, the coliform's generation time is estimated to be 12-24 hours but the generation time for *E. coli* in the laboratory is 15-20 minutes (Todar, 2014). The lag phase of E. coli is most affected by the varying concentrations of ampicillin. The ampicillin is an inhibitor of transpeptidase, which is needed for bacterial cell wall formation, and leads to cell lysis (Don, 2008).

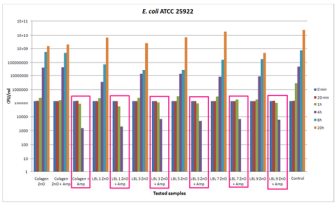

Figure 5. Graphic representation of CFU/ml values in order to evaluate the *Escherichia coli* ATCC 25922 ampicillin sensible strain growth in different time of incubation

In our experiment, in the first 60 minutes the number of the viable cells remained unchanged in the samples with the multifunctional materials containing ampicillin (figure.5). After this period, only multifunctional materials containing Zn O associated with ampicillin released the IC50 of ampicilline, expressed in the decrease of CFU.ml values (figure 5).

CONCLUSIONS

New antimicrobial materials were obtained strating from COLL/HA composite materials obtained by LbL, ZnO and ampicillin. It can conclude that during the synthesis procedure Ca^{2+} is partially substituted with Zn^{2+}. It was shown that the presence of ampicillin is usefull, all the samples obtained by LbL and containing

ampicillin had shown a very good antimicrobial activity. The regression of the *E. Coli* was quantitatively proved even at 1h while, practically these species were almost eradicated after 8h. The antimicrobial activity of the ZnO was not visible because the antimicrobial activity of these nanoparticles involves their presence in free form to be able to penetrate the cellular membrane of these microorganisms.

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