

## COLLAGEN NETWORKS OBTAINED BY LEATHER INDUSTRY BY-PRODUCTS

BOGDAN CURSARU<sup>1,2</sup>, MIHAELA-DOINA NICULESCU<sup>1</sup>, MARIANA DANIELA BERECHET<sup>1</sup>, MIHAI RADU<sup>3</sup>, M D LINA CAMELIA IGNAT<sup>1</sup>, LAURA-M D LINA POPESCU<sup>4</sup>, IOAN ALBERT TUDOR<sup>4</sup>

<sup>1</sup>*INCDTP - Division Leather and Footwear Research Institute, 93 Ion Minulescu st., District 3, Bucharest, 031215, Romania, bogdancursaru@yahoo.com*

<sup>2</sup>*National Research and Development Institute for Chemistry and Petrochemistry ICECHIM, Splaiul Independentei no. 202, Bucharest, 060021, Romania*

<sup>3</sup>*University POLITEHNICA of Bucharest, 1-7 Gheorghe Polizu St., Bucharest, 011061, Romania*

<sup>4</sup>*National R&D Institute for Non-ferrous and Rare Metals (INCDMNR-IMNR), 102 Biruintei Blvd., Pantelimon, Ilfov County, 077145, Romania*

Chemically cross-linked collagen polymeric networks with different structures and properties were obtained from collagen hydrolysates and glutaraldehyde and were designed for potential applications in bioeconomy. The swelling behavior of these networks was tested in distilled water at constant temperature and the equilibrium swelling degree was calculated. The influence of different cross-linking parameters used to tailor the structure and properties of these networks (collagen-glutaraldehyde ratio and the aminic nitrogen content of the collagen hydrolysate) upon the collagen networks was investigated by FTIR spectroscopy, thermal analysis, swelling tests and contact angle measurements. Different amounts of glycerol were added in order to further improve the general properties of the collagen networks. The general properties of these materials led to the conclusion that they could be used for potential applications in bioeconomy.

Keywords: Collagen hydrolysate, cross-linking, bioeconomy

## INTRODUCTION

Hydrogels are three-dimensional cross-linked polymeric networks which exhibit the ability to swell and retain large volumes of water in their structure, without dissolution. Their resistance to dissolution is given by the cross-links between network chains. Based on the type of these cross-links, hydrogels can be classified in two categories: chemical or permanent hydrogels, which exhibit chemical junctions between polymeric chains, and physical hydrogels which presents temporary junctions that arise from weak physical interactions, such as hydrogen bonds, chain entanglements, and ionic or hydrophobic interactions. Hydrogels can be also divided in two main categories, based on the nature of the polymeric chains: natural, synthetic polymers, or combination of these two categories. Natural hydrogels are made up from polymers like collagen, gelatin, starch, agarose, and alginate. Synthetic hydrogels are obtained from chemically synthesized polymers (Ahmed, 2015).

Collagen is one of the most important and abundant proteins present in animals and humans bodies and plays a key role in the extracellular matrix and in many tissues like skin, bone, cartilage, tendons (Niculescu and Gaidau, 2014; Zhang *et al.*, 2014; Fiorani *et al.*, 2014). Collagen is currently extracted from this tissues using chemical-enzymatic or chemical methods (Niculescu and Gaidau, 2014; Fiorani *et al.* 2014). Collagen hydrolysate is a polypeptide compound made by further hydrolysis of collagen, which displays the ability to form thin films on different surfaces, has a hydrophilic character and is not dangerous for human health. Due to these remarkable properties, it has been used in various applications, including as a natural fertilizer in agriculture due to the release of free amino acids with a considerable nitrogen content (Niculescu and Gaidau, 2014; Pei *et al.*, 2013). Other applications of collagen hydrolysates are in regenerative medicine, in pharmaceuticals, in

cosmetics, as a food additive and as a biodegradable packing material (Pei *et al.*, 2013). Unlike gelatin, collagen hydrolyates displays a lower molecular weight and better biodegradability and absorbency (Pei *et al.*, 2013). The mechanical and thermal stability of the collagen physical hydrogels can be further improved by employing various cross-linking agents, such as: formaldehyde (Zhang *et al.*, 2014; Tan *et al.*, 2015), glutaraldehyde (Zhang *et al.*, 2014; Osborne *et al.*, 1997; Tan *et al.*, 2015; Rault *et al.*, 1996), glycosaminoglycan (Osborne *et al.*, 1997), chondroitin-6-sulphate (Osborne *et al.*, 1997), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Osborne *et al.*, 1997; Sionkowska *et al.*, 2010; Rault *et al.*, 1996), 1,1-carbonyldiimidazole (Osborne *et al.*, 1997), hexamethylene diisocyanate (Zhang *et al.*, 2014; Rault *et al.*, 1996), cyanamide (Rault *et al.*, 1996), N-hydroxysuccinimide (Sionkowska *et al.*, 2010; Tan *et al.*, 2015), diepoxypropylether (Sionkowska *et al.*, 2010), hydrazine (Rault *et al.*, 1996), diphenylphosphorylazide (Rault *et al.*, 1996), dialdehyde carboxymethyl cellulose (Tan *et al.*, 2015) and genipin (Zhang *et al.*, 2014; Biswal *et al.*, 2016). Among these cross-linkers, glutaraldehyde was widely used for the collagen/gelatine hydrogels preparation due to its high reactivity, high water solubility and low cost. The reaction mechanism of collagen cross-linking by glutaraldehyde consists of the chemical reaction between the free amino functional groups of different amino acids from collagen and the carbonyl functional group from glutaraldehyde, with the formation of a Schiff base type structure (Tian *et al.*, 2016).

The collagen hydrogels display high biocompatibility and biodegradability, whereas their mechanical and thermal properties were relative low (Nistor *et al.*, 2015). In order to overcome this disadvantages, the collagen hydrogels were additivated with various compounds, such as cellulose (Pei *et al.*, 2013), glycerol or poly(ethylene glycols) of different molecular weights (Langmaier *et al.*, 2008).

The aim of this research is to obtain new 3-D structures by reusing collagen by-products from the leather industry. Moreover, the target of this research is to transform low-economical value by-products from leather industry into value-added products for bioeconomy, following the concept of economical and environmental sustainability.

## EXPERIMENTAL

The bovine collagen hydrolysates were obtained according the procedure described (Niculescu and Gaidau, 2014), and their characteristics are presented in Table 1.

Table 1. Physico-chemical characteristics for the bovine collagen hydrolysates employed for the 3-D collagen structures synthesis

No.	Characteristics, MU	HCN2	HCN3	Standard
1	Dry substance, %	9.80	9.59	SR EN ISO 4684:2006
2	Total ash, %	5.92	7.09	SR EN ISO 4047:2002
3	Total nitrogen, %	16.52	16.79	SR EN ISO 5397:1996
4	Free amino nitrogen, %	1.84	1.55	ICPI internal standard
5	Organic substance, %	92.90	94.36	SR EN ISO 5397:1996
6	Calcium oxide, %	4.90	3.43	ICPI internal standard
7	Chromium oxide, %	Values below the detection limit (0,3%)		SR EN ISO 5398-1:2008
8	pH	8.50	8.77	STAS 8619/3:1990

The bovine collagen hydrolysate HCN3 was employed for the formation of HCG-5 and HCG-9 hydrogels, whereas HCG-11 was obtained from the HCN2 collagen hydrolysate

(which displays a higher free amino N<sub>2</sub> content). In all cases the crosslinking agent was a 25% aqueous solution of glutaraldehyde. The HCG-9 and HCG-11 hydrogels were additivated with 25% and 2.5% glycerol, respectively, whereas HCG-5 was not additivated.

The FTIR spectra of the collagen xerogels were recorded in the transmission mode (FTIR-ATR) on a ABB MB3000 spectrometer. The IR spectra was recorded in the wavenumbers range of 4000-550 cm<sup>-1</sup> and consists of 64 scans at 4 cm<sup>-1</sup> spectral resolution. The DSC measurements of the collagen xerogels were performed on a Netzsch DSC200 F3 Maia thermal analysis system. The measuring conditions were the following: temperature range: -20 - 400°C, the heating rate was 10°C/min, and the Argon flow rate was 1Lh<sup>-1</sup>. The contact angle of a water droplet on the xerogel surface was measured with the VCA Optima XE device. The surface energy was determined by the means of the VCA Optima XE device software. For the swelling tests, the xerogel thickness was measured using a micrometer.

## RESULTS AND DISCUSSION

The free amino nitrogen content provides valuable information for the free amino groups available for the cross-linking reaction with glutaraldehyde. The HCN2 hydrolysate had a free amino nitrogen content of 1.84%, whereas HCN3 had 1.55%. After pH adjustment at the value of 7.4, the reaction mixture (collagen hydrolysate, glutaraldehyde and glycerol) was very well stirred and injected through a 2.5 mm thick circular rubber gasket between two parallel glass square plates (as described in *Cursaru et al.*, 2008), as one can see in Figure 1A. This device was kept in an oven at 40°C for 1 hour. At the end of the time, the device was opened and the hydrogel circular plate was cut into 10 mm diameter disks. These disks were after immersed into a determined volume of distilled water (considered in excess), and kept 6 days at room temperature with water changing every day, in order to remove the unreacted species. Figure 1B presents the increased volume of the collagen hydrogel at equilibrium swelling, as compare with the initial dry disk (xerogel).

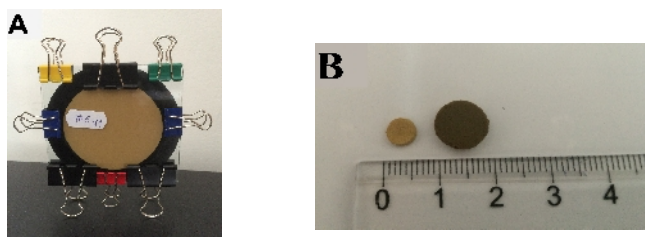


Figure 1. The device employed in the synthesis of the collagen networks (A); Hydrogel disks at the equilibrium swelling at 28°C (B)

Figure 2 comparatively presents FT-IR spectra of collagen hydrolysate (HCN3) and of the corresponding hydrogel (HCG-5). In these spectra, only HCG-5 displayed the two characteristic peaks of a Schiff base: at 3325 cm<sup>-1</sup> (assigned to stretching vibration of N-H group) and at 1670 cm<sup>-1</sup> (assigned to carbonyl groups). The presence of these peaks demonstrates the occurrence of the cross-linking reaction between collagen hydrolysate and glutaraldehyde. Both the collagen hydrolysate (HCN3) and its corresponding collagen network (HCG-5) displayed the following peaks, respectively:

O-H stretching vibration at 3069 and 3204  $\text{cm}^{-1}$ ; asymmetric stretching vibrations of  $-\text{CH}_2$  group at 2947-2874  $\text{cm}^{-1}$  and 2945-2872  $\text{cm}^{-1}$ ; characteristic bands of amide I (1637-1657  $\text{cm}^{-1}$  in the case of HCN3 and 1655  $\text{cm}^{-1}$  for HCG-5) due to  $\text{C}=\text{O}$ ; amide II (at 1540 and 1560  $\text{cm}^{-1}$ ) due to  $\text{C}-\text{N}$  and  $\text{N}-\text{H}$ ; amide III at 1248  $\text{cm}^{-1}$  for HCN3 and 1240  $\text{cm}^{-1}$  in the case of HCG5 (due to  $\text{C}-\text{N}$  and  $\text{N}-\text{H}$ ).

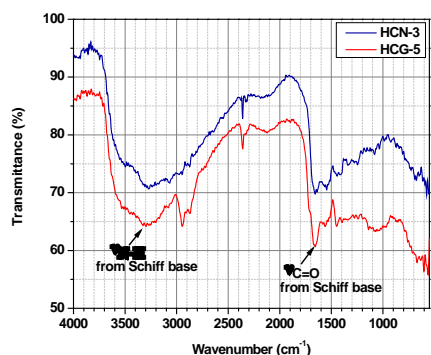


Figure 2. FT-IR spectra of collagen hydrolysate (HCN3) and of the corresponding hydrogel (HCG-5)

Swelling studies were done, in order to comparatively analyse the swelling behavior of the 3-D collagen networks. The swelling degree was determined by immersing a weighted xerogel disk ( $W_0$ ) (with a diameter around 6 mm and a thickness in the range of 1.25-1.5 mm) into a determined volume of distilled water at a constant temperature (28°C). The disks were extracted from the water at certain time intervals, superficially wiped with filter paper, weighted ( $W_t$ ) and sank again. The swelling experiments were carried out until the swelling degree of the hydrogel reached a constant value, corresponding to the equilibrium swelling degree. The swelling degree was determined according to equation (1) (Cursaru *et al.*, 2008):

$$\text{SD} = (W_t - W_0) / W_0 \quad (1)$$

Evolution of the swelling degree in time for the HCG-5, HCG-9 and HCG-11 hydrogels is presented in Figure 3.

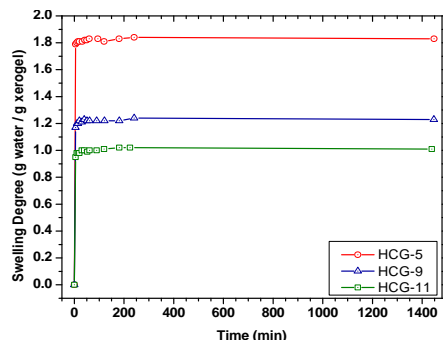


Figure 3. Evolution of the swelling degree in time for the HCG-5, HCG-9 and HCG-11 hydrogels

The equilibrium swelling degree (ESD) values were 1.83 for HCG-5, 1.23 in the case of HCG-9 and 1.02 corresponding to HCG-11.

Both the contact angle of a water droplet on a xerogel surface and the calculated surface energy are depicted in Figure 4, and together reveals the hydrophilic character of the hydrogels analysed.

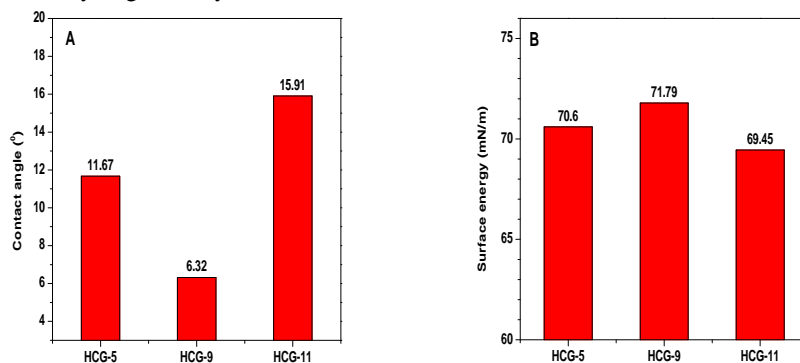


Figure 4. Contact angle of collagen networks (A) and the calculated surface energy (B) on HCG-5, HCG-9 and HCG-11 disks surface

From DSC curves depicted in Figure 5, one can observe a superior thermal stability of the xerogel (HCG-11) compared with the precursor hydrolysate (HCN2). This could be due to the cross-links present in the hydrogel network. Moreover, thermal degradation occurs in only two steps (at 180° and 333.8°C), comparing to collagen hydrolysate precursor, where more endothermic peaks can be observed.

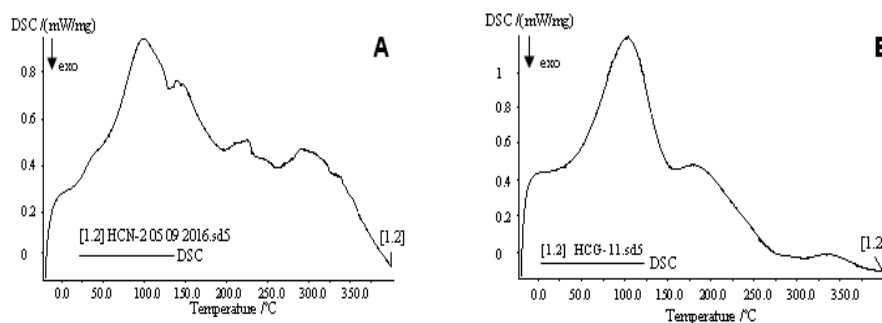


Figure 5. DSC curve for HCN2 hydrolysate and for HCG-11 xerogel (plasticized with 2.5% glycerol)

## CONCLUSIONS

Some new collagen 3-D networks with potential applications in bioeconomy were obtained from two different bovine collagen hydrolysates and glutaraldehyde.

The FT-IR spectra revealed the characteristic peak of a Schiff-base compound only on collagen hydrogels spectra, which demonstrated the occurrence of cross-linking

reaction between free amino groups present in collagen hydrolysates and the carbonyl function of glutaraldehyde.

The DSC thermograms revealed higher thermal stability in the case of the collagen hydrogels additivated with glycerol in comparison with the precursor collagen hydrolysate.

The contact angle determination demonstrated the hydrophilic character of the collagen hydrogels.

The HCG-5 hydrogel exhibited a higher swelling degree in comparison with the two others hydrogels, which contains glycerol.

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