DOI: 10.2478/9788367405805-002

ANTIBACTERIAL COATING FOR SHOE INSOLES WITH TITANIUM DIOXIDE NANOPARTICLES

DESISLAVA S. ANGELOVA[1], DARINA ZHELEVA[1],
YANA GOCHEVA[2], EKATERINA KRUMOVA[2]

[1]*Department of Textile, Leather and Fuels, University of Chemical Technology and Metallurgy, Sofia, Bulgaria, E-mail: darinajeleva@abv.bg*

[2]*Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria*

To protect the human foot from the attack of bacteria and fungi, it is extremely important to choose an appropriate antimicrobial agent. The main component building skin tissue is collagen. Hydrolyzed collagen is widely used in medicine due to its properties of biodegradability, biocompatibility and non-toxicity. It is characterized by excellent film-forming properties. TiO_2 nanostructures have been widely studied as antimicrobial agents due to their photocatalytic activity under UV light and possess excellent antibacterial properties. The application of nanomaterials to treated leather surfaces resists the growth of microorganisms as well as exhibiting a self-cleaning effect. In this study, TiO_2 nanoparticles were *in situ* synthesized using oxalic acid as a precursor. These particles were deposited on the cross-linked gelatin hydrogel as finishing layer applied to shoe insoles. SEM, UV-Vis, FTIR and antibacterial tests for Gram-positive and Gram-negative bacteria were performed. Microscopic observations prove a distribution of titanium dioxide nanoparticles and enveloped by the collagen film. The presence of chemical and physical bonds between the different components of the biocomposite film has been demonstrated. The antimicrobial activity of the investigated shoe insole samples was evaluated by the reduction of bacterial growth. Shoe insoles coated with gelatin-titanium dioxide nanocomposite show high antibacterial activity against the strains used. Consequently, incorporating metal particles such as TiO_2 into the cross-linked collagen hydrogel and fixing it to the treated leather shoe material is a good alternative for antimicrobial treatment.

Keywords: antibacterial finishing, insole, TiO_2 nanoparticles

INTRODUCTION

Natural leather is the most commonly used material for shoe insoles. Because of its absorbent and breathable properties, leather has unique characteristics, climate-balancing, and good hygienic effects on the shoe. The main component of leather is protein; therefore, it creates conditions for breeding microorganisms, including bacteria and fungi during the storage and use process, which may affect the structure and properties of leather and damage the health of users. Moreover, the human foot is in a continuous condition of increased temperature, pH and humidity. High levels of hydration, humidity and ambient temperatures, lead to an increase in the number of microorganisms, especially *Staphylococcus aureus* (Healy *et al*., 2010). Commonly used protection chemicals are mostly volatile organic compounds, due to their carcinogenic effect and environmental toxicity, it is prone to become unacceptable (Bai *et al*., 2022). This has led to the idea of developing antimicrobial material leather for insoles with a more durable and effective treatment without the use of harmful chemicals.

Considerable research has been focused on the treatment of leather with nanometal oxides during the pre-tanning process, where nanomaterials are added to the tanning solution. However, there are still few studies that deal with the nanoprocessing of leather in the final finishing step without adding the nanomaterial to the tanning solution. Several nano-metal oxides (Ag_2O, ZnO, MnO_2, CuO) have great, causing destruction of the cell wall and

https://doi.org/10.2478/9788367405805-002

considerable binding affinity toward thiol -SH- containing groups, showing significant antimicrobial effects (Muthukrishnan *et al*., 2019; Wang *et al*., 2017).

A sole leather loaded with nano-zinc oxide has been developed and the treated leather samples showed significant resistance against fungus and bacteria (Habib *et al*., 2023). Titanium dioxide nanoparticles (TiO_2-NPs) are already used in various practical applications, such as water and air purification, self-cleaning and self-sterilizing surfaces, and optical and dielectric devices. One of TiO_2 uses is as a pigment because of its brightness, high refractive index, and resistance to discoloration. Because of its interesting photocatalytic properties, TiO_2 has been used in decontamination, purification, and deodorization of air and wastewater but it also has the ability to kill cancer cells, bacteria and viruses under mild UV illumination. TiO_2 proves to be the most suitable safe and broad-spectrum antimicrobial agent (Sunada *et al*., 2003). Studies have shown an increase in the antimicrobial activity of leather coated with Ag–TiO_2 NPs (Carvalho *et al*., 2018; Khalid, 2022; Gaidău *et al*., 2016; Kaygusuz *et al*., 2016; Marques *et al*., 2022; Ignat *et al*., 2020). Others are considering the possibility of use in the leather treatment process with the addition of the TiO_2-SiO_2 nanocomposite (Kaygusuz *et al*., 2017).

The finishing film of leather can vary greatly, mainly depending on the its purpose. The sol-gel method is one of the most widely used techniques for the synthesis of functional coating films for substrate surface modification and the improvement of material properties which are affected by surface conditions (Tan *et al*., 2021). This technique possesses a number of advantages over conventional film formation techniques, including a relatively low processing temperature, ease of applying homogeneous multicomponent oxide films over large surfaces, and good control of the composition and properties of the final material.

In this study, a finish coating was successfully obtained by modifying leather samples with cross-linked gelatin containing TiO_2 particles, which were synthesized *in situ*.

MATERIALS AND METHODS

Materials

Natural pig leather from a local producer, chrome-tanned, no finish, 0.89 mm thick. The test pieces are 100/50 mm in size, and the average weight of the samples is 2.4 g. TiO_2 from Sigma-Aldrich (Darmstadt, Germany); Glutaraldehyde (25% aqueous solution) from Sigma-Aldrich (Darmstadt, Germany); Gelatin from Merck KGaA (Darmstadt, Germany); and oxalic acid ($H_2C_2O_4$) from Merck KGaA (Darmstadt, Germany).

Three variants of modification of leather materials were applied: *LT-1* (Le-TiO_2_1), *LT-2* (Le-TiO_2_2), and *LT-3* (Le-TiO_2_3). The finishing films were obtained by immersion in the different solutions in equal concentrations. For all samples, aqueous solutions were used in the following concentrations: 5% gelatin solution, 2.5% GA (glutaraldehyde), 0.1 M TiO_2, and 0.1 M $C_2H_2O_4$.

LT-1 (Le-TiO_2_1)

The leather samples were immersed in a solution of 0.1 M TiO_2 and a 0.1 M $C_2H_2O_4$ for 30 min at t=60°C. TiO_2 particles were synthesized *in situ*. After that, the samples removed from the solution and immediately immersed in solution of gelatin and finally the glutaraldehyde solution was added for crosslinking. The leather samples were dried for 2 h at t=60°C. The samples were rinsed with distilled water and left to dry at room temperature.

LT-2 (Le-TiO_2_2)

The leather samples were immersed in a gelatin solution for 2 h at t=60 °C. After that, the samples were immersed in the solution of TiO_2 and oxalic acid for 30 min at t=60°C. The next stage was adding the glutaraldehyde. Finally, the samples were washed and dried.

LT-3 (Le-TiO_2_3)

The leather samples were submerged into previously prepared solution of gelatin and glutaraldehyde. After crosslinked samples were immersed in solution of TiO_2 for 30 min and heated at t=60°C. The next stage was immersion in a solution of oxalic acid for 30 min. Then the samples are rinsed with distilled water and dried at 60 °C for 2 h.

Analysis

The surface morphology of the modified materials and the formation of TiO_2 particles were analyzed using a scanning electron microscope (SEM) Philips ESEM XL30 FEG. FTIR analysis was performed on a Fourier transform infrared spectrometer (IRAffinity-1, Shimadzu, Japan), the spectral range of 4000÷600 cm^{-1}. Spectral characteristics are taken using UVA/VIS/NIR spectrophotometer Lambda 750S PerkinElmer, USA, in range of length λ=2500÷250 nm. The leather physical and mechanical tests: Determination of water vapour permeability (ISO 14268:2023) was carried out with apparatus SATRA STM 473. The water vapour absorption indicator is determined by calculation (ISO 17229:2016).

The antimicrobial activity of the tested leather samples was evaluated using the Kirby–Bauer disc diffusion method (Hudzicki, 2009). The test bacterial strains used in this study included *Pseudomonas aeruginosa* 1390 (Gram-), *Bacillus subtilis* 168 (Gram-) and *Escherichia coli* W 1655 (Gram-): sourced from the National Bank of Industrial Microorganisms and Cell Cultures, Bulgaria, along with *Erysipelothrix rhusiopathiae* B40 (Gram+) from the culture collection of The Stephan Angeloff Institute of Microbiology. Bacterial cultures were first grown overnight in Nutrient Broth (HIMedia, India) at 37 °C, and the cell density was then standardized to McFarland 0.5. Sterile Mueller-Hinton agar plates were inoculated with the standardized bacterial suspensions. Antibiotics known to be effective against the test microorganisms were used as a positive control. The antimicrobial activity was determined after 24 h of incubation by measuring the diameter (mm) of the inhibition zones (ZOI) around each disc.

RESULTS AND DISCUSSION

Morphological Properties of Modified Leather Samples with TiO_2

The SEM micrographs in Fig. 1 show the surface of a treated and untreated leather samples. It can be seen that the control leather sample (C-control) has the characteristic fibrillar collagen structure. TiO_2 particles appear clearly on the treated surface: LT-1, LT-2, LT-3, indicating the success at their deposition. In addition, the pores are clearly visible on the surface of treated leather, suggesting that the surface coating is thin. Microscopic studies showed that TiO_2 particles were impregnated into the structure of the gelatin hydrogel at LT-1 and LT-2, and were distributed into small spherical particles, while in LT-3 the particles are on the surface of the film. TiO_2-NPs has polyhedral shape with rounded edges. It is worth mentioning that small bright spots can also be identified in LT-1 and LT-2 images, which probably are non-agglomerated. Greater aggregations were observed on the leather surface in LT-3, compared to the other variants studied.

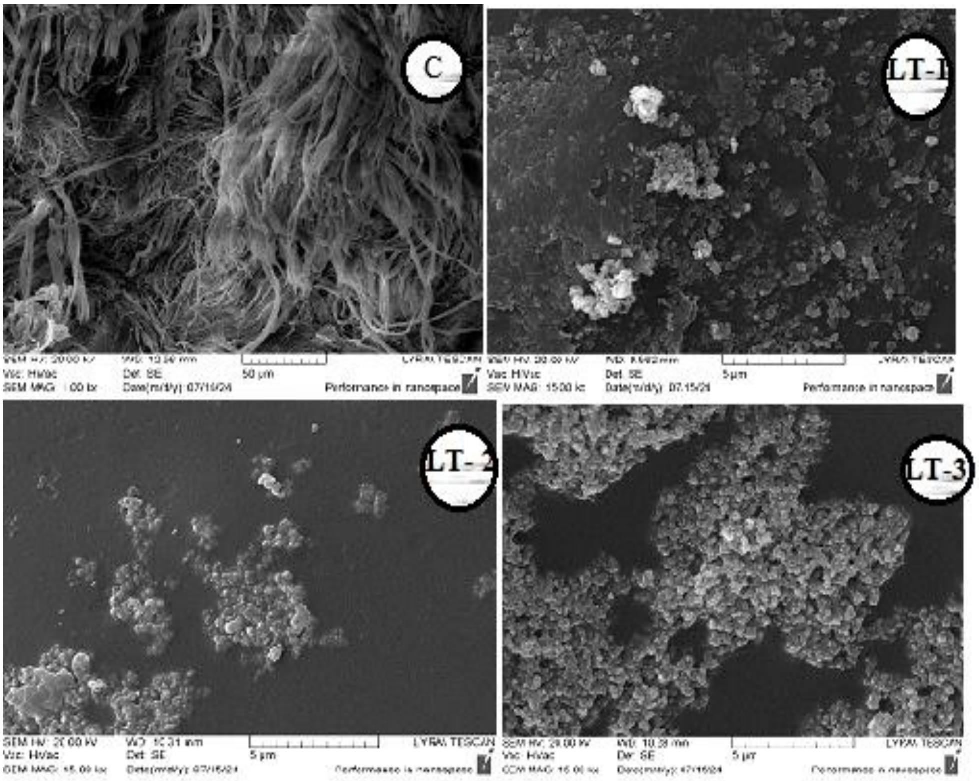

Figure 1. SEM images of the surface of the leather: C – control sample; LT-1, LT-2, LT-3 are modified leather samples

Determination of Water Vapour Permeability and Water Vapour Absorption

Table 1 shows how the various treatment options affect water vapour absorption and permeability. The samples LT-2 have reduced vapour permeability and water vapour adsorption, the samples LT-1 have increased vapour permeability and water vapour absorption as a result of the coating. The LT-3 provided the highest results, consequently these materials have the best absorbent properties and climate-balancing impact for shoe insoles.

Table 1. Vapour permeability and absorption tests of leather samples

Sample	Water vapour absorption W_1 *mg/cm²*	Water vapour permeability W_3 *mg/(cm².h)*	Vapour permeability coefficient W_2 *mg/cm²*
Le (Control)	2.17	17.41	141.45
LT-1	2.89	16.12	131.85
LT-2	1.47	13.20	107.07
LT-3	3.13	18.42	150.49

FTIR and UV-VIS Analysis

Fig. 2 represents the UV-VIS spectra of TiO_2 that showed the maximum absorption peak at 365 nm, which was a primary sign of the successful formation of TiO_2-NPs (Anandgaonker *et al*., 2019).

Fig. 3 shows absorption peaks of the FTIR spectrum of the obtained materials: 3290, 2918, 2843, 1629, 1536, 1449, 1226, 1025 cm^{-1} were assigned to –NH, $–CH_3$, $=CH_2$, –C=O, –NH, –C=O (in amide III) and C–N (in amine) groups (Carvalho *et al.*, 2018). The collagen macromolecules in the leather and in the gelatin hydrogel contain polar groups in the side chains of their constituent amino acid residues, which are amino or amide groups and carboxyl groups and can bind to metal atoms. The peaks appearing in the range of 1000–1300 cm^{-1} can be associated with Ti-O-Ti vibrations, which clearly indicate the O-Ti-O bond (Irshad *et al.*, 2021).

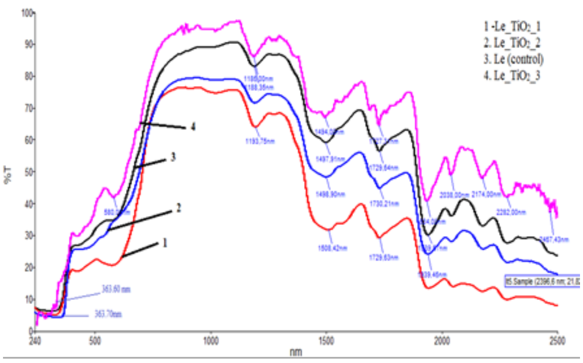

Figure 2. UV-VIS spectra of leather samples

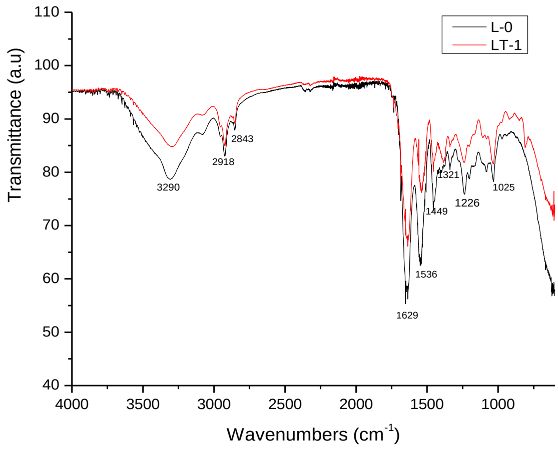

Figure 3. FTIR spectra of leather samples

Antibacterial Analysis

Fig. 4 shows the antibacterial tests for insole leather loaded with titanium oxide nanoparticles performed with the four bacterial strains. Pure leather (Le) was used as a negative control and the antibiotic discs were used as positive control. The results demonstrated antimicrobial potential of two modified samples LT-2 and LT-3.

Figure 4. The ZOI of modified leather samples against bacterial strains

The modified leather sample LT-2 exhibited the highest bactericidal effect on the both gram positive and gram-negative strains investigated. At the same time modified sample LT-3 inhibited Gram negative (*Ps. aeruginosa*) strains growth (see Table 2).

Table 2. Antimicrobial Activity Test

	Sample	*E. coli* (Gram-)	*B. subtillis* (Gram-)	*Ps.aeruginosa* (Gram-)	*Erysipelothrix rhysiopatiae* (Gram+)
1	Le (control)	-	-	-	-
2	LT-2	+3 mm	+3 mm	+4 mm	+2 mm
3	LT-3	-	-	+4 mm	-
7	Antibiotic	+7 mm	+17 mm	+18 mm	+10 mm

One possible explanation of the modified samples bactericidal effect is that it is due to the presence of Ti particles with a positive charge of titanium ions (Ti+) on the surface of the leather, which can cause oxidative stress and destruction of the bacterial cell wall.

CONCLUSIONS

A finish coating was successfully obtained by modifying leather samples with cross-linked gelatin containing TiO_2 particles, which particles were synthesized *in situ*. Three variants of synthesis of TiO_2 NPs were investigated. It was proved that LT-2 shows significant resistance against 4 types of bacteria. Consequently, these finishes can be very effectively used as protective antibacterial coatings for shoe leather materials, protecting the human foot from the effects of microorganisms.

Acknowledgment

This study is funded by the European Union-Next Generation EU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0002-C01, “BiOrgaMCT”.

REFERENCES

Anandgaonker, P., Kulkarni, G., Gaikwad, S. & Rajbhoj, A. (2019). Synthesis of TiO_2 Nanoparticles by Electrochemical Method and Their Antibacterial Application. *Arabian Journal of Chemistry*, *12*(8), 1815-1822. https://doi.org/10.1016/j.arabjc.2014.12.015

Bai, Z., Wang, X., Zheng, M., Yue, O., Xie, L., Zha, S., Dong, S., Li, T., Song, Y., Huang, M. & Liu, X. (2022). Leather for Flexible Multifunctional Bio-Based Materials: A Review. *Journal of Leather Science and Engineering*, *4*, 16. https://doi.org/10.1186/s42825-022-00091-6

Carvalho, I., Ferdov, S., Mansilla, C., Marques, S.M., Cerqueira, M.A., Pastrana, L.M., Henriques, M., Gaidău, C., Ferreira, P. & Carvalho, S. (2018). Development of Antimicrobial Leather Modified with Ag–TiO_2 Nanoparticles for Footwear Industry. *Science and Technology of Materials*, *30*, 60-68. https://doi.org/10.1016/j.stmat.2018.09.002

Gaidău, C., Petică, A., Ignat, M., Iordache, O., Ditu, L.M. & Ionescu, M. (2016). Enhanced Photocatalysts Based on Ag-TiO_2 and Ag-N-TiO_2 Nano-Particles for Multifunctional Leather Surface Coating. *Open Chemistry*, *14*(1), 383–392. https://doi.org/10.1515/chem-2016-0040

Habib, M.A. & Housseiny, M.M. (2023). Antimicrobial Performance of Sole Leather Loaded with Zinc Oxide Nanoparticle. *Journal of Natural Fibers*, *20*(2). https://doi.org/10.1080/15440478.2023.2226415

Healy, A., Dunning, D.N. & Chockalingam, N. (2010). Materials Used for Footwear Orthoses: A Review. *Footwear Science*, *2*(2), 93-110. https://doi.org/10.1080/19424280.2010.486045

Hudzicki, J. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, American Society for Microbiology, Retrieved from: https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-

Ignat, M., Miu, L., Hadîmbu, E., Șendrea, C., Micu, M.C., Păunescu, Caniola, I.M. & Badea, E. (2020). The Influence of TiO_2 and N-TiO_2 Nanopowders in Natural Leather Finishing for Heritage or Modern Binding. In L. Alexandrescu & G. Coară (Eds.), Proceedings of the 8^{th} International Conference on Advanced Materials and Systems – ICAMS 2020, Bucharest, Romania. https://doi.org/10.24264/icams-2020.V.3

Irshad, M.A., Nawaz, R., Rehman, M.Z., Adrees, M., Rizwan, M., Ali, S., Ahmad, S. & Tasleem, S. (2021). Synthesis, Characterization and Advanced Sustainable Applications of Titanium Dioxide Nanoparticles: A Review, *Ecotoxicology and Environment Safety*, *212*, 111978. https://doi.org/10.1016/j.ecoenv.2021.111978

Kaygusuz, M., Lkhagvajav, N., Yaşa, I. & Çelik. E. (2016). Antimicrobial Nano-Ag-TiO_2 Coating for Lining Leather. *Romanian Biotechnological Letters*, *21*(5), 11866-11874

Kaygusuz, M.K., Meyerb, M. & Aslanc, A. (2017). The Effect of TiO_2-SiO_2 Nanocomposite on the Performance Characteristics of Leather. *Materials Research*, *20*(4), 1103–1110. https://doi.org/10.1590/1980-5373-mr-2017-0180

Khalid, L.K. (2022). Modification, Characterization and Evaluation of Anti-Microbial Property of Ag-TiO_2 Nanoparticles Coated Traditional Leather. *Bayero Journal of Pure and Applied Sciences*, *13*(1), 53-59.

Marques, S.M., Carvalho, I., Leite, T.R., Henriques, M. & Carvalho, S. (2022). Antimicrobial TiN-Ag Coatings in Leather Insole for Diabetic Foot. *Materials*, *15*(6), 2009. https://doi.org/10.3390/ma15062009

Muthukrishnan, L., Chellappa, M. & Nanda, A. (2019). Bio-engineering and Cellular Imaging of Silver Nanoparticles as Weaponry against Multidrug Resistant Human Pathogens. *Journal of Photochemistry and Photobiology B: Biology*, *194*, 119–27. https://doi.org/10.1016/j.jphotobiol.2019.03.021

Sunada, K., Watanabe, T. & Hashimoto, K. (2003). Bactericidal Activity of Copper-Deposited TiO_2 Thin Film under Weak UV Light Illumination. *Environmental Science & Technology*, *37*(20), 4785–4789. https://doi.org/10.1021/es034106g

Tan, W.K., Muto, H., Kawamura, G., Lockman, Z. & Matsuda, A. (2021). Nanomaterial Fabrication through the Modification of Sol-Gel Derived Coatings. *Nanomaterials* (Basel), *11*(1), 181. https://doi.org/10.3390/nano11010181

Wang, L., Hu, C. & Shao, L. (2017). The Antimicrobial Activity of Nanoparticles: Present Situation and Prospects for the Future. *International Journal of Nanomedicine*, *12*, 1227-1249. https://doi.org/10.2147/IJN.S121956