Romanian Biotechnological Letters *Vol. 21, No. 5, 2016*

ORIGINAL PAPER

Antimicrobial Nano-Ag-TiO_2 Coating for Lining Leather

Received for publication March 18, 2015
Accepted, October 3, 2015

M. (KOIZHAIGANOVA) KAYGUSUZ[1*], N. LKHAGVAJAV[2], I. YAŞA[3], E. ÇELIK[4]
[1]Denizli Vocational School of Technical Sciences, Pamukkale University, Kınıklı, Denizli 20013, Turkey; [2]Leather Engineering Department, Engineering Faculty, Ege University, Bornova, Izmir 35100, Turkey; [3]Basic and Industrial Microbiology Department, Basic Science Faculty, Ege University, Bornova, Izmir 35100, Turkey; [4]Department of Metallurgical and Materials Engineering, Faculty of Engineering Dokuz Eylul University, Izmir 35160, Turkey
[*]Tel.: +902482123877; Fax: +902582118065; Email: meruyertk@pau.edu.tr

Abstract

In this study, the characterization and the antimicrobial properties of nanosized Ag-TiO_2 coatings on leather were investigated. For this purpose, turbidity, viscosity and pH of nAg-TiO_2 solutions prepared by the sol-gel method were measured. The surface morphology of treated leathers was observed using Scanning Electron Microscopy (SEM) and the elemental analysis was performed by energy dispersive spectrometer (EDS). The antimicrobial performance of nAg-TiO_2 coatings on leather materials to the test microorganisms as Escherichia coli, Staphylococcus aureus, Candida albicans and Aspergillius niger was evaluated by the application of qualitative (Agar overlay method) and quantitative (percentage of microbial reduction) tests. According to qualitative test results it was found that 2% and higher concentrations of nAg-TiO_2 on the leather samples were effective against all microorganisms tested. Moreover, quantitative test results showed that leather samples treated with 5% of nAg-TiO_2 demonstrated the highest antibacterial activity against E. coli with 93.50% bacterium removal, whereas 2% of nAg-TiO_2 on leather was enough to exhibit the excellent percentage reduction against S. aureus of 99.99%. The results are promising for the use of colloidal nano Ag-TiO_2 solution on lining leather as antimicrobial coating.

Keywords: Nano Ag-TiO_2, Antimicrobial activity, Lining leather, Coating.

1. Introduction

The use of antimicrobial agents has greatly contributed to material preservation and improvements in health quality of life. Such antimicrobial agents have been introduced for decades to treat and prevent bacteria, mold and yeast development (1). To obtain the final leather products which will have the desired properties on the surface certain functional additives can be applied to the surface of leather during the finishing process. Therefore, addition of suitable antimicrobial agents to leather surfaces can provide powerful antimicrobial functions. It was reported that to avoid or control cross infection and to extend the lifetime of the product, by stopping microbial growth, application of antimicrobial finishes and treatments on leather material can be performed (2). Antimicrobial treatment can be used in a number of ways including coating to the finished leather. However, normal leather fungicides, such as 2-(thiocyanomethylthio) benzothiazole are not suitable for use in shoe lining leather due to the disparate antimicrobial spectrum and the problem of toxicity (3). Thus, in order to enhance the antimicrobial performance of leather, a new antimicrobial agent which can be used to inhibit microorganism species need to be found. Silver or silver ions have long been known to possess strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities (4, 5), and therefore, they may have potential commercial

application in areas such as medical tools, appliances and health care products (6, 7). Silver, being a convenient and nontoxic element, improves TiO_2 bioactivity because of its inherent antibacterial activity against different microorganisms (8). Titania is an appropriate matrix for a silver titania antibacterial agent due to good distribution of silver on titania (9) and it was reported that the silver doped TiO_2 nanoparticles inhibite the growth and multiplication of microorganisms at a very low concentrations (10). Production of silver particles in nano sizes results in formation of a high surface area which is a significant property to inhibit microbial growth. Also, titania nanosize particles create more hydroxyl groups than microsized particles (11). In this study, to create leather with enhanced antifungal and antimicrobial properties, colloidal nAg-TiO_2 solutions prepared by the sol-gel method were applied on shoe lining leather. The colloidal solutions were characterized using a turbidimeter, a pH meter, and a digital rheometer. Characteristics of the films obtained from the nAg-TiO_2 solutions were determined by Scanning Electron Microscopy (SEM) and the elemental analysis was performed by energy dispersive spectrometer (EDS). The antimicrobial effects of nAg-TiO_2 coatings on lining leathers against the tested microorganisms, the bacteria *Escherichia coli* and *Staphylococcus aureus* and the fungi *Candida albicans* and *Aspergillius niger*, were investigated by qualitative (Agar Overlay Technique) and quantitative tests (Percentage of microbial reduction).

2. Experimental

2.1. Preparation and characterization of nano Ag-TiO_2 solution

The nAg-TiO_2 and TiO_2 solutions were prepared by the sol-gel method according to the following method, as explained by Yaşa et al. (10). In order to evaluate solution characteristics, which influence the structure of the thin film on leather, turbidity, pH values and rheological properties of the prepared sols were measured using a turbidimeter, a pH meter and a rheometer, respectively. VELP TB1 turbidimeter (Ustimate, Italy) was used to obtain the turbidity properties of the solutions in the range of 0–1000 ntu (nephelometric turbidity units). After preparation of solutions, their pH values were determined via a standard pH meter (WTW Inolab, Weilheim, Germany). The viscosity of the solutions was measured by means of a CVO 100 Digital rheometer (Bohlin Instrument, Worcestershire, UK). The surface morphology of treated leathers was observed using Scanning Electron Microscopy (SEM, Philips XL 20 Series) and the elemental analysis was performed by energy dispersive spectrometer (EDS).

2.2. Application of nAg-TiO_2 solutions to lining leather

The shoe lining leathers (crust) used in this study were manufactured by a conventional process without any treatment by neither bactericide nor fungicide. Leather samples measuring 2 x 2 cm were cut under sterile conditions and, except the control sample, different concentrations (0%-5%) of nAg-TiO_2 solutions were applied to the grain side of these samples. After application, the leather specimens were passed through a drying process at 105°C for 15 min and ironed at 100°C. Thus, a thin film containing 0%-5% of nAg-TiO_2 was formed on the leather samples.

2.3. Antimicrobial activity

Test microorganisms used in this study were *Escherichia coli* ATCC 12228, *Staphyloccocus aureus* ATCC 6538-P, *Candida albicans* ATCC 10239 and *Aspergillus niger* (TEM). Bacteria and *C. albicans* were activated in Muller Hinton Broth (MHB) in a shaking water-bath at 37°C for 24 h. *A. niger* was activated in Potato Dextrose Agar at 27°C for 5 days.

In Agar Overlay Technique, a qualitative test, the control leather sample and the leather samples treated with 0%-5% of nAg-TiO_2 were placed at the center of Petri dishes. 7 ml of

MHA containing 0.75% agar inoculated with bacteria and *C. albicans* (105 CFU/ml) was poured on to the leather samples on Petri dishes. To visualize microbial growth 50 ppm of triphenyl tetrazolium chloride (TTC) solution had been added to the agar. In the case of *A. niger*, the leather samples were put on Petri dishes inoculated with fungal spores (105 spore/ml) prepared in physiological saline solution (0.85% NaCl) by the spread plate technique. Plates inverted in plastic bags were incubated at 37°C for 24 h for bacteria and *C. albicans*, and at 27°C for 5 days for *A. niger*. The plates were visually assessed for zones of inhibition around and on the leather samples. The size of the inhibition zone was determined at two cross sectional point and the average was taken (12, 13). All experiments were carried out in triplicate. In percentage of microbial reduction test, a quantitative analysis, the Gram-negative bacterium *E. coli* (4.0x106 CFU/ml) and the Gram-positive bacterium *S. aureus* (7.3x105 CFU/ml) were utilized. The control leather sample and 0-5% of nAg-TiO_2 treated 2 x 2 cm leather samples were placed in 250 ml Erlenmeyer flasks with 50 ml physiological saline solution (0.85% NaCl) containing above-mentioned cell numbers of the test strains. The mixtures were cultured at 37°C in a shaking incubator for 24 h. After incubation, 1 ml of the bacteria containing mixture was serially diluted and 0.1 ml of each dilution was poured on MHA containing 0.5% TTC solution. After 24 h of incubation at 37°C, viable bacteria were counted based on colony forming units and the mean value of the cells at the lowest dilution was assessed. The reduction rate (%) of the specimen was computed according to the following equation (12-14):

Reduction rate (%) = ((A – B)/A) x 100, where A is the number of bacteria after 24 h in the control (non-treated) sample, and B is the number of bacteria after 24 h in the treated sample.

3. Results and Discussion

3.1. Solution characteristics

To reveal the complete dissolution of the powder-based precursors in the solutions turbidimetric measurements were performed. Table 1 presents the turbidity values of the prepared colloidal solutions. The turbidity values were in the range of 4.27–4.86 nephelometric turbidity units, which indicated that the powder-based chemical precursors had completely dissolved in the solutions. The turbidity values are significant in obtaining of very thin films from Ag-doped TiO_2 solutions on various substrates. So that very low turbidity values were determined, the solutions had very small particles. (Surface topography was conducted on films obtained from colloidal Ag-TiO_2 solutions by Atomic Force Microscopy in our previous work and the size of particles was detected to be in the range ~ 3-15 nm (10).

The pH value of sols is a factor which affects formation of the polymeric three-dimensional structure of the gel during the gelation process. This important issue should be taken into consideration when preparing solutions. While a ramified structure is randomly formed in acidic conditions, separated clusters are formed from solutions showing basic characters (15). The pH values of the solutions were measured via a standard pH meter. The pH values of the solutions varied from 1 to 2.

Table 1. Turbidity values of 0-5% nAg-TiO_2 colloidal solutions

Solutions	Turbidity (ntu)
TiO_2	4,32
0.25% Ag-TiO_2	4,54
0.50% Ag-TiO_2	4,27
0.75% Ag-TiO_2	4,40
1% Ag-TiO_2	4,29
2% Ag-TiO_2	4,63
5% Ag-TiO_2	4,45

The characteristic property of many sol-gel solutions is dependence of the viscosity on the shear rate or test time. Figure 1 displays the obtained viscosity for 0%-5% Ag-TiO_2 solutions. The viscosity curve presents the viscosity as a function of increasing test time. However, when the subsequent decline in the test time was probed (not shown here), no essential hysteresis effects were detected or the up-ramp curve and down-ramp curve practically intersected. The decrease in viscosity with increasing test time may be referred to the breakup of association complexes or network junctions; in other words, the rate of disruption of the complexes surpassed the rate at which associations were re-formed (10). It was determined that the viscosity of the nano-sized TiO_2 and Ag-TiO_2 colloidal solutions with different concentrations of Ag were in the range of 2–3 mPa.s.

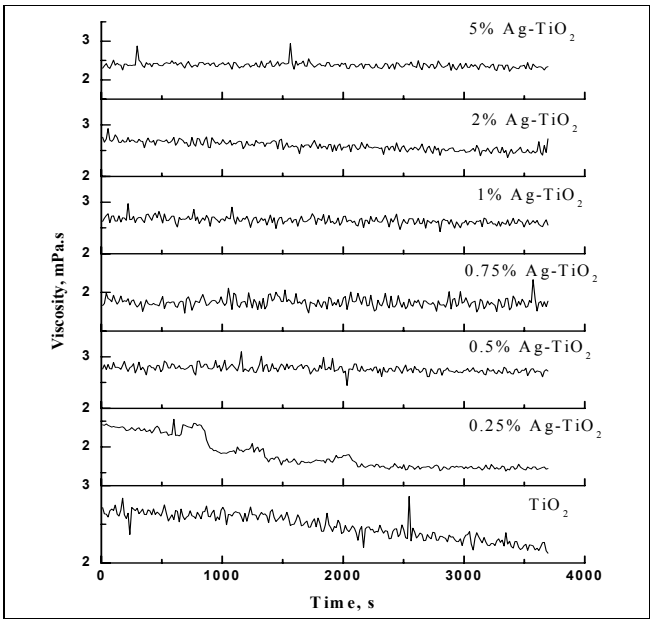


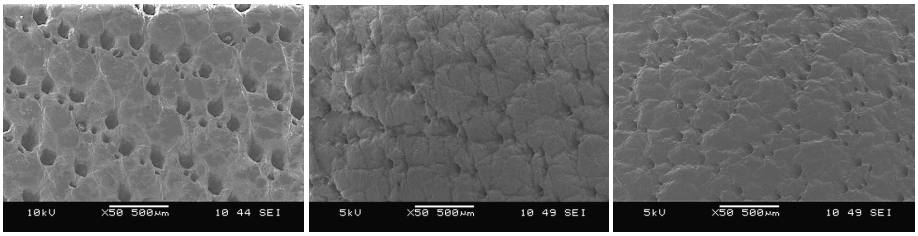
Figure 1. Viscosity values of the prepared 0%-5% Ag-TiO_2 colloidal solution

The viscosity values of every solution were close to the values for diluted solutions, suggesting that this was a key factor in controlling the film thickness. These results were reasonable for sol–gel processing since the thin films were formed from diluted solutions. In our case, nano TiO_2 and Ag-TiO_2 films were obtained with low-viscosity solutions. Furthermore, the fact that there was practically no change in the viscosity upon the addition of Ag to the solutions exhibited that the network was not significantly reinforced. In this regard, it is interesting to note that similar results were obtained in the turbidity study, in which smaller differences were observed between the solutions. A small decrease in the viscosity of solutions depending on the test time possibly signals fragmentation of the network as strong association complexes are formed (10).

3.2. Film characteristics

Figure 2a shows the grain surface of the control leather sample (without any treatment). The SEM micrograph of the grain surface of the leather sample treated with TiO_2 solution (Figure 2b) reveals that a thin, bright, transparent film was obtained on the leather substrate.

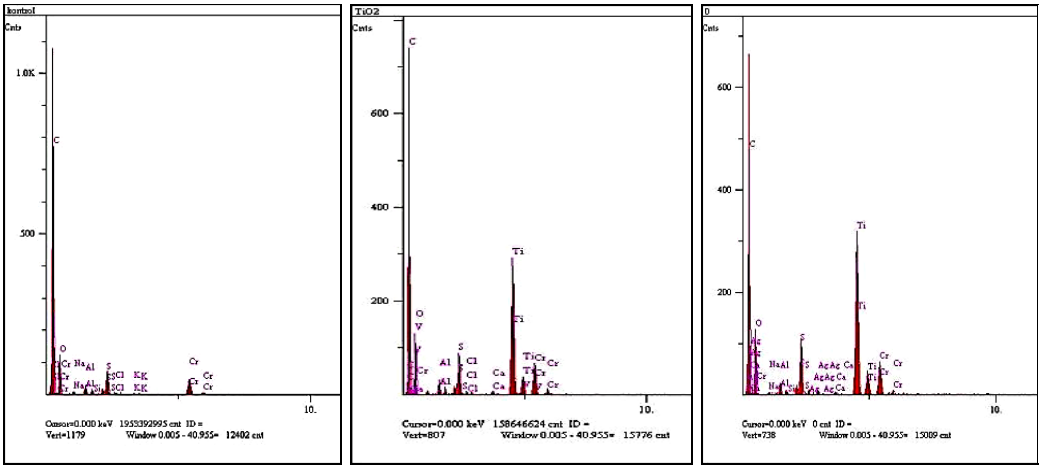
Actually, the nAg-TiO_2 coating was evenly distributed throughout the grain surface and homogenously penetrated through the pores. The pore structure of the treated leather sample was tighter than the control and TiO_2 treated sample; the film did not completely fill the leather pores (Figure 2c).



a) **b)** **c)**

Figure 2. SEM micrographs of control sample (a), samples treated with TiO_2 (b) and Ag-TiO_2 solutions (c)

The elemental composition of the control leather and the leather samples treated with pure TiO_2 and Ag-TiO_2 are shown in Figure 3. SEM-EDS microanalysis showed C, O, S and Cl contents in all leather samples. Predominant element from metals in all leather samples was Cr, followed by Al, Si and tiny amounts of Na and K. In addition to these elements a small amount of Ca was detected in the leather samples treated with TiO_2. Besides, the elemental analysis ascertained the presence of Ti in the leather samples (Figure 3b). SEM-EDS microscopy also confirmed the use of the Ag-TiO_2 as coating, identifying Ag and Ti on the leather samples treated with Ag-TiO_2 (Figure 3c).



a **b** **c**

Figure 3. SEM/EDS spectra of control sample (**a**) and leather samples treated with TiO_2 (**b**) and Ag-TiO_2 solutions (**c**)

3.3. Antimicrobial properties of nAg-TiO_2 coatings on leather material

The agar overlay test results of the leather samples against test bacteria and fungi are given in Table 2. According to the results, after appropriate incubation periods, no inhibition

zone was seen on the surface of the control sample and the leather sample treated with pure TiO_2, furthermore they were entirely covered by test microorganisms. Contrary to this, even the inhibition zones were seen around the leather samples treated with 0.25% of nAg-TiO_2 against tested bacteria, there were no inhibition zones around these samples when tested against fungi, but there were no microbial growths on the surfaces of the samples as well. The treatment of the leather samples with 0.75% - 5.0% of nAg-TiO_2 was effective and displayed good inhibition against both bacteria and fungi, as pronounced and clear inhibition zones were observed around these samples. It is obviously that greater concentrations of nAg in TiO_2 used resulted in an increase in antimicrobial activity in the treated leather samples, as indicated by the area of the associated inhibition zones around the samples (Table 2).

Table 2. Mean zones of inhibition (mm) of the leather samples

	Mean zones of inhibition (mm) against microorganisms			
Specimens	***E. coli***	***S. aureus***	***C. albicans***	***A. niger***
Control	x^a	x^a	x^a	x^a
TiO_2	x^a	x^a	x^a	x^a
0.25% Ag-TiO_2	22±1.0	23±0.6	o^b	o^b
0.75% Ag-TiO_2	24±1.0	26±0.5	22±1.0	22±0.6
1.0% Ag-TiO_2	26±0.5	29±0.7	23±1.5	23±0.5
2.0% Ag-TiO_2	29±1.0	32±0.5	25±1.0	24±0.6
5.0% Ag-TiO_2	33±0.5	34±0.7	27±1.5	25±0.5

a^x – no inhibition zone, microorganism growth on sample
b^o – no inhibition zone, but no microorganism growth on sample

All tested microorganisms were found to be sensitive at the concentrations 0.75% - 5.0% of nAg-TiO_2, because the leather specimens treated with such concentrations of nAg-TiO_2 displayed strong antimicrobial effects and zones of inhibition were in the range of 22-34 mm. The fact that no growth of any tested bacteria and fungi was determined on the surfaces of the leather samples containing concentrations of 0.75% - 5.0% of nAg-TiO_2 was considered as the result of the good spread of nAg-TiO_2 particles on the surface of leather samples. The greater size of inhibition zones around the samples with 2% - 5.0% of nAg-TiO_2 particles can be explained by the homogenous diffusion of nAg-TiO_2 into the leather samples. Table 3 lists the antibacterial properties of leather samples treated with different concentration of the nano sized Ag-TiO_2 colloids against the bacteria *E. coli* and *S. aureus*. At the initial stage, the number of *S. aureus* cells was 7.3 x 105 in all samples; after 24 h, the number of bacterial cells for the control sample and the leather sample treated with pure TiO_2 and 0.25% of nAg-TiO_2 were uncountable. The specimens treated by 2.0% and 5.0% of nAg-TiO_2 exhibited an excellent percentage reduction against *S. aureus* of 99.99% for both. However, in the case of *E. coli* there was a clear effect of the concentrations of Ag-TiO_2 nanoparticles. The antibacterial properties increased as the concentration of the nano-sized Ag-TiO_2 colloids increased. Contrary to *S. aureus*, specimens treated with pure TiO_2 and 0.25% - 0.75% of nano Ag-TiO_2 displayed weak antibacterial property against *E. coli* as 18.00%, 39.25% and 46.75% reduction, respectively. The leather samples treated with 5% of nAg-TiO_2 showed the highest antibacterial activity against *E. coli* with 93.50% of bacteria removal (Table 3).

Table 3. Bacterial reduction on leather samples treated with 0%-5% of nAg-TiO_2 solution

Initial number of bacteria	***E. coli*** 4×10^6		***S. aureus*** 7.3×10^5	
Specimens		Reduction rate (%)		Reduction rate (%)
Control	4.0×10^8	NA*	8.1×10^7	NA*
TiO_2	3.3×10^8	18.00%	1.6×10^8	NA*
0.25% Ag-TiO_2	2.4×10^8	39.25%	9.4×10^7	NA*
0.75% Ag-TiO_2	3.1×10^8	46.75%	7.3×10^7	10.30%
1.0% Ag-TiO_2	1.8×10^8	54.00%	3.7×10^3	95.45%
2.0% Ag-TiO_2	8.2×10^7	79.50%	6.3×10^4	99.99%
5.0% Ag-TiO_2	2.6×10^7	93.50%	<10	99.99%

*NA – non-active

It is well known that Ag ions and Ag-based compounds, including silver nanoparticles and metal oxides (TiO_2), are highly toxic to microorganisms, such as virus, fungus and bacterium. Gaidau et al. revealed that fungitoxic effect of the colloidal silver solution treated leather was evidenced in the case of immersion treatment (applied treatments were: immersion, spraying, tanning, retanning, etc.) and more pronounced in the presence of 50 g/L TiO_2 (16). In other study, wet-blue leather and metal-free leathers were treated by immersion in the electrochemically obtained Ag-TiO_2 dispersed solutions containing 10 g/L TiO_2 and 45 ppm Ag, with 19.7 nm diameter. The treated leathers especially wet-blue exhibited a strong antifungal activity with inhibition area up to 25 mm for mould growth and presented inhibitory action against *S. aureus* (ATCC 6538) (17). In this study, nano-sized Ag-TiO_2 colloidal solutions synthesized via the sol-gel method were applied to the grain side of the lining leather and the minimum level of nAg-TiO_2 (3-15 nm) for obtaining antimicrobial leather was established as 0.75% at which the inhibition area was in the range of 22-26 mm.

Maleknia et al. performed a study to assess the antibacterial properties of nanosized silver colloids on wool fabric (18). They reported that nano silver coating on wool fabrics not only had an antibacterial effect against *S. aureus* and *E. coli*, but also that its efficiency was still 96% after 20 washings. Yang et al. treated sheepskin with Ag nanoparticles (26 nm) at the concentration of 4.8x10-3 wt% (19). The antibacterial effect of the treated sheepskin against *S. aureus* and *E. coli* was evaluated after repeated perspiration treatments. Authors found that the treated sheepskin had an antibacterial inhibition ratio of 99.9% against the two tested bacteria without perspiration treatment. In our work, the leather samples treated with 5% of nAg-TiO_2 demonstrated the highest antibacterial activity against *E. coli* with 93.50% bacterium removal, while 2% of nAg-TiO_2 concentration applied to leather was enough to exhibit the excellent percentage reduction of *S. aureus* of 99.99%. Synthesis of Ag/TiO_2 nanocomposites has been carried out through different techniques. For practical applications, the sol–gel process is the most attractive method to introduce foreign metal ions into TiO_2 particles and films (20). It has several advantages such as high purity, homogeneity, low processing temperatures (21). In this work, this method was effective not only in preparation of Ag-TiO_2 nanoparticles, but also in easy application on leather and in forming a coating which was effective in killing the tested bacteria and fungi. Previous studies revealed high antimicrobial activity of silver nanoparticles against a broad spectrum of microorganisms. The advantage of the silver antimicrobial mechanism is the ability to produce an antibacterial effect at very low concentrations (22). Silver being better deposited in the matrix of TiO_2 provides a better interaction with leather and potentiates synergic biocidal effects (17). The quantitative test results indicating that the Ag-TiO_2 nanoparticles are responsible for the antibacterial activity of the coating on leather and this antibacterial activity is quite strong above 2% concentration of nAg-TiO_2 against both bacteria tested. Relatively, the antibacterial

effect against *E. coli* is lower than that against *S. aureus*, probably because of the difference in cell walls between Gram-positive and Gram-negative bacteria. The cell wall of *E. coli*, which consists of lipids, proteins and lipopolysaccharides (LPS), provides effective protection against biocides. However, the cell wall of Gram-positive bacteria, such as *S. aureus*, does not consist of LPS as observed by Speranza et al. (23). From the test results, it was established that the effective inhibitory concentration of nAg-TiO_2 coating on leather toward all tested microorganisms was 5%. At this concentration, not only reductions of 93.50%-99.99% in bacteria but also inhibitions of bacteria and fungi were seen. The antimicrobial performance of nAg-TiO_2 coatings on leather which was performed by a small amount can be considered as a promising application approach for the leather industry.

4. Conclusions

In summary, the colloidal nano Ag-TiO_2 solutions successfully synthesized by us had the turbidity values in the range of 4.27-4.86 ntu, the pH values of 1 to 2 and the viscosity approximately equal to 2-3 mPa.s. The thin and transparent coatings obtained from these solutions were evenly distributed. In an investigation of the applicability of nAg-TiO_2 solution produced by using the sol-gel method as an antimicrobial agent for shoe lining leather, it was found that nAg-TiO_2 solutions provide effective antimicrobial properties to leather material. Furthermore, their performances were increased by increasing the concentration of nAg-TiO_2. According to the results of the agar overlay method in vitro, the minimum level of nAg-TiO_2 for obtaining antimicrobial leather was established as 0.75%. In addition to this, the leather samples treated with 5% of nAg-TiO_2 demonstrated the highest antibacterial activity against *E. coli* with 93.50% bacterium removal, while 2% of nAg-TiO_2 concentration applied to leather was enough to exhibit the excellent percentage reduction of S. aureus of 99.99%. The results are promising for colloidal nano Ag-TiO_2 solution use in antimicrobial applications as coatings on lining leathers.

5. Acknowledgment

We would like to thank TÜBITAK (The Scientific and Technological Research Council of Turkey) for the financial support (Project №107M201).

References

1. EC. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market, Official Journal of European Communities 24.4.98 EN L 123/1 (1998).
2. BUNCE K, KHAN N, The Layman's guide to antimicrobial fabrics and testing methods. UK: Shirley Technologies Ltd (2004).
3. GU H, ZHAO C, WANG L, GONG Y, CHEN W, A New Combined Antimicrobial Agent: Development and Application in Shoe Lining Leather. XXX Congress IULTCS, China, Beijing (2009).
4. GRIER N, Silver and Its Compounds in Disinfection, Sterilization and Preservation. Philadelphia, USA: S.S. Block, Lea &Febiger (1983).
5. UCHIDA M, Antimicrobial zeolite and its application. *ChemInd* 46: 48-54, (1995).
6. KAWASHITA M, TSUNEYAMA S, MIYAJI F, KOKUBO T, KOZUKA H, YAMAMOTO K, Antibacterial silver-containing silica glass prepared by sol-gel method. *Biomaterials*, 21: 393-398 (2000).
7. KOKKORIS M, TRAPALIS CC, KOSSIONIDES S, VLASTOU R, NSOULI B, GROTZSCHEL R et al. RBS and HIRBS studies of nanostructured $AgSiO_2$ sol-gel thin coatings. *NuclInstr Methods Phys Res* B. 188: 67 (2002).
8. LEE MS, HONG SS, MOHSENI M, Synthesis of photocatalytic nanosized TiO_2–Ag particles with sol–gel method using reduction agent. *J MolCatal A Chem*. 242: 135–140 (2005).

9. KIM HY, KIM JH, KWON SC, JEONG SH, A study on multifunctional wool textiles treated with nano-sized silver. *J Mater Sci* 42: 8020–8024 (2007).
10. YAŞA I, LKHAGVAJAV N, KOIZHAIGANOVA M, ÇELIK E, SARI Ö, Assessment of antimicrobial activity of nano-sized Ag-TiO_2 prepared by sol-gel method. *World J MicrobiolBiotechnol*, 28: 2531–2539 (2012).
11. AMIN SA, PAZOUKI M, HOSSEINNIA A, Synthesis of TiO_2–Ag nanocomposite with sol–gel method and investigation of its antibacterial activity against *E. coli*. *Powder Technology*, 196: 241–245 (2009).
12. LKHAGVAJAV N, KOIZHAIGANOVA M, YAŞA I, ÇELIK E, SARI Ö, Characterization and antimicrobial performance of nano silver coatings on leather materials, *Brazilian Journal of Microbiology*, 46(1): 41-48 (2015).
13. BALOGH L, SWANSON DR, TOMALIA DA, HAGNAUER GL, MCMANUS AT, Dendrimer-silver complexes and nanocomposites as antimicrobial agents. *J Nano Letters*, 1(1): 18-21 (2001).
14. KIM S, KIM H, Antibacterial performance of colloidal silver-treated laminate wood flooring. *J Intern BiodeterBiodegrad*, 57: 155-162 (2006).
15. BRINKER CJ, SCHERER GW, Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing. San Diego, USA: Academic Press (1990).
16. GAIDAU C, PETICA A, CIOBANU C, MARTINESCU T, Investigation on antimicrobial activity of collagen and keratin based materials doped with silver nanoparticles. *Romanian Biotechnological Letters*, 14(5):4665-4672 (2009).
17. GAIDAU C, PETICA A, DRAGOMIR T, IOVU H, ANDRONESCU C, Ag and Ag/TiO_2 nano-dispersed systems for treatment of leathers with strong antifungal properties. Journal of the American Leather Chemists Association, 106(3):102-109 (2011).
18. MALEKNIA L, AALA A, YOUSEFI K, Antibacterial properties of nanosized silver colloidal solution on wool fabric. *Asian Journal of Chemistry*, 22: 5925-5929 (2010).
19. YANG W, WANG X, GONG Y, LI H, CHEN W, GAIDAU C, Preparation of antibacterial sheepskin with silver nanoparticles: potential for use as a mattress for pressure ulcer prevention. *Journal of the American Leather Chemists Association*, 107(3): 85-91 (2012).
20. YU B, LEUNG KM, GUO Q, LAU WM, YANG J, Synthesis of Ag–TiO_2 composite nano thin film for antimicrobial application. *Nanotechnology*, 22(11): 5603 (2011).
21. AHLIAH ISMAIL W, ABIDIN ALI Z, PUTEH R, Transparent Nanocrystallite Silver for Antibacterial Coating. *Journal of Nanomaterials*, 2013: 1-6 (2013).
22. SÁNCHEZ-NAVARRO MM, PÉREZ-LIMIÑANA MA, CUESTA-GARROTE N, MAESTRE-LÓPEZ MI, BERTAZZO M, MARTÍNEZ-SÁNCHEZ MA, ORGILÉS-BARCELÓ C, ARÁN-AÍS F, Latest Developments in Antimicrobial Functional Materials for Footwear. Microbial pathogens and strategies for combating them: science, technology and education. Formatex Research Center, Spain, 1, 102-113 (2013).
23. SPERANZA G, GOTTARDI G, PEDERZOLLI C, LUNELLI L, CANTERI R, PASQUARDINI L, CARLI E, LIU A, MANIGLIO D, BRUGNARA M, ANDERLE M, Role of chemical interaction in bacterial adhesion to polymer surfaces. *Biomaterials*, 25(11), 2029-37 (2004).