Antimicrobial Electrochemically Obtained Nanosilver Solutions for Leather and Furskin Treatment

AURORA PETICA[1], CARMEN GAIDAU[1]*, JA MA[2], DEMETRA SIMION[1], QU XU[2], MIHAELA NICULESCU[1]
[1]INCDTP- Leather and Footwear Research Institute Division, 93 I.Minulescu Str., 031215, Bucharest, Romania
[2]Shaanxi University of Science and Technology, Institute of Creative Materials Division, Xian, Shaanxi, R.P.China

The silver nanoparticles (AgNps) with antibacterial and antifungal properties against a great number of bacteria and fungi may be used in form of colloidal sols or doping agents for a lot of composite materials with polymer matrix. AgNps as ecological alternative for organic biocides represent an innovative challenge for treatment of collagen and keratin based materials such as leather and furskin. Using an efficient and innovative electrochemical method, colloidal silver solutions (CSSs) containing up to 45 ppm Ag with AgNps smaller than 10 nm and Zeta potential values between – 40 ÷ - 50 mV, which indicates very stable solutions, were obtained and used for treatment of leathers and furskins. Bacteriostatic and bactericidal effect of the CSSs was evaluated by measuring minimal concentration with bacteriostatic effect – MCBs and minimal concentration with bactericidal effect – MCBc against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. Also, the fungistatic properties were evaluated and the results were very good. Leather and furskin treated by immersion in CSSs, with a content of 820 ppm Ag for chromium-tanned, 760 ppm Ag for metal-free leathers and 160 ppm for furskin have displayed resistance against some important bacteria such as E.coli, P. aeruginosa or S. aureus. The exposure of the leathers and furskins to a mix of fungi (A.niger, T.viride, P. glaucum, S. brevicaulis and P. variotii) has shown a good resistance after 7 days, better in the case of leathers. To demonstrate the way in which AgNps are present and distributed on treated leather surface, as a result of the interaction with processed leather, and of the correlation with antimicrobial resistance, Ag content and valence state were analyzed by atomic absorption spectroscopy (AAS) and X–ray photoelectron spectroscopy (XPS).

Keywords: electrochemical syntheses, silver nanoparticles, colloidal silver solution, antimicrobial leather and furskin.

Due to the presence of microorganisms such as bacteria, mould, fungi and viruses in the living environment and the development of antibiotic resistance the pharmaceutical companies and the researchers are searching for new antibacterial materials [1,2]. At present, nanoscale materials have stood out as novel antimicrobial agents owing to their high surface area to volume ratio and their unique chemical and physical properties. Among different types of nanomaterials, silver nanoparticles (AgNps) have been proved to be most effective, presenting strong inhibitory and bactericidal effects against a great number of bacteria and fungi and, at the same time, represent an ecological alternative for organic biocides [3, 4]. It is reported that when silver ions bind to electron donor groups of the biological molecules containing thio, amino, carboxylate, imidazole or phosphate groups, they inhibit vital activities of the bacteria and cause bacterial inactivation [3]. L. Feng and co-workers [5] showed that the mechanism of the antibacterial effect of silver ions (Ag^+) involves interaction with the thiol groups of proteins, blocking the S-H bounds, which induces the inactivation of bacterial proteins. AgNps may be used in form of colloidal sols or doping agents for a lot of composite materials with polymer matrix. Silver at nanometric scale, with diameter less than 10 nm, is very reactive and it can be combined with macromolecules which contain –RN-CO- structures like polyvinyl pyrrolidone [6], polyurethanes [7] or proteins [8]. While Ag ions from salts have only limited usefulness over time, because of [7] the fast diminishing of the Ag ion concentration, AgNps allowed growth of the contact surface of Ag with microorganisms and Ag ions are released gradually [3]. Recently, AgNps have been used in many fields, such as coatings of stainless steel medical devices, water treatment, products for medicine, cosmetics, fabrics, textile, and consumer goods. We extend its application for treatment of collagen and keratin based materials such as medical leather and furskin. Furthermore, because fungal growth is a common problem in the leather industry and because of the restrictive legislation, many fungicides such as the phenolic or heterocyclic compounds that are often used in the tanning industry [9] are susceptible to become unacceptable, it is extremely important to find new compounds that have no toxicity and are capable of exhibiting a prolonged antifungal effect.

This paper presents some original results regarding the investigation of antimicrobial activity for electrochemically obtained AgNps in aqueous media and for leather and sheepskin treated with them.

Experimental part

Material and Methods

Synthesis and characterization of colloidal silver solutions-CSS

AgNps were electrochemically obtained as CSSs using so-called "sacrificial anode method" [10], by anodic dissolving of Ag electrodes in an aqueous solution containing two stabilizer agents, namely: polyvinyl pyrrolidone (PVP) as steric stabilizer and Na-lauryl sulfate (SDS) as electrostatic co-stabilizers, dosed in a well defined weight ratio. Electrochemical equipment includes a

* email: carmen_gaidau@icpi.ro

constant current pulse generator with stirring and alternating polarity and electrodes of 99.999% Ag with sizes of 155 / 27 mm.

The experiments were carried out using the following materials:

- deionized water with conductivity < 1μS, resistivity of 18 μΩ·cm and *p*H = 5 – 7;
- poly [1-vinyl-2-pyrrolidone] $(C_6H_9NO)_n$ (PVP10 with M = 10,000 from Sigma – Aldrich);
- Na-lauryl sulfate, provided from Sigma – Aldrich.

The working parameters for electrochemical process were: current intensity of 2..5 mA, time for 4-6 h and temperature at 25^{0}C.

The Ag concentration of the obtained CSSs has been determined through UV-Vis spectra analysis, involving a JASCO V 500 spectrophotometer. The nanoparticles sizes have been analyzed through DLS (Dynamic Light Scattering) technique using Zetasizer Nano equipment and their morphology has been evidenced by TEM micrographs with a Philips CM 100 electronic microscope.

Obtaining and characterization of leather and furskin treated with CSSs

Leather samples used were chromium tanned and metal-free types; furskins originated from raw sheepskins and were processed with specific technologies [11]. Leather samples were washed for 30 min with distilled water in a "Wacker" drum, and then were immersed three times in CSS at 30^{0}C for 1h, followed by free drying.

The silver content was analyzed by atomic absorption spectroscopy-AAS (Analitik Jena), the silver concentration, chemical state and binding energy on the leather surface were evidenced by X–ray photoelectron spectroscopy (XPS) with a K-Alpha Thermo Scientific device (mono-chromatized Al K_α X-ray source (1486.6 eV), 2 x 10^{-9} mbar pressure, compensation of surface charge with an Ar flood gun, pass energy of 200 eV).

To evaluate fungitoxic properties, the antibiogram method and standard methods [12] for leather materials were used. These methods are applied by using the following fungi: *Penicillium glaucum*, *Aspergillus niger, Stachybotris atra* and *Trichoderma viride*. Besides these, two other fungi with biodegradation potential for leather were inoculated, namely: *Scopulariopsis brevicaulis* and *Paecilomyces variotii.* Antibacterial properties of leather functionalized with CSSs were evaluated by antibacterial activity tests [13].

Antibacterial properties of leather and furskin treated with CSSs were assessed by difusimetric method, against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

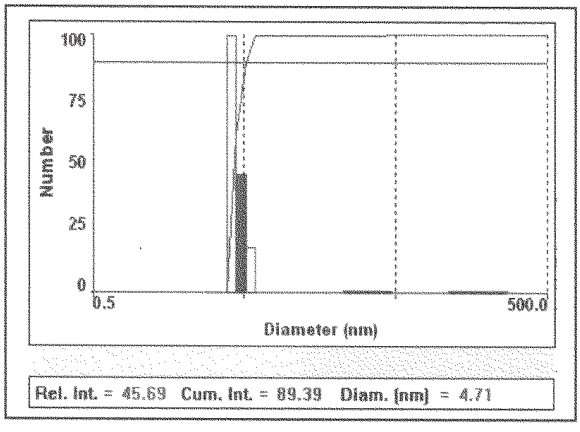

Fig 1. Histogram of AgNps grain size distribution

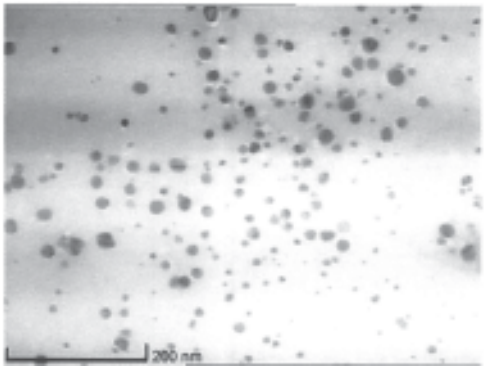

Fig. 2. TEM micography of AgNps

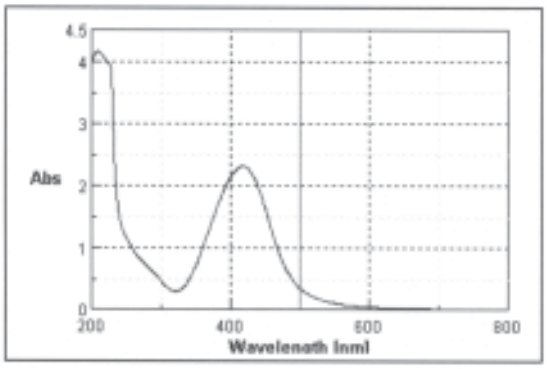

Fig.3 UV-Vis absorbance spectra

Results and discussions

Concentration, size, stability and morphology of electrochemically obtained CSSs

Colloidal silver solution (CSS) with 45 ppm Ag concentration was electrochemically obtained in deionized water which contains a mix of PVP and SDS, in a molar ratio of 10:1 at a current density of 1-5 mA. Electrosynthesis of AgNps in aqueous media is an efficient and ecological process which offers the advantage of a high purity of final formed solution and a broad spectrum of antibacterial and antifungal activity [10]. The CSSs obtained using this method showed very good antimicrobial activity. The characteristics of this solution in terms of grain size distribution, morphology and stability are presented in figures 1-4.

From grain size distribution histogram (fig.1) it can be seen that almost 90% of particles number is up to 4.71 nm and; Zeta potential is -51.46 mV, value which indicates a very stable CSS; TEM micrograph (fig.2) shows that CSS contains AgNPs smaller than 10 nm with spherical and uniform shape without agglomeration tendency; from UV-Vis spectra (fig.3) the absorbance peak at 426nm can be noticed.

Antibacterial effect of CSSs

The obtained CSSs have been tested to evidence their antibacterial and antifungal properties. According to the antibiogram method, the fungistatic effect is expressed by the presence and magnitude of inhibition area for mould growth around the filter paper padded with tested solutions. Photographic images of the inhibition area produced by a 45 ppm CSS, after 7 and 14 days of exposure at fungi mix, are presented in figure 4.

It can be seen that after 7 days of exposure the inhibition area around the filter paper is very well delimitated and, also, it is present even after 14 days.

The antibacterial activity was assessed against three frequent bacterial species (*E. coli*, *S. aureus* and *P. aeruginosa*), by measurement of the minimal

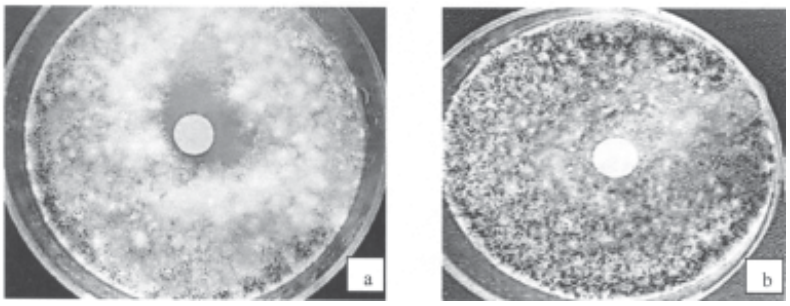

Fig.4. Inhibition zone produced by a CSS with 45 ppm Ag after: a) 7 days b) 14 days CSSs

Ag conc. (ppm)	*Staphylococcus aureus ATCC* 25923		*Escherichia coli ATCC* 25922		*Pseudomonas aeruginosa*	
	MCBs (ppm)	MCBc (ppm)	MCBs (ppm)	MCBc (ppm)	MCBs (ppm)	MCBc (ppm)
45	22.5 – 11.25	45	11.25 – 5.62	22.5	11.25 – 5.62	22.5

Table 1
MINIMAL CONCENTRATION WITH BACTERIOSTATIC EFFECT AND MINIMAL CONCENTRATION WITH BACTERICIDAL EFFECT OF THE TESTED

concentrations with bacteriostatic and bactericidal effects, using fractional dilutions (1; 1/2; 1/4; 1/8; 1/16). By their morphological,physiological and metabolic characteristics, these bacteria offer a useful diversity for obtaining of some pertinent results.

Bacteriostatic effect is achieved at a dilution of 1/2 from the initial CSS concentration, in the case of *S. aureus* and 1/4-1/8 for *E. coli* and *P. aeruginosa*, while the best bactericidal effect is at 1/2 from the initial CSS concentration, in the case of *E. coli* and *P. aeruginosa.*

It is noticed that *Staphylococcus aureus* is more resistant than the other two bacteria and this is due to its membrane which consists of a thick peptidoglycan layer which creates resistant biofilms; the results are in agreement with literature data [14].

Characteristics of leathers treated with CSSs

To demonstrate the way in which AgNps are present and distributed on treated leather surface, as a result of the interaction with processed leather, and of the correlation with antimicrobial resistance, Ag content and valence state were analyzed by atomic absorption spectroscopy (AAS) and X–ray photoelectron spectroscopy (XPS).

Ag content in the two types of leathers, determined by AAS is 820 ppm for chromium-tanned leather and 760 ppm for metal-free leather.

The chemical state of AgNps loaded on the surface of treated leathers was investigated by X-ray photoelectron spectroscopy (XPS), which is a sensitive technique for surface analysis regarding the composition and valence state of Ag.

Based on the total XPS spectra (fig.5 and 7), the relative contents of Ag (3 d) in the two types of leather were calculated as 0.18% in chromium-tanned leather and 0.11% in metal free leather, respectively. Ag^0 is preserved in a slightly greater proportion in the case of chromium-tanned leather than in the case of metal-free leather probably due to higher availability of Ag^0 of complexing by means of aminic groups, active in the case of chromium tanned leathers.

The binding energies of Ag 3 d5/2 and 3d3/2 electrons are 373.37 eV and 367.44 eV for chromium-tanned leather (fig.6) and 373.59 eV and 367.59eV for metal free leather (fig.8), respectively. From literature data, the binding energy of 367.44 - 367.59 eV is attributed to the AgO and Ag_2O state of Ag and the binding energy of 373.38 – 373.59 eV is more specific for Ag^0 state [15].

Additionally, from the high resolution spectra, the slitting of the 3d doublet of Ag is 5.93 in the case of chromium-tanned leather (fig.6) and 6.00 eV in the case of metal-free leather (fig.8), indicating the presence of zero valence nanosilver [16].

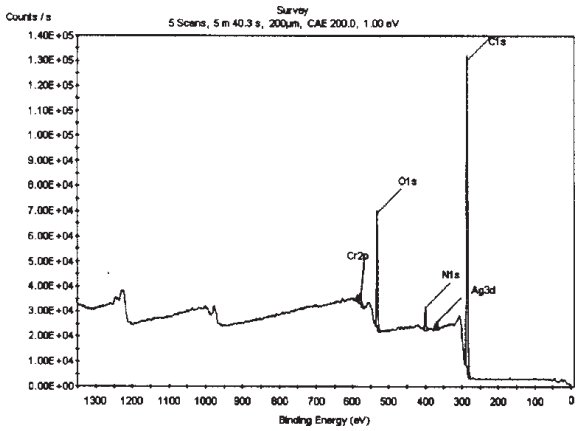

Fig.5. The total XPS spectra of chromium tanned leather surface, treated with CSS.

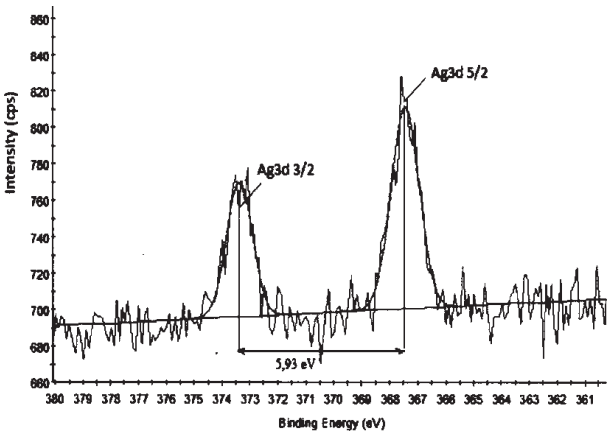

Fig.6. The Ag3d XPS spectra on the chromium tanned leather surface, treated with CSS.

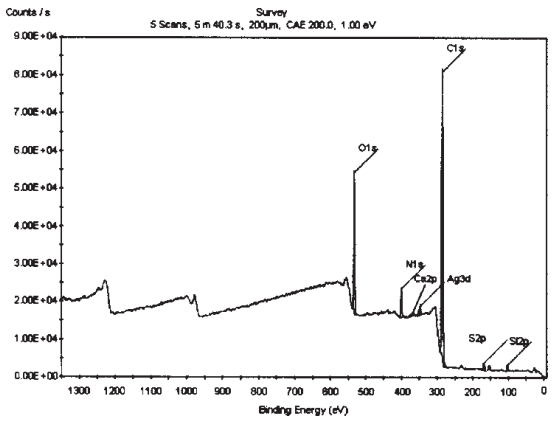

Fig.7. The total XPS spectra of metal-free leather surface, treated with CSS.

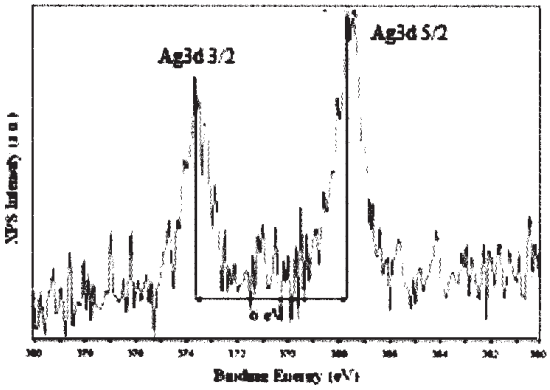

Fig.8. The Ag3d XPS spectra on the metal-free leather surface, treated with CSS.

Samples	Ag conc, ppm	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Chromium-tanned leather	820	+	+	+
Metal-free leather	760	+	+	+
Furskin	160	-	+	+

"+" – with antibacterial activity; "-"– without antibacterial activity

Table 2
ANTIBACTERIAL ACTION OF LEATHER AND FURSKIN TREATED WITH CSSs

Sample	**Exposed surface**	**Marks 0-5***		**Remarks**
		7days	**14 days**	
Furskin FSI	wool	0	2	*Penicillium*-14 days, *Stachybotris atra, Chaetomium globosum*
	leather	0	0	
Furskin FSII	wool	0	1	
	leather	0	0	*Stachybotris atra*
Furskin FSIII	wool	0	1	*Stachybotris atra* Trace of inhibition zone
	leather	0	0	
Control furskin CFSI	wool	1	3	*Penicillium*
	leather	1	2	*Aspergillus niger*
Control furskin CFSII	wool	1	3	
	leather	1	3	
Control furskin CFSIII	wool	1	2	*Stachybotris atra*
	leather	1	3	*Trichoderma viridae*
Chromium Leather LSI	grain side	0	4	Trace of inhibition zone of 1mm in all LS samples on the grain side after 7 days of exposure
	flesh side	0	4	
Metal-free Leather LSII	grain side	0	5	
	flesh	0	5	
Metal-free Leather LSIII	grain side	0	5	
	flesh side	0	5	
Control leather CLSI	grain side	5	5	In all control samples mark 5 is due to massive growth of *Aspergillus niger*
	flesh side	5	5	
Control leather CLSII	grain side	5	5	
	flesh side	5	5	
Control leather CLSIII	grain side	5	5	
	flesh side	5	5	

*where 0 mark means the highest resistance and 5 mark the weakest resistance.

Table 3
EVALUATION OF LEATHER AND FURSKIN SAMPLES TREATED WITH 45 PPM CSS AGAINST FUNGI MIX, COMPARING WITH CONTROL SAMPLES

Antibacterial and antifungal effect of leather and furskinn treated with CSSs

The chromium-tanned leather and the metal-free leather treated by immersion in 45 ppm CSS, with a content of 820 ppm and, respectively, 760 ppm Ag in dermal surface have displayed resistance against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (table 2), assessed by difusimetric method. An explanation generally accepted is that the nucleophilic Ag nanoparticles adhere to the electrophilic bioactive sites of bacterial cell

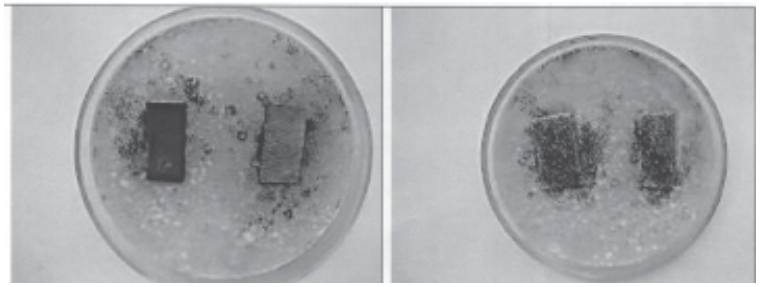

Fig.9 a) LSI-Metal-free leather treated with CSS after 7 days of exposure to fungi mix action b) CLSI – Metal-free untreated leather after 7 days of exposure to fungi mix action.

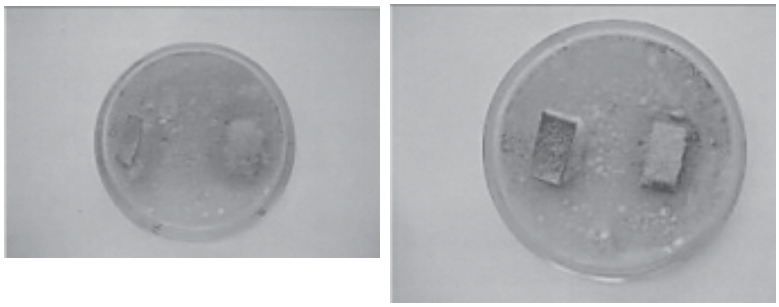

Fig.10 a) FSII – Chromium-tanned furskin treated with CSS, after 7 days of exposure to fungi mix action b)CFS II – Chromium-tanned untreated furskin, after 7 days of exposure to fungi mix action

membrane and continuously supply the Ag^+ ions which bond the -S-H groups of bacterial cell enzymes [17].

Furskin with a content of 160 ppm Ag has antibacterial action against the *E. coli* and *P. aeruginosa* and not against the *S.aureus*, due to its higher resistance [14] and, also, to the less content of Ag in furskin.

In the case of fungi, according to literature [18], their destruction is slower than in the case of bacteria because of their chemical stability. The exposure of the same leather and furskins, according to the antibiogram method, to a fungi mix used for inoculation, which contains: *Aspergillus niger*, *Paecilomyces variotii*, *Trichoderma viride*, *Stachybotris atra, Scopulariopsis brevicaulis, Chaetomium globosum* and *Penicillium glaucum* is presented in table 3.

There are 3 samples of treated furskin, marked FSI, FSII, FSII, 3 control untreated furskin samples, marked CFSI,CFSII, CFSIII, 3 samples of treated leather, marked LSI, LSII, LSIII and 3 control samples of untreated leather, marked CLSI, CLSII and CLSIII. Furskin samples were chromium-tanned and leather samples were chromium-tanned (LSI) and metal-free (LSII, LSIII). The results indicate that after 7 days of exposure, both treated furskin and leather are marked with "0" but after 14 days, only furskins have good marks. The furskin and leather samples showed improved resistance after 7 days of exposure to a mix of fungi in comparison with control samples. Chromium-tanned leathers showed inhibition zone after 7 days of exposure and just slightly improved resistance after 14 days of exposure in comparison with control samples.

In figure 9 are presented pictures of LSI-Metal-free leather treated with CSS after 7 days of exposure to fungi mix action (a), comparing with control sample (b), where the invasion of *Trichoderma viride* can be seen.

From images of treated furskin (fig. 10 (a)), comparing with untreated furskin (fig. 10 (b)) it can be noticed that derma side has very good resistance.

Conclusions

Using an efficient and simple electrochemical method, stable and high purity nano-Ag based colloidal solutions containing 45 ppm Ag, with AgNps smaller that 10 nm and – 51.46 mV zeta potential were obtained. A good antifungal effect was proved after 7 and 14 days of exposure to fungi mix with an inhibition area very well delimited. For tested CSSs, MIC showed a very good efficiency against both Gram positive and Gram negative cocci. Bacteriostatic effect is achieved at a dilution of 1/2 from the initial CSS concentration, in the case of *S. aureus*, and 1/4-1/8 for *E. coli* and *P. aeruginosa*, while the best bactericidal effect is at 1/2 from the initial CSS concentration, in the case of *E. coli* and *P. aeruginosa.* Analysis regarding concentration and valence state of Ag on the leather surface by XPS technique has allowed the identification of Ag^0 (atomic silver) and the relative contents of Ag (3 d) in the two types of leather were calculated as 0.18% in chromium-tanned leather and 0.11% in metal-free leather, respectively. Ag^0 is preserved in a slightly greater proportion in the case of chromium-tanned leather than in the case of metal-free leather, probably due to higher availability of Ag^0 of complexing by means of aminic groups, active in the case of chromium tanned leathers.

Assessment of the antibacterial action of leather and furskin treated with CSSs by difusimetric method indicates that the chromium-tanned leather with a content of 820 ppm Ag and the metal-free leather with 760 ppm Ag have displayed resistance against *S.aureus, E.coli* and *P.aeruginosa.* Furskin with a content of 160 ppm Ag has resistance to the *E. coli* and *P. aeruginosa* and not to *S.aureus*, due to its stronger structure of outer membrane and, also, to the less content of Ag in furskin. Antifungal effect tested using antibiogram method, by the exposure of the same leathers and furskins to a fungi mix (*A.niger*, *P.variotii*, *T.viride*, *S.atra, S.brevicaulis, C.globosum* and *P.glaucum)* showed that after 7 days, both treated furskins and leathers are marked with "0" but after 14 days, only treated furskins showed improved resistance in comparison to control samples.

The results are promising for the CSSs use in antimicrobial applications, including antibacterial finishes and disinfecting techniques for all types of leathers, textiles for food and health industry and, especially, for bio-medical application.

Acknowledgements: The work was funded by UEFISCDI, contract PN-II-PT-PCCA-167, and bilateral contract Romania-China PNII-616. The authors are grateful to Prof. Dr. Horia Iovu for the help with X–ray photoelectron spectroscopy analyses.

References

1. WHO/CDS/CSR/DRS/2001.5, 2002, - Surveillance standards for antimicrobial resistance.
2. SIRVAITYTE, J., SIUGZDAITE, J., VALEIKA, V., REV. CHIM. (Bucharest), 62, nr. 9, 2011, p.884.

3. YEO, S.Y., LEE, H. J., JEONG, S.H., J.Mater. Sci. 38, 2003, p. 2199.
4.KARANDIKAR et al. US Patent Appl. Pub. No. 0003603 A1, Jan. 4, 2007.
5. FENG, Q.L., WU, J., CHEN, G.Q., CUI, F.Z., KIM, T.N., KIM, J.O., J.Biomed.Mater Res. 52, 2003, p. 662.
6. HONGSHUI, W., XUELIANG, Q., JIANGUO, C., XIAJIAN, W., SHIYUAN, D., Materials Chem. and Phys., 94, 2005, p. 449.
7.CIOBANU, C., KONCSAG, C., Compozite si nanocompozite polimerice, Ed. Pim, 2007.
8.BHANU, P. , CHAUHAN, S. , RAJESH, S. , LATIF, U., MONI C., LAMOREAUX W.J., Acta Chim. Slov 52, 2005, p. 361–370.
9.ADMINIS U., HUYNH C., MONEY C.A., J. Soc. Leather Technol.Chem, 86, 3, 2002, p. 118.
10. PETICA, A., GAVRILIU, S., LUNGU, M., BURUNTEA, N., PANZARU, C., Materials Science and Engineering B, 152, 2008, p. 22.
11. GAIDAU, C., PLAVAN, V., LUPULESCU, D., CRUDU, M., MIU, L., Buletinul Institutului Politehnic Iasi LIII (LVII), 5, 2007, p. 407.
12. *** SR CEI60068-2-10/2006; Pr. PI – 14, 2007; ASTM D: 4576-86, 1996
13. *** Document no. FTTS-FA-002.
14. GILL, S.R., FOUTS, D.E., ARCHER, G.L., MONGODIN, E.F., J. Bacteriol, 187, 7, 2005, p. 2426.
15. WANG.H., NIU J., LONG X., HE Y., Ultrasonic Sonochemistry 15, 2008, p. 386.
16. MOULDER, J.F., STICKLE, W.F., SOBOL, P.E., BOMBEN K.D., (Eds.), Hand Book of X-ray Photoelectron Spectroscopy, Perkin–Elmer Corporation Physical Electronics Division, 1992.
17. ELECHIGUERRA, J.L., BURT, J.L., MORONES, J.R., CAMACHO-BRAGADO, A., GAO, X., LARA, H.H., YACAMAN, M.J., Journal of Nanobiotechnology, 3, nr.6, 2005, p. 1.
18. ERICCSSON, H.M., SHERRIS, J.C., Acta Pathol. Microbiol. Scand B Microbiol Immunol. Suppl. 217B 1971, p.64.

Manuscript received: 27.05.2013