Investigation of Antimicrobial Properties of Leather and Sheepskin for Medical Use Treated with Colloidal Silver Solutions

Aurora Petica[1], Carmen Gaidau[1], Viorel Floristean[2]

[1]Leather and Footwear Research Institute, Bucharest, 031215, Romania mailto:carmen_gaidau@icpi.ro
[2]Agricoles Sciences and Veterinary Medicine University, Iassy, Romania, vfloristean@univagro-iasi.ro

Abstract

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 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 leather and furskins.
Bacteriostatic and bactericidal effect of the CSSs was evaluated 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 leather and 160 ppm for furskin have displayed resistance against S. *aureus*, *E.coli* and *P. aeruginosa.* 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 leather.

Keywords: silver nanoparticles, colloidal silver solution, antimicrobial leather and furskin

1.Introduction

Due to the presence of microorganisms such as bacterium, 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 (WHO/CDS/CSR/DRS/2001.5, 2002). In the present, nanoscale materials have remarked as novel antimicrobial agents owing to their high surface area to volume ratio and the 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, in the same time, represent an ecological alternative for organic biocides (Yeo et al. 2003, Karandikar et al. US Patent 2007). 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 (Yeo et al. 2003). L. Feng and co-workers (Feng et al. 2003) 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 composites 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 (Hongshui et al. 2005), polyurethanes (Ciobanu and Koncsag 2007) or proteins (Bhanu et al. 2005). While Ag ions from salts have only limited usefulness in time, because of 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 (Yeo et al. 2003). 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 restriction of legislation, many fungicides such as the phenolic or heterocyclic compounds that are often use in the tanning industry (Adminis et al. 2002) are susceptible to become unacceptable, is extremely important to find new compounds that have no toxicity and are capable of exhibiting a prolonged antifungal effect.

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

2. Material and Methods

2.1. Synthesis and characterization of colloidal silver solutions-CSS

AgNps were electrochemically obtained like CSSs using so-called “sacrificial anode method” (Petica et al. 2008), 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 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 pH = 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 was: current intensity: 2..5 mA, time 4-6 hours and temperature 25^0C.

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

2.3. Obtaining and characterization of leather and sheepskin treated with CSSs

Leathers samples used were chromium tanned and metal-free types; furskin was originating from raw sheepskins and were processed with specific technologies (Gaidau et al. 2007). Leather samples were washed for 30 minutes with distillated water in a "Wacker" drum, and then were immersed tree times in CSS at 30^0C for 1h, followed by free drying.

The silver content was analyzed by atomic absorption spectroscopy-AAS, 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 (monochromatized Al K_α X-ray source (1486.6 eV), 2×10^{-9} mbar pressure, compensation of surface charge with an Ar flood gun, pass energy of 200 eV).

To evaluate fungitoxic properties, the antibiogram method was used and standard methods (SR CEI60068-2-10/2006; Pr. PI – 14, 2007; ASTM D: 4576-86, 1996) for leather materials. These methods are applied by using 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 CSS were evaluated by antibacterial activity tests (FTTS-FA-002, 2000).

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

3. Results and Discussion

3.1 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 (Petica et al. 2008). The CSSs obtaining using this method, showed very good antimicrobial activity. The characteristics of this solution from grain size distribution, morphology and stability point of view 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 (fig.2) is -51.46 mV, value which indicates a very stable CSS; TEM micrograph (fig.3) shows that CSS contains AgNPs smaller than10 nm with spherical and uniform shape without agglomeration tendency; from UV-Vis spectra (fig.4) is remarks absorbance peak at 426nm.

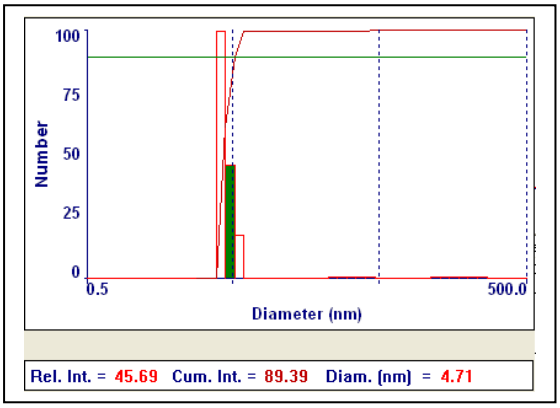


Fig1. Histogram of AgNps grain size distribution

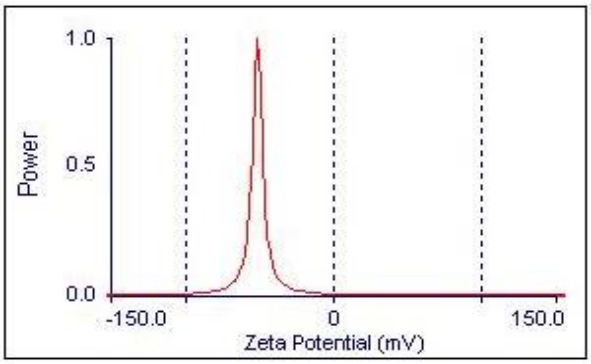


Fig.2 Zeta potential recording

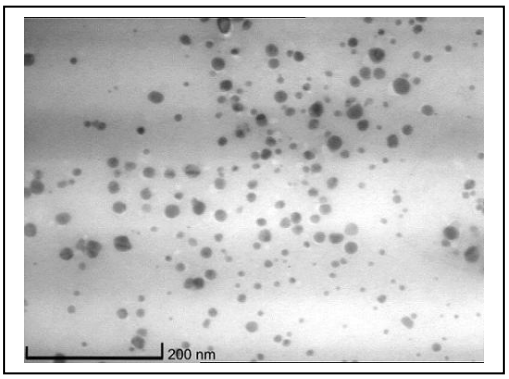


Fig3. TEM micography of AgNps

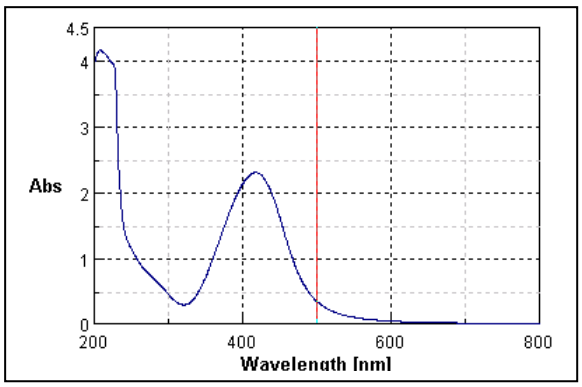


Fig.4 UV-Vis absorbance spectra

3.2 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 Fig.5.

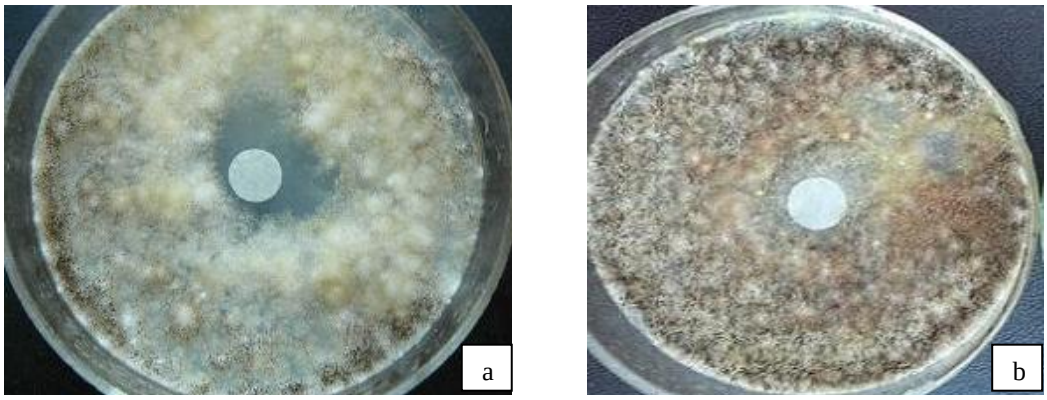


Fig.5 Inhibition zone produced by CSS with 45 ppm Ag after: a) 7 days b) 14 days.

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 frequently bacterial species (*E. coli*, *S. aureus* and *P. aeruginosa*), by measurement of the minimal concentrations with bacteriostatic and bactericidal effects, using fractional diluting (1; 1/2; 1/4; 1/8; 1/16). These bacteria offer by their morphological, physiological and metabolic characteristics a useful diversity for obtaining of some pertinent results.

Table 1-Minimal concentration with bacteriostatic effect and minimal concentration with bactericidal effect of the tested 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

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 remarks 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 (Gill et al., 2005).

3.3. 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 comparable for both types of treated leather and 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 highly sensitive technique for surface analysis regarding the composition and valence state of Ag.
Based on the total XPS spectra (Fig.6 and Fig.8), 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. 7) and 373.59 eV and 367.59eV for metal free leather (Fig.9), respectively. From literature data, the binding energy of 367.44 - 367.59 is attributed to the AgO and Ag_2O state of Ag and the binding energy of 373.38 – 373.59 is more specific for Ag^0 state (Wang et al. 2008).

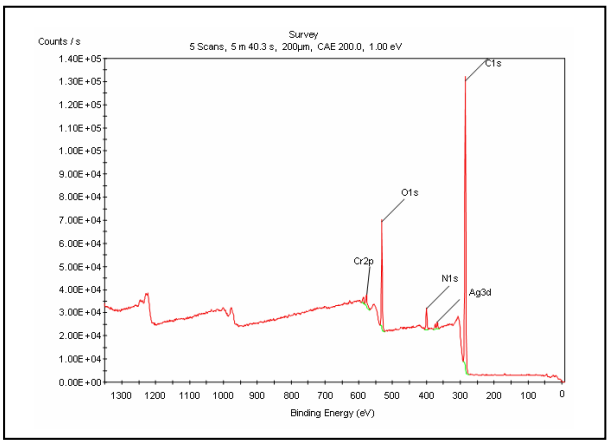


Fig.6 The total XPS spectra of chromium tanned leather surface, treated with CSS

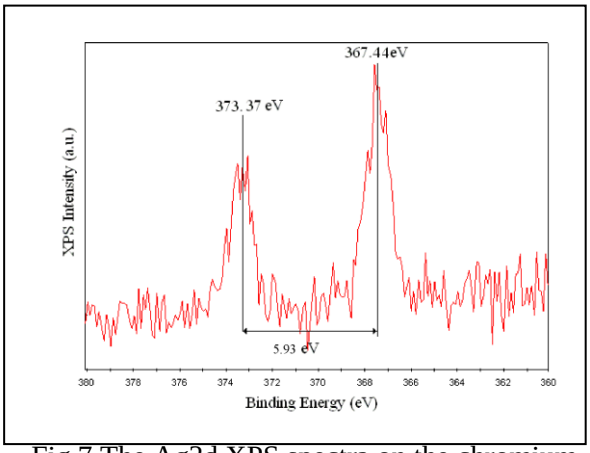


Fig.7 The Ag3d XPS spectra on the chromium tanned leather surface, treated with CSS

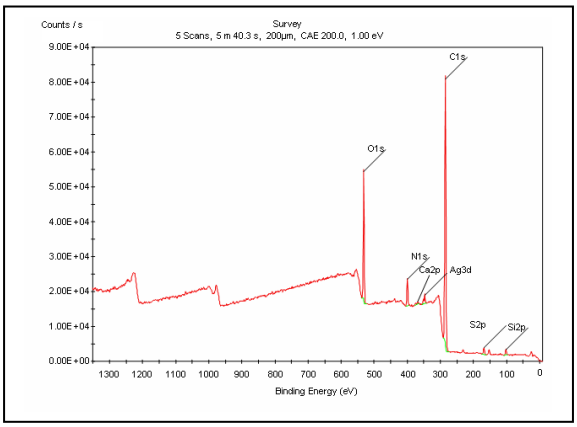


Fig.8 The total XPS spectra of metal-free leather surface, treated with CSS

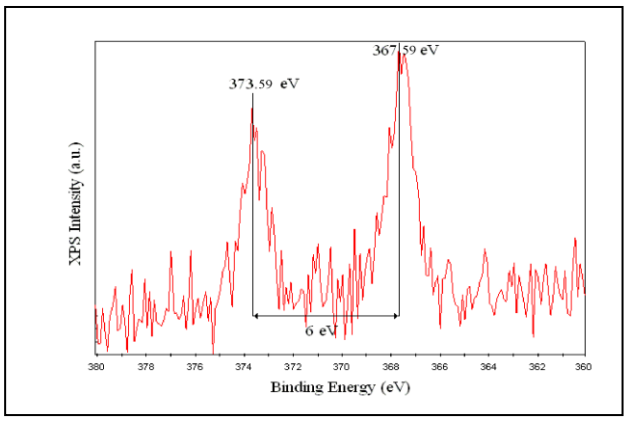


Fig.9 The Ag3d XPS spectra on the metal-free leather surface, treated with CSS

Moreover, from the high resolution spectra, the slitting of the 3d doublet of Ag is 5.93 in the case of chromium-tanned leather (Fig.7) and 6.00 eV in the case of metal free leather (Fig.9), indicating the presence of metallic silver (Moulder et al. 1992).

3.4 Antibacterial and antifungal effect of leather and sheepskin treated with CSSs

The chromium-tanned leather and the metal-free leather treated by immersion in 45 ppm CSS, with a content of 820 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 membrane and continuously supply the Ag^+ ions which bond the -S-H groups of bacterial cell enzymes (Elechiguerra et al. 2005).

Table 2- Antibacterial action of leather and furskin treated with CSS's

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

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

Furskin with a content of 160 ppmAg, has resistance to the *E. coli* and *P. aeruginosa* and not to the *S.aureus*, due to its higher resistance (Gill et al., 2005) and, also, to the more little content of Ag.

In the case of fungus, according to literature (Ericcsson et al. 1971), 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, noted FSI, FSII, FSII, 3 control untreated furskin samples, noted CFSI,CFSII, CFSIII, 3 samples of treated leather, noted LSI, LSII, LSIII and 3 control untreated leather, noted CLSI, CLSII and CLSIII. Furskin samples were chromium-tanned and leather samples were metal-free. The results indicate that after 7 days of exposure, both treated furskin and leather are noted with "0" but after 14 days, only derma side of treated furskin has good marks.

In Figure 10 is presented LSI-Metal-free leather treated with CSS after 7 days of exposure at fungi mix action, comparing with control sample from Figure 11, where it could be seen the invasion of *Trichoderma viride.*

Table 3- Evaluation of leather and furskin samples treated with 45 ppm CSS against fungi mix, comparing with control samples

Sample	Exposed side	Marks 0-5		Remarks
		7days	14 days	
FSI	wool	0	2	*Penicillium*-14 days, *Stachybotris atra, Chaetomium globosum*
	flesh	0	01-1	
FSII	wool	0	1-2	
	flesh	0	01-1	*Stachybotris atra*
FSIII	wool	0	1	*Stachybotris atra* Trace of inhibition zone
	flesh		01	
CFSI	wool	01	2-3	*Penicillium*
	flesh	01	2	*Aspergillus niger*
CFSII	wool	01	3	
	flesh	01	3	
CFSIII	wool	01	1-2	*Stachybotris atra*
	flesh	01	3+	*Trichoderma viridae*
LSI	grain	0	4	Trace of inhibition zone of 1mm at all LS samples on front face at 7 days of exposure
	flesh	0	4-5	
LSII	grain	0	5	
	flesh	0	5	
LSIII	grain	0	5	
	flesh	0	5	
CLSI	grain	5	5	At all control samples mark 5 is due to massive growing of *Aspergillus niger*
	flesh	5	5	
CLSII	grain	5	5	
	flesh	5	5	
CLSIII	grain	5	5	
	flesh	5	5	

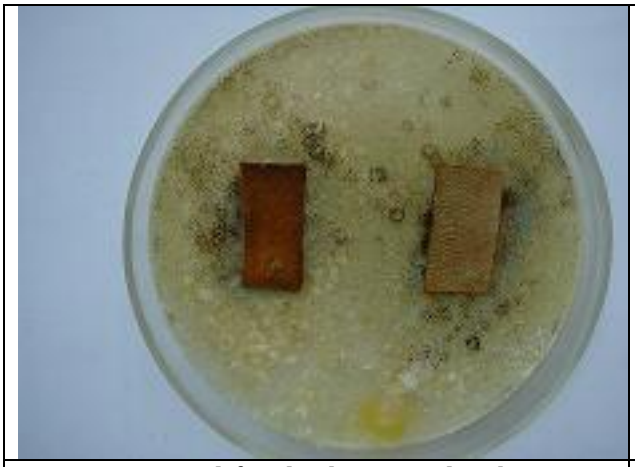
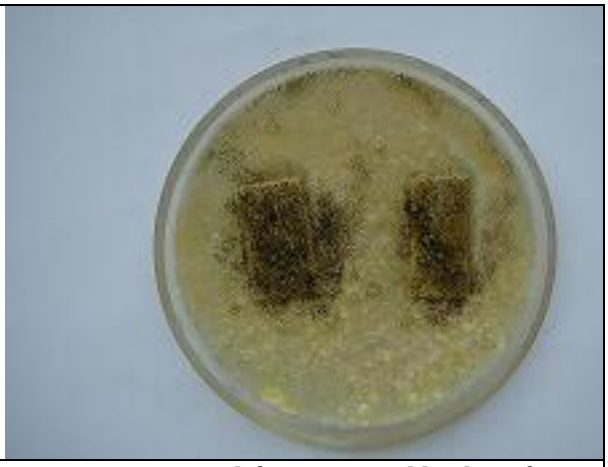
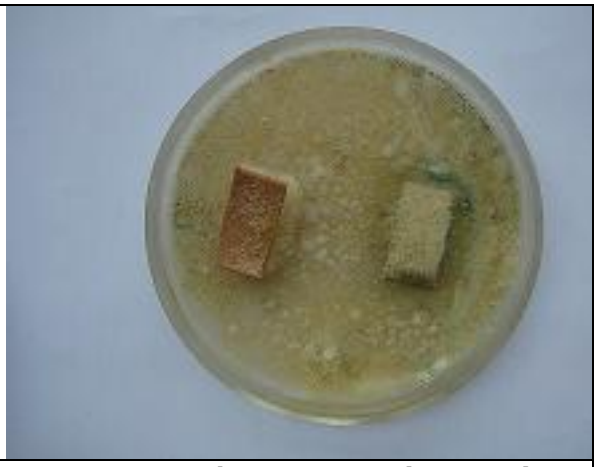
	

Fig.10 LSI-Metal-free leather treated with CSS after 7 days of exposure at fungi mix action	Fig.11CLSI – Metal-free untreated leather after 7 days of exposure at fungi mix action

From imagines of treated furskin presented in Figure 12, comparing with untreated furskin from Figure 13, can be noticed that derma site has o very good resistance.

	
Fig.12 FSII – Chromium-tanned furskin treated with CSS, after 7 days of exposure at fungi mix action	Fig.13 CFS II – Chromium-tanned untreated furskin, after 7 days of exposure at fungi mix action

4. 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 at fungi mix with an inhibition area very well delimited. For tested CSSs, MIC shows 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 (metallic 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 in dermal surface have displayed resistance against *S.aureus, E.coli* and *P.aeruginosa.* Furskin with a content of 160 ppmAg, has resistance to the *E. coli* and *P. aeruginosa* and not to *S.aureus*, due to its higher resistance (Gill et al., 2005) and, also, to the more little content of Ag. Antifungal effect tested using antibiogram method, by the exposure of same leather and furskins to a fungi mix (*A.niger*, *P.variotii*, *T.viride*, *S.atra*, *S.brevicaulis*, *C.globosum* and *P.glaucum)* shows that after 7 days, both treated furskin and leather are noted with "0" but after 14 days, only derma side of treated furskin has 01 mark.

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.

Acknowledgement

This work was supported by a grant of CNDI– UEFISCDI, project number PN167.

References

Adminis, U., Huynh C., Money, C.A., 2002, The need for improved fungicides for wet-blue, JSLTC, 86 (3), 118-121p.

Bhanu, P., Chauhan, S., Rajesh, S., Latif, U., Moni, C. and Lamoreaux, W.J., 2005, Nanoengineering of Metallic Solutions Through Silicone Constructs, Acta Chim. Slov., 52, 361–370p.

Ciobanu, C., Koncsag, C., 2007, Biomateriale Avansate pe baza de structuri proteice bioactive, nanostructurate dopate cu nanoparticule metalice, Editura PIM, 25-35p.

Document no. FTTS-FA-002, 2000, Test method of specified requirements of antibacterial textiles for medical use.

Elechiguerra**,** J.L., Burt, J.L., Morones, J.R., Camacho-Bragado A., Gao, X., Lara, H.H., Yacaman, M.J., 2005, Interaction of silver nanoparticles with HIV-1, Journal of Nanobiotechnology, **3**(6)

Feng, Q.L., Wu, J., Chen, G.Q., Cui, F.Z., Kim, T.N.,.Kim, J.O., 2003, A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus, J.Biomed.Mater Res., 52**,** 662-668p.

Ericcsson, H.M., Sherris, J.C., 1971, Antibiotic sensitivity testing, Report of an international collaborative study, Acta Pathol. Microbiol. Scand. Suppl., 217B (64).

Gill, S.R., Fouts, D.E., Archer, G.L., Mongodin, E.F., 2005, Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain, J.Bacteriol. 187, **7**, 2426-38p.

Hongshui, W., Xueliang, Q., Jianguo, C., Xiajian, W., Shiyuan, D., 2005, Mechanisms of PVP in the preparation of silver nanoparticles, Materials Chemistry and Physics, 94, 449-453p.

Gaidau, C., Plavan, V., Lupulescu, D., Crudu M. and MIU, L., 2007, New treatments for quality improvement in medical sheepskins, Buletinul Institutului Politehnic Iasi LIII (LVII), **5**, 407-410p.

Karandikar and al., US Patent Appl. Pub. No. 0003603 A1, Jan. 4, 2007: Antimicrobial silver Compositions.

Moulder, J.F., Stickle, W.F., Sobol, P.E., Bomben K.D., 1992, (Eds.), Hand Book of X-ray Photoelectron Spectroscopy, Perkin–Elmer Corporation Physical Electronics Division.

Petica, A., Gavriliu, S., Lungu, M., Buruntea, N., Panzaru, C., 2008, Colloidal silver solutions

with antimicrobial properties, Materials Science and Engineering B, 152, 22-27p.
SR CEI60068-2-10/2006; Pr. PI – 14, 2007; ASTM D: 4576-86, 1996.
Wang.H., Niu J., Long X., He Y., 2008, Sonophotocatalytic degradation of methyl orange by nano-sized Ag/Ti O_2 particles in aqueous solutions, Ultrasonic Sonochemistry**, 15**, 386-392p.
S.Y.Yeo, H. J. Lee and S.H.Jeong., 2003, Antibacterial effect of nano- sized silver colloidal solution on textile fabrics, J.Mater. Sci., **38**, 2199p.
WHO/CDS/CSR/DRS/2001.5, 2002, - Surveillance standards for antimicrobial resistance.