Nanosilver Application for Collagen Based Materials Treatment

Carmen Gaidau [1,*], *Aurora Petica* [2], *Viorica Trandafir* [1],
Constantin Ciobanu [3], *Tamara Martinescu* [4]

[1] R&D National Institute for Textile and Leather-Division Leather and Footwear Research Institute,Bucharest, 031215, Romania
[2] INCDIE-ICPE CA, Bucharest, 03013, Romania
[3] Petru Poni"Institute of Macromolecular Chemistry, Iassy, 700487, Romania
[4] SC Nappa Com Ltd, Ploiesti, 100365, Romania

Abstract: Leather and sheepskins for medical use are natural materials based on collagen and keratin, well known for preventive and curative properties, suitable for treatment of orthopedic, diabetic or bedsore diseases. The microbiological resistance of leather and sheepskins for medical use is an important characteristics and could be obtained by using quaternary ammonium compounds in washing process, phenol active ingredients in wet or dry finishing stages. In our paper we have investigated the possibility of designing new materials based on nanosilver particles and new technologies for application on medical leathers and sheepskins. The new compounds, nanosilver colloidal solutions obtained by chemical and electrochemical synthesis routes were characterized by DLS (Dynamic Light Scattering), TEM (Transmission Electron Microscopy) and UV- vis spectroscopy. Nanosilver colloidal solutions with 20-40 ppm Ag concentration and particle size of 5 nm were obtained by electrochemical method and have shown a high stability when was prepared with additives based on polyvynilpirrolidone. Colloidal silver solutions were chemically synthesized by reduction of silver nitrate solutions with sodium citrate solution when 137 ppm Ag concentration and 5 nm particle size were obtained. The correlation of different kinds of treatments, silver concentration in leather with antifungal and antibacterial resistance of new collagen supports doped with nanosilver particles was done by using AAS, XPS, antibiogram method for Penicillium glaucum, Aspergillus niger, Trichoderma viride Scopulariopsis brevicaulis, Paecilomyces variotii and diffusimetric method for Staphylococcus aureus, Acinetobacter baumannii, Stenotrophomonas maltophilia and Pseudomonas aeruginosa.

Key words: colloidal silver solutions; antibacterial and antifungal characteristics; collagen doped with nanosilver; medical items, medical leathers and furs, nanosilver particles.

1. Introduction

The success of the introduction of silver nanoparticles (SNP) in different forms in bioscience, healthcare and consumer goods is already known [1 – 5].

*Corresponding author. Phone: 0040213235060. E-mail: carmen_gaidau@hotmail.com

SNP present a great interest due to theirs unique properties, such as high electrical and thermal conductivity, catalytic activity or optical properties that depend on the size and the shape of the particles. Special SNP properties and efficiency as antimicrobial and antifungal agent are due to a high surface area, very small size (<20nm) and high dispersion.

It is believed 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 [6]. As a result DNA molecules become condensed and lose their ability to replicate. In addition, Ag^+ from silver based solution is a long lasting biocide with high temperature stability and low volatility. The SNP may be used in form of colloidal sols or doping agents for a lot of composite materials with polymer matrix.

In this paper colloidal silver solutions (CSS) obtained with electrochemical or chemical methods are used to interact with collagen and/or keratin from medical leather and sheepskins to induce bioresistance properties at fungi or microbes action. This approach is an alternative at currently use of phenolic active ingredients or quaternary ammonium compounds with toxic and polluting potential, promoting "green chemistry". With this in view, different CSS with and without TiO_2 [7] were electrosynthesized and also CSS associated with polyhydroxiurethane (PHU) type polymers obtained by chemical methods. Paper presents some results concerning CSS and SNP characteristics, like concentration, stability, morphology, antifungal and antibacterial properties, the interactions with collagen and bioresistance effects conferred to medical leather and sheepskins.

2. Experimental

Different CSS were electrochemically obtained, with and without TiO_2 and also, using chemical methods, CSS combining with polyhydroxiurethanes compatible with collagen and keratin substrates, tanned with chrome salts or organic tannins were synthesized.

2.1 New nanomaterials and polymers for leather and furskins treatment

The electrochemical synthesis of CSS was performed by so-called "sacrificial anode method" [7], involving a constant current pulse generator, with stirring and alternating polarity, electrodes of 99.999 Ag with sizes of 155 / 27 mm.

CSS with 32 ppm Ag, such as or combining with 10g/l TiO_2, CSS electro synthesized in the presence of 10 g/l TiO_2 and 50 g/l TiO_2 were used for treatment of medical leather and furskins.

Also, CSS were chemically synthesized by reduction of silver nitrate solutions with sodium citrate solution. In this way a CSS with 137 ppm Ag concentration was obtained.

Polyhydroxiurethane synthesized to interact with collagen and SNP is perfectly compatible with collagen and keratin substrates conferring them a permanent softness with following characteristics: aqueous, homogeny solution, light yellow color, 45-65% concentration and 10000cP viscosity.

2.2 Characterization of new colloidal nanosilver solutions

The Ag concentration of the obtained CSS was determined by quantitative analysis and UV-vis absorbance spectra recording by a JASCO V 500 spectrophotometer. The nanoparticles sizes and Zeta potential were measured by DLS (Dynamic Light Scattering) technique using Brookhaven equipment. The silver nanoparticles morphology and dispersability were evidenced by transmission electron microscopy (TEM) measurements, using an electronic microscope Philips CM 100.

2.3 Treatment of leathers and furskins, evaluation of biological resistance

Experimental applications on collagen based materials, by immersion, by spraying, in tanning baths, in retanning or neutralization bath, using CSS with and without TiO_2 or in combination with special prepared polyhyoxiurethanes were done. Silver concentration from different treated derma of leather and furskins was analyzed by atomic absorption spectroscopy (Analitik Jena spectrometer) and was evidenced on surface by using X–ray photoelectron spectroscopy (XPS)[8] with an 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).

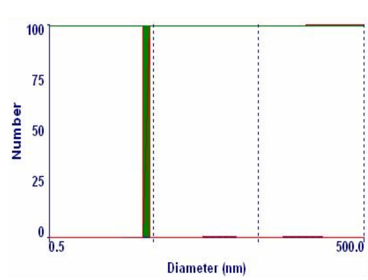
To analyze antifungal activity, the antibiogram method was used. The fungi mix with biodeteriogen potential for medical leather and furskins, used for inoculation, contained: *Penicillium glaucum*, *Aspergillus niger*, *Trichoderma viride Scopulariopsis brevicaulis*, *Paecilomyces variotii*. The evaluation of biological resistance of collagen based materials was performed by using standardizated methods [9-12].

To evaluate the antibacterial efficiency against *Staphylococcus aureus*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia and Pseudomonas aeruginosa*, diffusimetric method was used on leather and furskins treated with SNP [13].

3. Results and discussion

3.1 Size, stability and morphology of CSSs obtained by electrochemical method

Firstly, a CSS with 32 ppm Ag concentration, obtained in the presence of a mix of two stabilizers agents and with the characteristics presented in Figure 1 – 4 was electrosynthesized.



Rel. Int. = 100.00 Cum. Int. = 99.72 Diam. (nm) = 4.20

Fig. 1- Grain size distribution of SNP

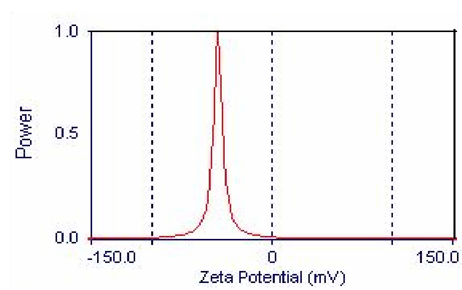


Fig. 2 -Zeta potential distribution

From grain size distribution diagram it can be seen that 99.72% from particles number is up to 4.20 nm and Zeta potential distribution is a monomodal one; -44.89 mV value indicates that particles are fully covered by the mix of stabilizers and thus, the solution is very stable.

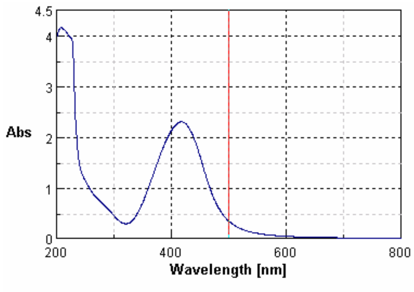


Fig. 3 UV-VIS absorbance

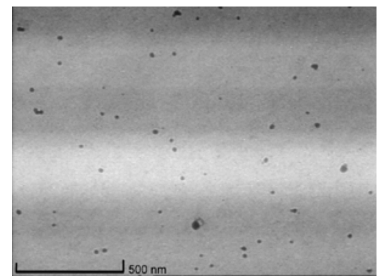


Fig. 4 - TEM micrograph

To increase antimicrobial activity of the CSS and to ensure a synergistic effect, 10g/l TiO_2 was added. This was added either in a CSS solution of 32 ppm Ag, either in water before electrosynthesis, when Ag was deposited on TiO_2 nanoparticles. TiO_2 is a type *n* semiconductor with a strong photocatalitic action, whose particles is positively charged and attracts the negatively charged silver nanoparticles. In consequence, a strong interaction between SNP and TiO_2 substrate takes place. Recently, the photoinduced bactericidal activity of TiO_2 thin films has been demonstrated [14].

3.2 *Size of CSS obtained by chemical method*

Chemically obtained CSS has 137 ppm Ag concentration and pH- 6.80 at 20 ℃. SNP size was measured by UV-vis spectroscopy and Zeta-sizer equipment; UV-Vis spectra are presented in figure 5 and size distribution in Figure 6. From figure 6 it can be seen that most of SNP have size up to 5 nm, in accord with UV-Vis spectra results (figure 5).

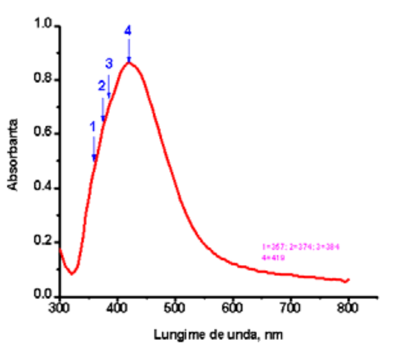


Fig. 5-UV-vis spectra for CSS with 137 ppm Ag

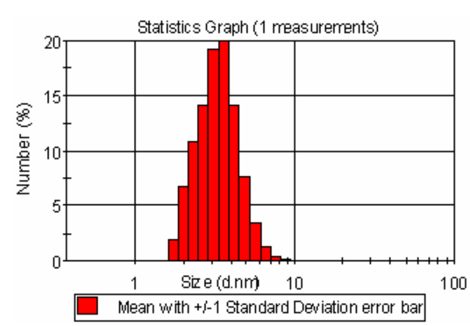


Fig. 6-Rate and size distribution of SNP

3.3 *Treatment of leather and furskins with CSS*

The realization of medical leather and furskins substrates with biological resistance characteristics involves finding an integration step of SNP treatment in the technological process of these. Many applications which take into account interaction possibilities with collagen or keratin, processed in different ways, were designed. Applied treatments were: immersion, spraying, tanning, retanning, using CSS with and without TiO_2 or in combination with special prepared polyhyoxiurethanes.

3.4 ***Microbiological assessment of collagen and keratin supports treated with CSS***

Based on standardized methods [8-11] for determination of collagen supports resistance at fungi (fig. 7,8) and bacteria (table 1, [12]) we concluded that improved microbiological resistances could be obtained by using SNP materials designed for leather and furskins use.

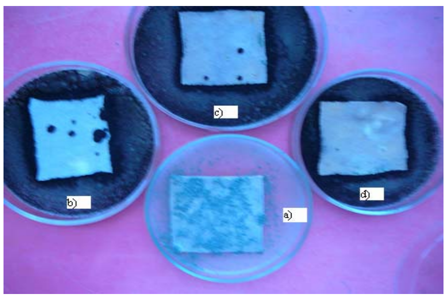


Fig. 7- Fungitoxic effect on leather support expressed by mould growth:
a) witness, b) 32 ppm Ag CSS,
c) Ag/ TiO_2 CSS with 10 g/l TiO_2,
d) Ag/ TiO_2 CSS with 50 g/l TiO_2,
after 7 days

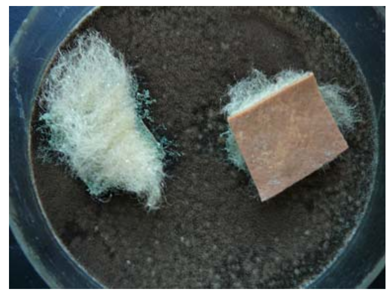


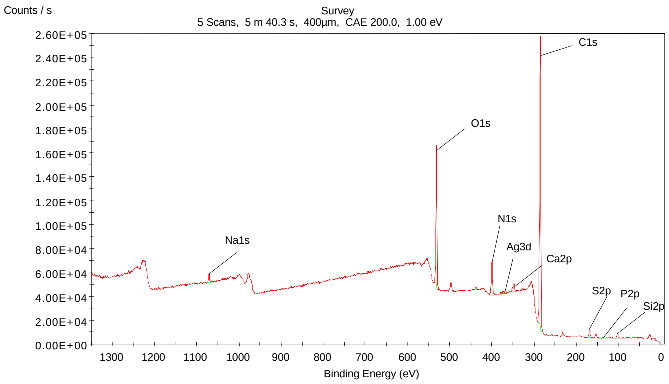
Fig.8 –Fungitoxic effect on furskins after 3 days 01 mark on fur and 0 mark on derma

Table 1 Antibacterial action of leathers treated with CSS by immersion

CSS	Ag conc in leather samples ppm	*Staphylococcus aureus*	*Acinetobacter baumannii*	*Stenotrophomonas maltophilia*
Electrochemical CCS with 32 ppm Ag	490	+	+	+
Electrochemical CCS with 10 g/l TiO2	4110	+	+	+
Electrochemical CCS with 50 g/l TiO2	3450	+	+	+

"+" – with antibacterial activity

The assessment of Ag on the surface of leathers treated with polyhyoxiurethanes and SNP obtained by chemical method was done by using a XPS device (fig.9) and showed clearly the possibility of using wet treatment of leathers for surface functionalization against different kind of bacteria (in this case the leather samples showed resistance against *Pseudomonas aeruginosa* ATCC 27853).



Name	Peak BE	FWHM eV	Q	At. %
C1s	284.46	3.29	1	76.09
O1s	531.25	3.42	1	14.29
N1s	399.36	3.16	1	5.17
S2p	167.83	3.34	1	1.24
Na1s	1070.92	3.10	1	0.49
Si2p	101.97	3.25	1	1.37
Ag3d	368.09	3.04	1	0.11
Ca2p	347.89	6.28	1	0.66
P2p	133.46	3.34	1	0.58

Fig.9 - Elemental ID and quantification of Ag on leather surface treated with polyhyoxiurethanes and SNP

4. Conclusions

- ❖ CSS with 5nm main diameters of SNP, by electrochemical and chemical approaches were synthesized;
- ❖ In order to use these CSS like antifungal and antibacterial agents for collagen based materials (leathers and furskins) treatment, different mixes were realized;
- ❖ The concentration of silver nanoparticles in collagen supports is highly influenced by CSS type and technology of application. The improvement of fungitoxic and antibacterial activity of collagen supports treated with CSS was obtained;
- ❖ Leather treated by immersion presents a good antibacterial action, even at lower Ag concentration.

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