Progresses in treatment of collagen and keratin-based materials with silver nanoparticles

Carmen Gaidau1*, Aurora Petica1, Marin Micutz2, Mihai Danciu3, Todorka Vladkova4
1INCDTP-Leather and Footwear Research Institute (ICPI), 031215 Bucharest, Romania
2Department of Physical Chemistry, University of Bucharest, 030018 Bucharest, Romania
3Morphopathology Department, "Grigore T. Popa" University of Medicine and Pharmacy, 700115 Iasi, Romania
4Polymer Engineering Department, University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria

Abstract: The influence of different chemical systems on the interaction of collagen at the molecular level with silver nanoparticles in collagen-based materials is a very important source of information for designing leather technologies and treatments as ecological alternatives to organic preservatives currently used. The UV circular dichroism as a scientific tool to assess mainly the secondary structure of proteins in solution proved that silver nanoparticles are able to interact with collagen in aqueous medium in the presence of some classical cross-linkers and other chemical additives similar to those occurred in collagen-based materials. The most important influence on secondary structure of collagen was observed in the case of interaction with chemically and electrochemically synthesized silver nanoparticles in the presence of basic chromium salts with or without poly(hydroxy urethane).

The treatment of chromium tanned sheepskins with poly(hydroxyl urethane) and dispersion of chemically synthesized nanosilver showed the best resistance to fungi and bacterial exposure tests. These collagen-based materials containing different amounts of nanosilver were investigated by Atomic Absorption Spectroscopy, Atomic Force Microscopy and Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy. The influence of sheepskins treated with silver nanoparticles on the wound healing process was assessed and the nanoparticles concentration seems to have a positive effect up to 370 ppm and does not influence the inflammatory process above this concentration.

Keywords: Collagen treatment • Circular dichroism • Nanosilver • Leather processing • Preservatives

1. Introduction

Silver nanoparticle (AgNPs) synthesis attracts an increasing interest due to their new and different characteristics as compared with those at the macroscopic phase with interesting applications in various domains such as optics, microelectronics, catalysis, information storage or energy conversion. The success of the introduction of AgNPs in different forms in bioscience, healthcare and consumer goods is already known [1–5]. The AgNPs with antimicrobial and antifungal properties against many types of bacteria and fungi may be used as colloids or doping agents for a lot of composite materials with a polymer matrix. Thus, various antibacterial finishes and disinfecting techniques have been developed for different types of textiles, cellulosic and synthetic materials [6].

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 bonds, which induces the inactivation of bacterial proteins [7].

* E-mail: carmen.gaidau@icpi.ro
AgNPs as ecological alternative for organic biocides, which are mainly volatile organic compounds (VOC), represent an innovative challenge for treatment of collagen and keratin-based materials such as medical sheepskins. Medical sheepskins are a valuable natural material, compatible with the human body, able to absorb body pressure, friction and humidity (bedsore generating factors). Physiological heat transfer of medical sheepskins makes this material a unique product, unmatched by any other synthetic materials used for recovery from orthopaedic conditions. There is a limited number of publications regarding the interaction of collagen and keratin with AgNPs-based systems [8-12] or AgNPs in combination with benzalkonium bromide [13,14]. In the present study, we report our results on investigation of collagen-AgNPs in aqueous media and the effect of some additives on collagen-AgNPs interaction as a starting point to a better understanding of the influence of nanosilver upon a more complex substrate such as sheepskins (mainly based on two proteins: collagen and keratin). These results, reported herein, will eventually allow for the proper selection of AgNPs-based systems and technological conditions for effective interactions with highly porous materials like sheepskins and assessing the potential phlogogen effect of AgNps on skin as well.

2. Experimental procedure

2.1. Experimental design

In order to find out the proper conditions (composition, pH) within which an optimum interaction between collagen and nanosilver takes place, several systems containing collagen and AgNPs as primary components were investigated through ultraviolet circular dichroism (UV-CD). Briefly, the influence of the additional ordering of crosslinking agents (basic chromium salt and water-soluble poly(hydroxy urethane)) for collagen and the type of AgNPs upon aqueous collagen-nanosilver mixtures were studied by UV-CD to select those systems which preserve the collagen triple helix almost intact. Based on this original constraint, a convenient biocidal action of collagen-AgNPs interaction as a starting point to a better understanding of the influence of nanosilver upon a more complex substrate such as sheepskins (mainly based on two proteins: collagen and keratin). These results, reported herein, will eventually allow for the proper selection of AgNPs-based systems and technological conditions for effective interactions with highly porous materials like sheepskins and assessing the potential phlogogen effect of AgNps on skin as well.

2.2. Materials used

The following aqueous AgNPs-based systems were used:

- chemically synthesized colloidal silver dispersion with a concentration of 140 ppm and an average diameter of 10 nm, designated PP. The silver nanoparticles were synthesized by Ag⁺ reduction from a dilute AgNO₃ solution with sodium citrate in the presence of polyvinylpyrrolidonone (PVP) as stabilizer in the first step, followed by the reduction with sodium borohydride solution in order to increase AgNPs concentration [8];

- chemically synthesized colloidal silver dispersion with a concentration of 50 ppm and an average diameter of 10 nm (G). Chemical synthesis of AgNPs was performed by Ag⁺ reduction from a dilute AgNO₃ solution in the presence of sodium citrate and PVP as electrostatic and steric stabilizer [8];

- electrochemically synthesized colloidal silver dispersion with a concentration of 17 ppm and an average diameter of 5 nm (SC). The electrochemical synthesis of AgNPs was performed by so-called “sacrificial anode method” and consists of an anodic dissolution in an Ag electrode in a dispersion media of compatible mixture of a stabilizer (PVP) and a co-stabilizer (sodium lauryl sulfate), using a constant current pulse generator with changing polarity and continuous stirring [8];

- dispersed systems of silver nanoparticles (electrochemically synthesized) deposited on TiO₂ with a nanosilver concentration of 32 ppm and an average diameter of 5 nm (Ag/TiO₂). The silver nanoparticles deposited on TiO₂ were obtained by in situ electrosynthesis of Ag in a dispersed system of TiO₂ nanopowder and sodium polyacrylate as stabilizer and dispersant agent, using the method aforementioned [8]. As crosslinkers for collagen, two chemical compounds were used:

- basic chromium salt (Chromosal, Lanxess, with Cr₂O₃ content of 25%), designated Cr;

- water-soluble poly(hydroxy urethane) – aqueous solution of 32%, viscosity 10,000 cP (PHU) [15].

The collagen component (as collagen type I, Col) used in all studied aqueous systems was extracted from bovine hide having the same concentration of 5×10⁻⁶ mol L⁻¹ throughout the samples [16]. Sheepskins utilized to test the selected technology (consistent with the results on aqueous collagen-based systems) were processed in a pilot unit according to the technologies for medical use [17].

2.3. Methods of analysis

Collagen-AgNPs interaction in aqueous systems regarding possible secondary structure modifications of protein were studied by means of UV-CD spectroscopy

Progresses in treatment of collagen and keratin-based materials with silver nanoparticles
using a Jasco-810 spectropolarimeter. To record UV-CD spectra, the following working parameters were set: wavelength range 190-250 nm; scanning speed of spectra – 50 nm min\(^{-1}\) with 0.2 nm pitch and 2 s response time; number of spectra accumulation for each sample – 4; continuous feeding of measuring chamber of apparatus with high purity nitrogen (99.9995%) to suppress the oxygen absorption mainly within the range of 190-200 nm. The UV-CD spectra were corrected by subtracting the baseline recorded for the correspondingly collagen-free mixtures. All UV-CD measurements were performed at room temperature (23°C) using a quartz cuvette (Helma, Suprasil Quartz) of 0.2 mm path length.

Microbiological resistance tests of sheepskins treated with silver nanoparticles were carried out in accordance with the national [18] and international [19,20] standards and methods used for antifungal and antibacterial treated materials. In this respect, triplicate samples were taken into account.

Silver concentration in the sheepskins was determined by Atomic Absorption Spectroscopy (AAS) on an Analitik Jena spectrometer, by detaching the wool of sheepskins from the dermis and by triplicate analytical measurements.

In order to identify the presence of silver on the dermis surface of sheepskins and the manner of its deposition within the dermis structure and on the wool surface, Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDS) (FEI Quanta 200) and Atomic Force Microscopy (AFM) (S-3400N Hitachi) were used.

Histological response in rat skin in contact with sheepskin treated with AgNPs-based mixtures was evaluated using in vivo experiments on Sprague-Dawley rats. The skin samples retrieved through biopsy were fixed in buffered formalin, at neutral pH, included in paraffin and stained by Hematoxylin-eosine and van Gieson mixtures.

Histopathological diagnosis of skin fragments (retrieved by biopsy) was done after 48 and 96 hours of skin interaction with sheepskins treated with various concentrations of AgNPs in comparison with the untreated sheepskins. The induction of inflammatory reactions by epicutaneous contact enabled the evaluation of irritant potential of sheepskin treated with AgNPs. The “per primam” healing was also investigated, by connective tissue repair of the surgical wound resulting from the biopsy. Histopathological observations have enabled to estimate the influence of AgNPs on the animal skin sensitization.

### 2.4. Formulation of the aqueous collagen-based mixtures to investigate silver nanoparticles-collagen interaction through UV-CD spectroscopy

CD is a powerful technique that allows the investigation of secondary structure of proteins in solution state. Aside from the majority of proteins, native collagen in solution exhibits a particular shape of its CD spectra very similar to that obtained for poly-L-proline II: an intense negative peak located near 200 nm and a weaker positive one at 220-225 nm. This is considered strong evidence for a triple helical conformation of collagen [21-26]. A very important dimensionless feature regarding the helicity degree of collagen in aqueous solution is the absolute value of the ratio of positive peak intensity to that of the negative peak (Rpn) in its CD spectrum [25-27]. An Rpn value of about 0.12 is typical for the non-denatured collagen in aqueous solution [28]. Collagen denaturation triggers intramolecular conformational changes which give rise to a more or less important modification of CD spectrum shape: partial denaturation progressively induces declining Rpn and a redshift of cross point, while the positive peak completely disappears during full denaturation and the negative one lowers and shifts to larger wavelengths [25,29,30].

A number of 26 collagen-based aqueous mixtures having the same concentration of collagen (0.15% \(\approx 5\times10^{-4}\) mol L\(^{-1}\)) were prepared. Each of these systems contains one or more noncollagenous components of different but constant amounts within 100 g of final collagen-based mixture in accordance to the following: 0.072 g Cr, 0.036 g PHU, 0.90 g SC, 0.90 g Ag/TiO\(_2\), 0.72 g PP, 0.72 g G at pH = 5.5 (Table 1). All UV-CD spectra recorded for the 26 aqueous systems were compared to that for the aqueous collagen solution of 0.15%. An Rpn value of 0.12 obtained for the last system (sample 1, Table 1) certifies the integrity of native conformation (triple helix) of collagen used for sample preparation.

### 2.5. Assessment of the efficiency of the silver nanoparticles treatment in the medical sheepskin processing technology

Verifying the efficiency of medical sheepskin treatment with AgNPs-based mixtures was done for sheepskins tanned with basic chromium salt, re-tanned with poly(hydroxy urethane) and additivated with PP (sample 5, Table 1), according to the technological flow chart shown in Table 2. Two samples of the
Table 1. Particular compositions of the aqueous collagen-based mixtures investigated through UV-CD.

| Sample number | Composition expressed as noncollagenous components | Sample abbreviation |
|---------------|---------------------------------------------------|---------------------|
| 1             | -                                                 | Col                 |
| 2             | Chromium basic salt                               | Col-Cr              |
| 3             | Poly(hydroxy urethane)                            | Col-PHU             |
| 4             | Chromium basic salt and poly(hydroxy urethane)    | Col-Cr-PHU          |
| 5             | Chromium basic salt, poly(hydroxy urethane) and PP | Col-Cr-PHU-PP       |
| 6             | Poly(hydroxy urethane) and PP                      | Col-PHU-PP          |
| 7             | PP and poly(hydroxy urethane)                     | Col-PP-PHU          |
| 8             | SC                                                | Col-SC              |
| 9             | Chromium basic salt and SC                         | Col-Cr-SC           |
| 10            | SC and chromium basic salt                         | Col-SC-Cr           |
| 11            | Ag/TiO$_2$ and chromium basic salt                 | Col-Ag/TiO$_2$      |
| 12            | Ag/TiO$_2$ and chromium basic salt                 | Col-Ag/TiO$_2$-Cr   |
| 13            | Chromium basic salt and Ag/TiO$_2$                 | Col-Cr-Ag/TiO$_2$   |
| 14            | Chromium basic salt, poly(hydroxy urethane) and G | Col-Cr-PHU-G        |
| 15            | Poly(hydroxy urethane) and G                       | Col-PHU-G           |
| 16            | G and poly(hydroxy urethane)                       | Col-G-PHU           |
| 17            | Poly(hydroxy urethane) and chromium basic salt     | Col-PHU-Cr          |
| 18            | Poly(hydroxy urethane), chromium basic salt and PP | Col-PHU-Cr-PP       |
| 19            | Poly(hydroxy urethane), PP and chromium basic salt | Col-PHU-PP-Cr       |
| 20            | Poly(hydroxy urethane), chromium basic salt and G  | Col-Cr-PHU-G        |
| 21            | Poly(hydroxy urethane), G and chromium basic salt  | Col-PHU-G-Cr        |
| 22            | Chromium basic salt and PP                         | Col-Cr-PP           |
| 23            | PP and chromium basic salt                         | Col-PP-Cr           |
| 24            | PP                                                 | Col-PP              |
| 25            | G                                                  | Col-G               |
| 26            | G and chromium basic salt                          | Col-G-Cr            |
| 27            | Chromium basic salt and G                          | Col-Cr-G            |

Table 2. Parameters of medical sheepskins technology using silver nanoparticle-based systems.

- % with respect to tanned sheepskin weight
- Washing: H$_2$O – 600%; temperature – 40°C; duration– 30 min; run – continuously.
- Drain
- Neutralization: H$_2$O – 600%; temperature – 40°C; 1% HCOONa; duration– 30 min; 1.4% NaHCO$_3$ ; duration– 30 min; run – continuously; control pH=5.5.
- Drain
- Re-tanning: H$_2$O – 600%; temperature – 40°C; 6% PHU; duration– 30 min; run – continuously.
- Drain
- Washing
- AgNPs treatment: H$_2$O – 200%; PP – 2.5%; temperature – 40°C; duration– 12h; run – continuously for 60 min and 11h statically.
- Drain
- Rest for 24 h. Free drying, Staking, Wool finishing.
treated sheepskins were marked PB1 and PB2. Control sheepskins (BM1 and BM2, respectively) were sampled from areas symmetrically located to the test samples.

3. Results and discussion

3.1. The UV-CD measurements on the interaction of AgNPs with collagen

The influence of AgNPs and other chemical additives as well as of their addition ordering on the native helical structure of collagen available into the prepared aqueous mixtures was studied through UV-CD spectroscopy. The main features regarding the corresponding UV-CD spectra are shown in Table 3. All the parameters for the collagen reference sample strongly confirm the integrity of the native helicity of collagen (see Table 3, sample 1). At the same time, it is necessary to mention that none of the UV-CD data exhibit a certain degree of collagen denaturation for the entire set of the systems investigated (see Table 3). On the contrary, some enhanced helicity of collagen was observed. This is supported by the Rpn values, the amplitude of the positive peaks and the wavelengths at which the negative and positive peaks and the cross point are located. Thus, compared to reference sample 1, the presence of the basic chromium salt has the greatest influence upon Rpn values which can be associated with a further stabilization of the collagen triple helix (samples 2, 4, 5, 9, 10, 18, 20, 22, 27, Table 3). Indeed, some investigations performed on collagen showed that an enhancement of its triple helix stability is in line with an increase of both its Rpn and denaturation temperature [26]. It was proved that Cr(III) may bind to the collagen molecule through coordinative bonds mainly involving carboxyl groups belonging to the aspartic and glutamic acid residues of the primary structure of collagen [28,33]. That is why basic chromium salts play a major role as cross-linkers in tanning technology. As a consequence, the interchains cross-linking within the collagen triple helix most likely brings enhanced stability of the native secondary structure of collagen.

On the other hand, poly(hydroxy urethane) (sample 3, Table 3) barely influences (in fact there is a tiny increase) the collagen helicity what demonstrates a lower strength of crosslinking power of PHU exerted onto collagen molecule through hydrogen bonds. Going further, all kinds of AgNPs used practically do not affect the triple helical structure of collagen (samples 8, 11, 24, 25, Table 3). In the case of the multicomponent systems (containing at least two noncollagenous components), it is easy to observe how both noncollagenous component and addition ordering influence conformational stability of collagen at the molecular level. For example, taking into account the tricomponent systems containing collagen, basic chromium salt and poly(hydroxy urethane), the maximum enhancement of collagen helicity (measured by Rpn value) is attained when Cr is the second added component into the system. Thus, the Rpn value found for Col-Cr-PHU (sample 4, Rpn = 0.14) is identical with that for Col-Cr (sample 2, Rpn = 0.14). At the same time, the Rpn value for Col-PHU-Cr (sample 17, Rpn = 0.12) is equal to that for Col alone (sample 1, Rpn = 0.12), which is pretty close to that for Col-PHU (sample 3, Rpn = 0.13) (see Table 3 and Fig. 1).

Such behaviour seems to be caused by the sensitive balance between the two major routes whereby Cr and PHU components act upon collagen as cross-linkers: coordinate bonds in the case of Cr and hydrogen bonds for PHU. So, once Cr (as the second component added, sample 4) crosslinks collagen, it is hard to believe that PHU (as the third component added) is able of further noticeable crosslinking of collagen. Conversely, the crosslinking effect of PHU (weaker than that of Cr), as a second component added (sample 17), is entirely counterbalanced by the presence of Cr, as the third component added, probably due to a great affinity of Cr-PHU to form complexes. An identical situation to that described above was found for the systems consisting of collagen, basic chromium salt and nanosilver PP (see Fig. 2 and Table 3) which, in turn, is very similar to that for the tricomponent mixtures composed of collagen, basic chromium salt and nanosilver G (samples 25-27, Table 3). Replacing nanosilver G with nanosilver PP (in fact an increase of chemically synthesized nanosilver
Progresses in treatment of collagen and keratin-based materials with silver nanoparticles concentration from 50 to 140 ppm) for the last three systems (25-27) leads to the corresponding mixtures (samples 22-24, Table 3) that display a similar behaviour, except the fact that a higher nanosilver amount induces a slight crosslinking effect onto collagen (Rpn = 0.13 for sample 24 compared to Rpn = 0.12 for sample 25, Table 3). This tendency to enhance collagen helicity is somehow emphasized when the silver nanoparticles SC are used (samples 8-10, Table 3), probably due to their surface characteristics resulting from synthesis. On the contrary, the use of silver nanoparticles deposited on TiO₂ exhibits a lack of influence onto collagen secondary structure (sample 11, Table 3) and a weak crosslinking action onto collagen irrespective of the addition ordering into the tricomponent systems 12 and 13 (see Table 3).

### Table 3. The main characteristics of the UV-CD spectra for the collagen contained within the investigated mixtures.

| Sample number and abbreviation | Minimum | Maximum | Cross point, nm | Rpn |
|-------------------------------|---------|---------|-----------------|-----|
|                              | λ, nm   | θ, mdeg | λ, nm           | θ, mdeg |
| 1 Col                         | 198.2   | -130.2  | 222.2           | 15.5  |
| 2 Col-Cr                      | 198.6   | -118.5  | 221.4           | 16.0  |
| 3 Col-PHU                     | 197.2   | -128.9  | 221.8           | 16.3  |
| 4 Col-Cr-PHU                  | 198.4   | -120.7  | 221.0           | 16.6  |
| 5 Col-Cr-PHU-PP               | 198.4   | -129.7  | 221.6           | 19.0  |
| 6 Col-PHU-PP                  | 196.4   | -122.1  | 221.4           | 15.4  |
| 7 Col-PPHU                    | 197.0   | -134.7  | 221.4           | 15.9  |
| 8 Col-SC                      | 198.0   | -126.3  | 221.4           | 16.9  |
| 9 Col-Cr-SC                   | 199.2   | -119.0  | 221.0           | 17.3  |
| 10 Col-SC-Cr                  | 197.8   | -116.0  | 220.8           | 16.6  |
| 11 Col-Ag/TiO₂                | 196.6   | -132.4  | 221.4           | 16.4  |
| 12 Col-Ag/TiO₂-Cr             | 197.6   | -125.8  | 221.0           | 16.4  |
| 13 Col-Cr-Ag/TiO₂             | 198.4   | -119.4  | 221.4           | 15.7  |
| 14 Col-Cr-PHU-G               | 198.4   | -121.4  | 220.2           | 16.1  |
| 15 Col-PHU-G                  | 195.8   | -127.6  | 220.6           | 15.7  |
| 16 Col-G-PHU                  | 197.4   | -121.9  | 222.6           | 16.4  |
| 17 Col-PHU-Cr                 | 198.2   | -130.5  | 222.0           | 16.1  |
| 18 Col-PHU-Cr-PP              | 198.0   | -115.5  | 221.0           | 16.4  |
| 19 Col-PHU-PP-Cr              | 198.0   | -122.1  | 222.0           | 15.7  |
| 20 Col-PHU-Cr-G               | 198.6   | -107.2  | 220.8           | 15.3  |
| 21 Col-PHU-G-Cr               | 197.8   | -113.3  | 221.1           | 14.8  |
| 22 Col-Cr-PP                  | 198.0   | -115.5  | 221.4           | 16.5  |
| 23 Col-PP-Cr                  | 198.6   | -125.2  | 221.2           | 16.4  |
| 24 Col-PP                     | 197.0   | -130.4  | 221.2           | 16.6  |
| 25 Col-G                      | 197.6   | -133.4  | 221.0           | 16.4  |
| 26 Col-G-Cr                   | 198.0   | -125.9  | 220.9           | 16.7  |
| 27 Col-Cr-G                   | 196.6   | -125.1  | 221.0           | 16.9  |

**Figure 2.** CD spectra of collagen contained into Col (1), Col-PP (24), Col-Cr-PP (22) and Col-PP-Cr (23).
In regard to tetracomponent systems (samples 5, 14 and 18-21, Table 3), it is very important to mention that the collagen triple helix is well-preserved, even consolidated to a more or lesser extent. Thus, the mixtures composed of collagen, basic chromium salt, poly(hydroxy urethane) and nanosilver G (samples 14, 20 and 21, Table 3) behave pretty similar, even though the system having chromium salt as the second added component (sample 14) should have exhibited the maximum Rpn value, not sample 18 (which, in fact, have maximum Rpn of 0.14). Instead, the tetracomponent systems containing collagen, basic chromium salt, poly(hydroxy urethane) and nanosilver PP (sample 5, 18 and 19, see Table 3 and Fig. 3) show, as expected, a descending stabilization effect onto collagen helicity as Cr component appears to be the second, the third and the fourth added component, respectively.

Indeed, the highest value of Rpn (0.15) is shown by Col-Cr-PHU-PP (sample 5, Table 3), which means the maximum stabilization effect on the native conformation of collagen molecule. Based on that result, the mixtures identical to sample 5 were chosen for sheepskin processing technology previously presented (see Table 2).

3.2. The resistance of AgNPs treated sheepskins to fungi and bacteria

The assessment of the treatment efficiency using AgNPs-based materials as biocides was carried out by testing their resistance to a mixture of fungi according to the ASTM D 4576-86 standard. Thus, the sheepskin samples were placed into Petri dishes on inoculated nutrient agar-medium with a mixture of fungi \( \text{(Aspergillus niger, Penicillium glaucum, Paecilomyces variotii, Scopulariopsis brevicaulis, Trichoderma viride)} \) typical for leather materials. After 3, 7, 14 and 28 days of inoculation, the samples were examined under microscope and the fungi-covered surface was measured and rated on a scale from 0 to 5, where 5 corresponds to more than 75% surface coverage.

Very good resistance was observed in the case of treated sheepskins with the selected AgNPs-based system (sample 5, Table 3) as can be seen in Figs. 4,5 and Table 4.

Testing the biocidal effect of the sheepskins treated with AgNPs upon \text{Staphylococcus aureus (ATCC 6538)} and \text{Pseudomonas aeruginosa (ATCC 9027)} indicates bactericidal action against two of the strains specific for the hospital environment, with high resistance to many bactericides (Table 5).
3.3. Proving the presence of AgNPs deposited onto collagen and keratin structures of sheepskins

The contact of medical AgNPs-treated sheepskins with human skin is made through woollen side and the nanosilver concentration is quite important to assess the sensitization potential of nanoparticles. The correlation between AgNPs deposition onto sheepskin structure and its biological resistance is also important for a better understanding of nanosilver-based materials behaviour and the efficiency of such types of treatments. The efficiency of sheepskin resistance to the action of fungi and bacteria is strongly related with the presence of AgNPs within the structure of sheepskins and also on

Table 4. The resistance of sheepskins treated with AgNPs to a mix of fungi.

| Sample symbol | Collagen or keratin side | Estimating resistance to fungi [18,19] marks* | Exposure time, days |
|---------------|--------------------------|-----------------------------------------------|---------------------|
|               |                          | [3, 7, 14, 28]                               |                     |
| BP1           | wool                     | 0, inhibition zone of 4 mm wide               | 0                   |
|               |                          |                                               | 1                   |
|               |                          |                                               | 1                   |
| Control BM1   | wool                     | 1                                              | 2                   |
|               |                          |                                               | 3                   |
|               |                          |                                               | 3                   |
| BP2           | wool                     | 0                                              | 1                   |
|               |                          |                                               | 2                   |
|               |                          |                                               | 2                   |
| Control BM2   | wool                     | 1                                              | 2                   |
|               |                          |                                               | 3                   |
|               |                          |                                               | 3                   |

*mark 2 is the minimum acceptable level for treated material

Table 5. Testing the biocidal effect of the sheepskins treated with AgNPs against Staphylococcus aureus (ATCC 6538) and Pseudomonas aeruginosa (ATCC 9027).

| Sample symbol | Bacteria strain                        | CFU reduction after 24 h [20] |
|---------------|----------------------------------------|-------------------------------|
| BP1, BP2      | Staphylococcus aureus (ATCC 6538)      |                              |
|               | T0 = 0.8 x 10⁴ CFU mL⁻¹                |                              |
|               | T24 = 0 CFU mL⁻¹                       | 100% reduction               |
| BP1, BP2      | Pseudomonas aeruginosa (ATCC 9027)     |                              |
|               | T0 = 0.16 x 10⁵ CFU mL⁻¹               |                              |
|               | T24 = 0 CFU mL⁻¹                       | 100% reduction               |

Figure 6. SEM image of AgNPs deposited onto collagen fibrillar structure of dermal layer of sheepskin

Figure 7. SEM image of AgNPs deposited onto keratin fibres of sheepskin.

908
their surface (including dermis and wool surface).

To certify AgNPs deposition onto collagen and keratin structure of sheepskins, SEM-EDS and AFM techniques were employed. The presence of AgNPs (from sample 5, Table 3) both deep inside the sheepskin structure and onto its surface (including the keratin fibres as well), as can be observed in Figs. 6, 7, is a valuable indication of an adequate treatment performed upon sheepskin.

Thus, after the treatment, the semiquantitative elemental analysis (by EDS) of some areas located onto the dermal surface of sheepskin shows an average silver occurrence of 4% (by weight) (Fig. 8). At the same time, the size of the AgNPs deposited onto collagen fibrils from the dermal layer of sheepskin ranges from 30 to 47 nm in diameter as observed by AFM (Fig. 9).

### 3.4. The influence of AgNPs concentration in wool on sensitisation of skin

To assess the sensitisation induced by AgNPs to skin, sheepskin samples were processed according to the technology described in Table 2, with four different concentrations of AgNPs (P1-P4). The silver concentrations (by AAS) corresponding to each individual sample (P1-P4) are shown in Fig. 10.

According to the silver concentrations (Fig. 10), the histopathological results reported in Table 6 indicate a positive effect on skin healing in the case of sample P1 (AgNPs concentration of 369.5 ppm). Instead, samples 2-4 do not affect the skin or healing process in comparison to the control sample, which is equivalent to state that only a certain AgNps concentration (found to be ca. 370 ppm) may meet at least two desired conditions: to be skin-friendly (mainly non-inflammatory) and to improve skin healing.

### 4. Conclusions

The paper presents progress realised in optimizing the treatment of medical sheepskins with silver nanoparticles-based systems as an alternative to using organic biocides, with limited efficiency and adverse
effects for consumers and for the environment. The kinds of silver nanoparticle-based products and auxiliary materials which ensure an optimal crosslinking of the sheepskin structure as well as their addition ordering were chosen based on circular dichroism investigation to preliminarily assess the interaction of collagen with these noncollagenous components. The efficiency evaluation of the selected treatment was carried out by specific tests of resistance to fungi and bacteria. The tests have proved a high efficiency of the selected treatment and the possibility of using silver nanoparticles as fungicide and bactericide at the same time, which differentiates them from currently used organic biocides. This biocidal efficiency was directly related to the silver nanoparticles presence both inside the sheepskin structure (onto the collagen fibrils of the dermis layer) and on the sheepskin surface (including the surface of keratin fibres) as an indication of a well-done sheepskin treatment. Histopathological investigations performed on rat skin in interaction with sheepskin treated with silver nanoparticles allowed the identification of optimal concentrations to achieve the best beneficial effects on wound healing without sensitisation reactions towards skin.
Acknowledgements

This work was supported by the grants of the Romanian National Authority for Scientific Research (CNDI–UEFISCDI), project number 167 and Romanian National Authority for Scientific Research in bilateral cooperation with MEYS Bulgaria, project number 385.

References

[1] J.H. Fendler, Korean J. Chem. Eng. 18, 1 (2001)
[2] L.M. Liz – Marzan, Mater. Today 7, 26 (2004)
[3] J.L. Elechiguerra, J.L. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H.H. Lara, M.J. Yacaman, J. Nanobiotechnology 3, 1 (2005)
[4] R.J. Holladay, H. Christensen, W. Moeller, US Patent, No. 7,135,195 B2, Nov. 14 (2006)
[5] T. Yadav, A. Vecoven, US Patent Appl. Publ., No. 008868 A1, Jan. 13 (2005)
[6] H.J. Lee, S.Y. Yeo, S.H. Jeong, J. Mater. Sci. 38, 2199 (2003)
[7] Q.L. Feng, J. Wu, G.Q. Chen, F.Z. Cui, T.N. Kim, J.O. Kim, J. Biomed. Mater. Res. 52, 662 (2003)
[8] C. Gaidau, A. Petica, V. Plavan, C. Ciobanu, M. Micutz, C. Tablet, M. Hillebrand, J. Optoelectron. Adv. M 11, 845 (2009)
[9] C. Gaidau, A. Petica, C. Ciobanu, T. Martinescu, Rom. Biotech. Lett. 14, 4665 (2009)
[10] I. Ionita, A.M. Dragne, C. Gaidau, T. Dragomir, Rom. Rep. Phys. 62, (2010) http://www.infirm.ro/rrp
[11] C. Gaidau, M. Giurginca, T. Dragomir, A. Petica, W. Chen, J. Optoelectron. Adv. M 12, 2157 (2010)
[12] C. Gaidau, A. Petica, T. Dragomir, H. Iovu, C. Andronescu, J. Soc. Leath. Tech. Ch. 106, 102 (2011)
[13] Y. Weite, L. Hui, G. Ying, C. Wuyong, C. Gaidau, J. Optoelectron. Adv. M 13, 661 (2011)
[14] W. Yang, X. Wang, Y. Gong, H. Li, W. Chen, C. Gaidau, J. Soc. Leath. Tech. Ch. 107, 3 (2012)
[15] C. Ciobanu, I. Bestiuc, A. Farcas, D. Filip, An Stiint U Al I VIII,1, 21 (2000)
[16] S. Dasgupta (Ed.), Italian Leather Chemists’ Association, 9-12 March, Florence, Italy, XXVIIIth IULTCS Congress (AICC, Florence, Italy, 2005), 57
[17] C. Gaidau, V. Plavan, D. Lupulescu, M. Crudu, L. Miu, Bul. Instlt. Politec. din Iasi LIII (LVII) 5, 407(2007) (in Romanian)
[18] STAS 12697-88 (method B) Dessed leathers. Mildew action testing
[19] ASTM D: 4576-86 (Reapproved 1996) – Standard Test Method for Mold Growth Resistance of Blue Stock (Leather)
[20] H.M. Ericsson, J.C. Sherris, Acta Pathol. Microbiol. Scand. Suppl. 217B, 64 (1971)
[21] M.L. Tiffany, S. Krim, Biopolymers 11, 2309 (1972)
[22] M.G. Venugopal, J.A.M. Ramshaw, E. Braswell, D. Zhu, B. Brodsky, Biochemistry 33, 7948 (1994)
[23] N. Sreerama, R.W. Woody, Biochemistry 33, 10022 (1994)
[24] A. Tomadje, W.C. Johnson, Jr., J. Am. Chem. Soc. 117, 7023 (1995)
[25] Y. Feng, G. Melacini, J.P. Taulane, M. Goodman, J. Am. Chem. Soc. 118, 10351(1996)
[26] U. Freudenberg, S.H. Behrens, P.B. Welzel, M. Muller, M. Grimmer, K. Salchert, T. Taeger, K. Schmidt, W. Pompe, C. Werner, Biophys. J. 92, 2108 (2007)
[27] D. Barth, A.G. Milbradt, C. Renner, L. Moroder, ChemBioChem. 5, 79 (2004)
[28] R. Gayatri, A.K. Sharma, R. Rajaram, T. Ramasami, Biocem. Biophys. Res. Commun. 283, 229 (2001)
[29] M.L. Tiffany, S. Krim, Biopolymers 8, 347 (1969)
[30] D.D. Jennex, C. Sprecher, W.C. Johnson, Jr., Biopolymers 15, 513 (1976)
[31] J.R. Rao, B.U. Nair, T. Ramasami, J. Soc. Leather Technol. Chem. 81, 234 (1997)
[32] R. Gayatri, A. Rajaram, R. Rajaram, K. Govindaraju, J.R. Rao, B.U. Nair, T. Ramasami, Proc. Indian. Acad. Sci. (Chem Sci) 109, 307 (1997)
[33] R. Gayatri, R. Rajaram, B.U. Nair, F. Chandrasekaran, T. Ramasami, Proc. Indian Acad. Sci. (Chem Sci) 111, 133 (1999)