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Investigation on silver nanoparticles interaction withcollagen based materials

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This work investigate the possibility of designing new materials based on nanosilver particles and new approaches for application on collagen materials as ecological alternative to phenolic active ingredients or quaternary ammonium compounds. The new compounds, nanosilver colloidal solutions with 32-137 ppm Ag concentration and particle size of 5 nm were characterized by Dynamic Light Scattering, TEM and UV-vis spectroscopy. The possible interaction of collagen with nanosilver colloidal solutions and polyhydroxiurethanes doped with nanosilver particles was carried out by using circular dycroism spectrometry studies on model treated collagen solution and AAS on medical leather and furskins.

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Keywords: Colloidal silver solutions, collagen, circular dycroism spectrometry, medical leather and furskins

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]. 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. SNP have a high surface area, very small size (<20nm) and high dispersion.

Moreover, SNP have an increased interest due to their antimicrobial and antifungal properties against a great number of bacteria and fungi, with large applications, including medical leathers and sheepskins.

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 from medical leather and sheepskins to induce bioresistance properties at fungi or microbes action. This approach is an alternative at currently used of phenolic active ingredients or quaternary ammonium compounds with toxic and polluting potential, promoting "green chemistry". With this in view, different CSSs with and without TiO_2, [7] were electrosynthesized and also CSSs 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 CSSs were electrochemically obtained, with and without TiO_2 and also, using chemical methods, CSSs combining with polyhydroxiurethanes compatible with collagen substrates, tanned with chrome salts or organic tannins were synthesized.

CSSs obtained by electrochemical method

The synthesis of CSSs 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. To prepare stable CSSs with an optimum content of SNPs, a mix of stabilizer and co-stabilizer agents have been used, respectively PVP [poly (N-vinylpyrrolidone)] and Na-lauryl sulfate.

The experiments were carried out using the following materials:

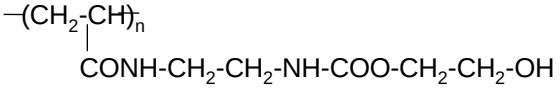
- deionized water with conductivity < 1μS, resistivity of 18 μΩ·cm and pH = 5 – 7;
- poly [1-vinyl-2-pyrrolidone] $(C_6H_9NO)_n$ (PVP10 with M = 10,000 from Sigma – Aldrich);
- Na-lauryl sulfate, provided from Sigma – Aldrich

- TiO_2, min. 99, 5%, provided Merck, Germany, grained to a d_{FSSS}=150-200 nm.

By this method CSSs with 32 ppm Ag, such as or combining with 10g/l TiO_2, CSSs of Ag/TiO_2 with 10 g/l TiO_2 and Ag/TiO_2 with 50 g/l TiO_2 were obtained and used for treatment of medical leather and furskins.

CSSs obtained by chemical method

CSSs 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 SNPs has following structure:



Polymeric solution is perfectly compatible with collagen and a solution of 2-4% reported at leather substrate weight conferring them a permanent softness and presents following characteristics: aqueous, homogeny solution, light yellow color, 45-65% concentration, 10000cP viscosity and min. 0, 2 kgf/cm^2 glass adherence.

CSSs characterisation

The Ag concentration of the obtained CSSs 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.

Studies regarding SNP's interaction with collagen molecule by circular dycroism analysis (CD). SNP's interaction with collagen based materials

In order to identify interaction mechanism between SNPs and collagen substrates, directly or together with TiO_2 / polyhydroxiurethanes, studies on model solutions of gelatins, regarding triple helix structure of collagen by circular dycroism spectrometry measurements were performed [8]. The use of this method is based on dycroic signal of collagen, due to his triple helix structure and characterized by the presence of a positive band with the peak at about 220 nm and of a negative band with the peak at about 200 nm. Usually, in his native structure, type I collagen exhibits a positive peak with a far lower intensity than the negative one. The ratio between these two intensities, in absolute value, defines the so called dycroic ratio (RPN), which is between 0.1-0.4. In the same time, in the collagen's dycroic spectra there could appear displacements of the two peaks, generally to higher wave lengths (the batocrom effect). This behavior is attributed to the higher ionic strength of the water based medium in which the collagen is dispersed. The decrease of the collagen's triple helix content reflects itself in lower positive and, as well, negative ellipticity. The general rule from which it can be appreciated the triple helix structure's integrity or modifications are the observation of the maximum positive ellipticity around 220nm.

Circular dycroism spectra were recorded at room temperature with spectrophotometer Jasco-815, in the far region (190-250), in shafts with optic ways by 0,2 mm and the following recording parameters: slot - 300 μm; responding tome – 1s; acquisitions number – 2. Considering band's modifications from the region around 220 nm of the CD spectra correlated with collagen structural modifications, results emphasized the effect of the used materials, of the adding order or of the pH reaction media. Interaction between SNPs with different additives and collagen macromolecule from leather and furskins supports was modeled by using a 10^{-5} M collagen solution [8], obtained from collagen gel with MW of 300000 Da.

Experimental applications on collagen based materials, by immersion, by spraying, in tanning baths, in retanning or neutralization bath, using CSSs 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, in flame and oven with Analitik Jena spectrometer.

To analyze antifungal activity, the antibiogram method was used. The fungi mix with biodeteriogen potential for medical leather and furskins, used for inoculation, containing: *Aspergillus niger*, *Paecilomyces variotii*, *Trichoderma viride*, *Scopulariopsis brevicaulis*, *Penicillium glaucum*. The evaluation of biological resistance of collagen based materials were performed by using standardizated methods [11-14].

To evaluate the antibacterial efficiency, the minimal inhibitorial concentration (*MIC*) upon the: *Staphylococcus aureus (ATCC)* - gram-positive cocci; *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* - Gram negative coccobacillus was determined [15].

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 5g/l PVP 10 and 0,5g/l Na-LS and with the characteristics presented in Figure 1 – 4 was electrosynthesized.

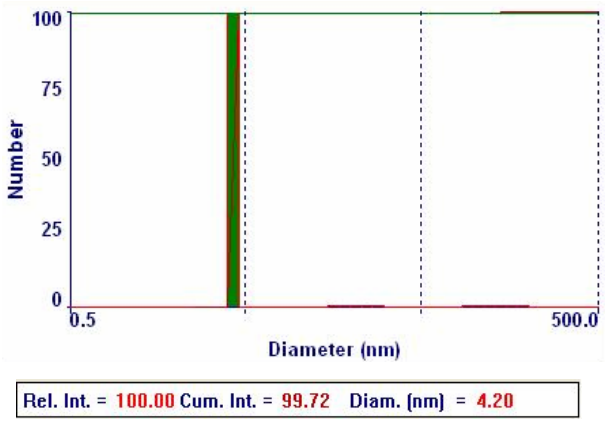


Fig. 1. Grain size distribution of SNP.

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 indicate that particles are fully covered by the mix of stabilizers and thus, the solution is very stable.

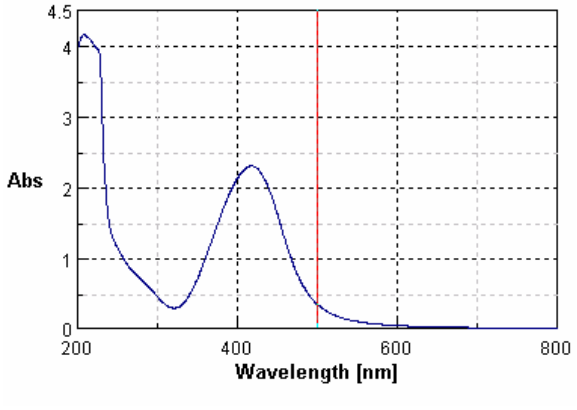


Fig. 3. UV-VIS absorbance.

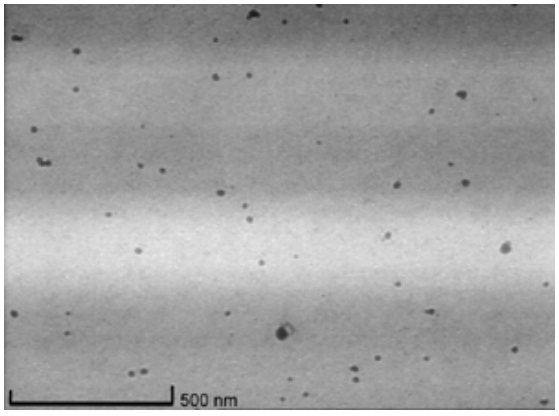


Fig. 4. TEM micrograph

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 [16].

3.2 Size of CSSs obtained by chemical method

Chemically obtained CSS has 137 ppm Ag concentration and pH- 6.80 at 20^0C. SNP's 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's have size up to 5 nm, in accord with UV-Vis spectra results (Fig. 5).

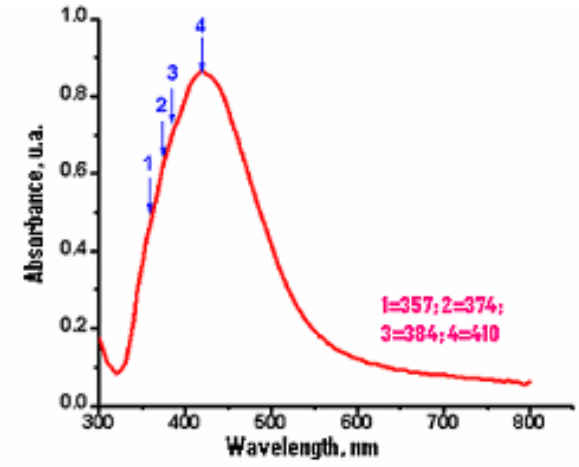


Fig. 5-UV-vis spectra for CSS with 137 ppm Ag.

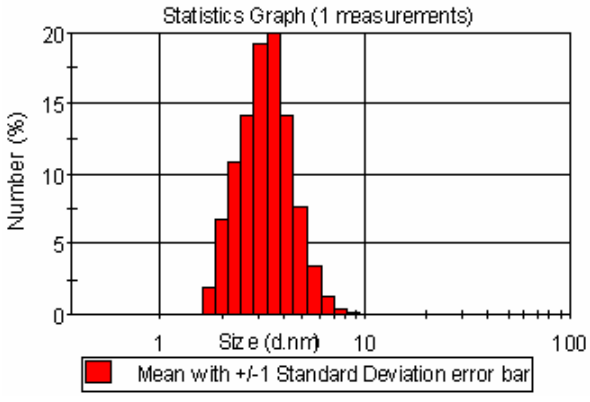


Fig. 6. Proportion and size distribution of SNP's

3.3 Collagen triple helix structure modification in interaction with SNPs and different additives by using circular dycroism spectrometry (CD)

Circular dycroism study was performed upon the following model compositions:

1. Collagen solution + 2% Cr_2O_3;
2. Collagen solution in neutral media + 4% PHU;
3. Collagen solution in neutral media + 2% Cr_2O_3 + 4% PHU;
4. Collagen solution in neutral media + 2% Cr_2O_3 + 4% PHU + 80% chemical CSS;
5. Collagen solution in neutral media + 4% PHU + 80% chemical CSS (137 ppm Ag);
6. Collagen solution at pH = 4 + 100 % electrochemical CSS (32 ppm Ag);
7. Collagen solution in neutral media + 80% chemical CSS (137 ppm Ag);
8. Collagen solution + 2% Cr_2O_3 + 100% electrochemical CSS (32 ppm Ag);
9. Collagen solution + 100% electrochemical CSS (32 ppm Ag) + 2% Cr_2O_3;
10. Collagen solution + 100% Ag/TiO_2 (10 g/l TiO_2);
11. Collagen solution + 100% Ag/TiO_2 (10 g/l TiO_2) + 2 % Cr_2O_3;
12. Collagen solution + 2 % Cr_2O_3 + 100% Ag/TiO_2 (10 g/l TiO_2).

The most significant modifications recording in triple helix configuration of collagen under the influence of chemical additives used in collagen substrate processing are presented in Figure 7. In the presence of Cr_2O_3, polyurethane solution effect is very strong, ellipticity being drastically lowed at just a few mdeg unities (Figure 7a and 7b).

From Fig. 7a it can be seen that pure collagen solution and collagen in the presence of Cr_2O_3 (1) or 4%PHU (2) presents similar spectra, with minor modifications, while for sample 3, in which both chemicals simultaneous exists, triple helix structure is broken. The same effect is also observed for sample 4, which additionally contains 80% chemical CSS (Fig. 7 b – 3 and 4 spectra presented on a large scale).

The adding order effect of chemical substances can be observed by comparing obtained spectra for 5 & 7, 8 &11 and 11 & 12 samples. Thus, comparing the collagen sample's spectra with the 5&7 samples spectra, which differ only in the adding order of polyurethane and CSS, it can be observed that the spectra of the sample 7 presents a greater ellipticity even regarding the reference collagen, while in the sample's 5 case the effect is insignificant for the 221 nm band and greater for the band corresponding to the negative ellipticity.

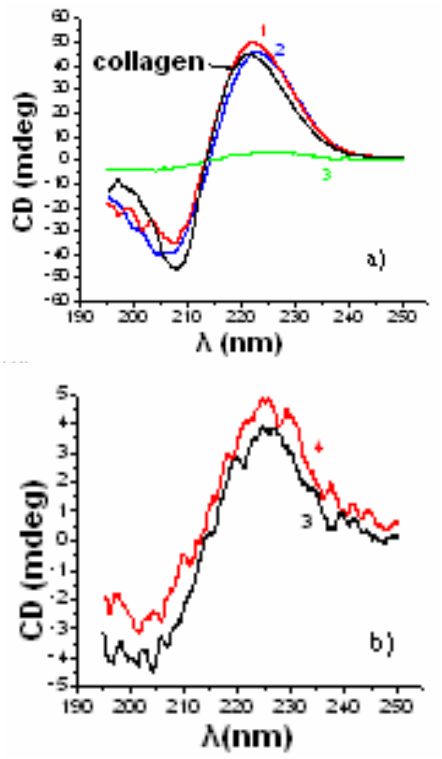


Fig. 7 a) and b)-CD spectra for collagen 1, 2, 3 and 4 samples.

As been shown, chemicals adding order effect it been seen in the case of samples 8 and 9, too, comparing with sample 1, all samples having in common Cr_2O_3 .

At the adding of 100% CSS (8 sample), can remark a helix structure stabilization as against sample 1, which contains only Cr_2O_3, while in the case of sample 9, at which first was added CSS and then Cr_2O_3, a decrease of ellipticity was observed.

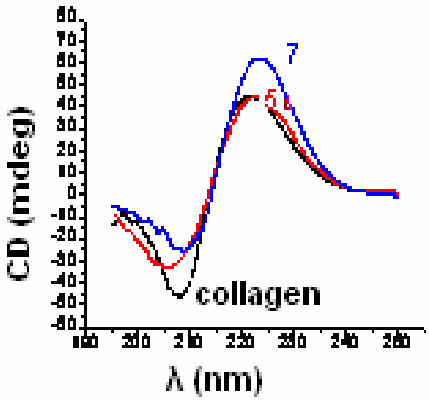


Fig. 8. CD spectra of the collagen, 5 and 7 samples

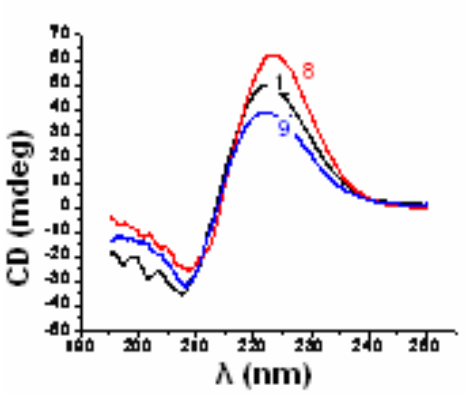


Fig. 9. CD spectra for the 1, 8 and 9 samples

The samples 10, 11 and 12 have in common a 100% CSS of Ag/TiO_2 (10 g/l TiO_2), in the absence of chromium oxide, in the case of sample 10, and in the presence of Cr_2O_3 for samples 11 and 12. The comparison between the sample 10 with the pure collagen solution evidence the effect of the auxiliary 100% Ag/TiO_2, and the comparison between samples 11 and 12, the effect of the adding order of the two components, 100 % Ag/TiO_2 (10g/l TiO_2) and Cr_2O_3. A comparison between the spectra of the samples 1 and 12 gives information about the influence of the added 100% Ag/TiO_2 (10g/l TiO_2) solution over a collagen solution which already contains chromic oxide. The circular dycroism spectra corresponding to the above mentioned samples pairs appear in the fig. 7 - 9.

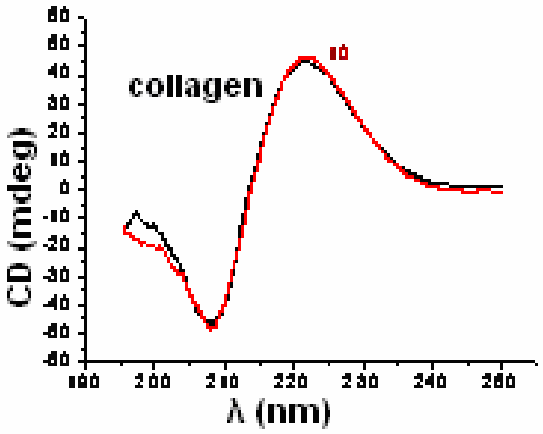


Fig.10. CD spectra of the 1 and 10 samples.

From fig.10 can observe that the dichroic spectra of the reference collagen and of the sample 10 are practically the same, in the limits of the experimental errors, proving that the addition of 100% Ag/TiO_2 (10g/l TiO_2) doesn't modify the collagen structure. Considering 1&12 samples' spectra it can be seen that adding 100% Ag/TiO_2 (10g/l TiO_2) in a collagen solution which already contains chromium oxide has the effect of reducing the positive ellipticity, so it can be associated with a decrease in the triple helix contain. With regard to the adding succession of the auxiliaries, which means the comparison of the 11&12 samples' spectra (fig.12) we find no major differences.

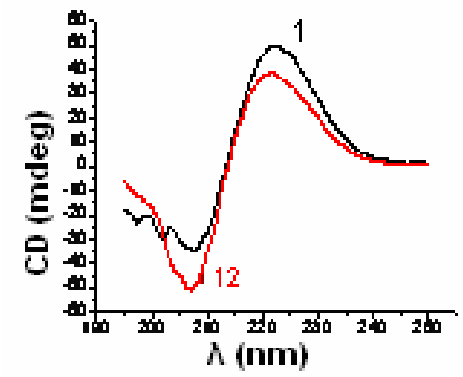


Fig.11- CD spectra for the 1 and 12 samples

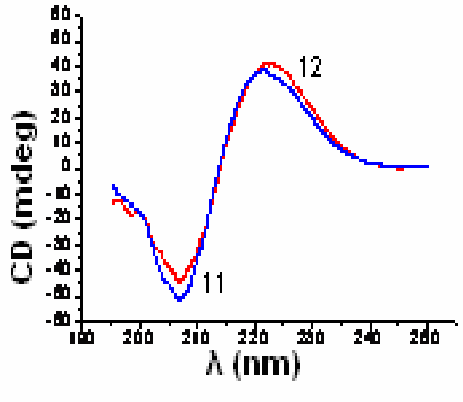


Fig.12 CD spectra for the 11 and 12 samples.

Obtaining results are synthetically presented in Table 1.

Table 1. Main characteristics of CD spectra of reference collagen and 1 -12 samples.

No.	Negative peak		Wave length at 0 ellipticity, nm	Positive peak		RPN
	Wave length, nm	Ellipticity, mdeg		Wave length, nm	Ellipticity mdeg	
Ref.	208	-46	213.5	221	45	0.98
1	207.4	-35.5	213.5	222.1	50.3	1.42
2	206	-39.4	214.3	222.7	46.5	1.18
5	205.8	-33.1	213.5	222.7	44.5	1.34
6	205.6	-34.7	214.3	224	27.9	0.80
7	208.7	-25.5	214.2	223.4	62.3	2.44
8	209.6	-21	214	223.2	62.1	2.96
9	208.2	-32.3	213.7	222.7	38.8	1.20
10	208	-48.6	213.9	221.8	46.2	0.95
11	207.1	-51.7	213.7	221.6	38.8	0.75
12	206.9	-44.7	214	222.3	41.2	0.92

From this models, it can be concluded that, due to the interaction of the collagen with SNPs and additional auxiliary materials, for all the studied samples (excepting samples 3 and 4 for which we can presume an almost complete collagen degradation) the RPN parameter (which is the absolute values ratio of the positive and negative peaks intensities) is abnormally high (over 0.7), this being a clue that the triple helix structure would be significantly modified (unwrapped), even though the positive ellipticity has pretty high values. From this reason, even for the reference collagen, there is a great probability to exist a rather high grade of micro degradation. The explanation, at least from qualitative point of view, of the presumed modifications at the triple helix structure level, was done on the bases of the comparative study of the maximum positive ellipticity of collagen in the analyzed samples. So, the fact that the samples 1, 7 and 8 have maximum positive ellipticities, notably greater than the reference collagen, could have, most probably, the following interpretation: the reference collagen presents a certain disorder degree (micro degradation) of the triple helix structure which in the conditions of 1, 7 and 8 can be partially diminished by the local restoration of the native structure. The way on which some additives could determine such a process depends on the specific action of the introduced active species, which can lead to covalent cross-linked, by ionic bridges or other kinds of interactions between the catena in the collagen structure.

3.4 Effect of CSS's and technological process on leather and furskins

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

3.4.1 AAS determination of SNP from leathers and furskins

From the table 2 it can be seen that the concentration of SNP from collagen based materials is highly influenced by the technology of leather processing and the type of CSS's. The immersion treatment by using the electrochemical CSS with 10 g/l TiO_2 assures the highest level of silver concentration in collagen based materials.

Table 2. Ag concentration in leather and furskins, determined by AAS method.

No.	CSS composition	Leather substrate	Treatment type	Ag concen-tration in leather, ppm
1	32 ppm Ag	Without Cr	Immersion	490
2	Ag/TiO_2/ with 10 g/l TiO_2	Without Cr	Immersion	4110
3	Ag/TiO_2/ with 50 g/l TiO_2	Without Cr	Immersion	3450
4	32 ppm Ag	With Cr	Pickling	647
5	32 ppm Ag	With Cr	Tanning	160
6	Ag/TiO_2/ with 50 g/l TiO_2	With Cr	Finishing by spraying	10
7	Ag/TiO_2/ with 10 g/l TiO_2	With Cr	Finishing by spraying	12
8	32 ppm Ag	With Cr	Finishing by spraying	4
9	PHU, chemical CSSs (137 ppm Ag)	Without Cr	Retanning	100
10	PHU, chemical CSSs (137 ppm Ag)	With Cr	Retanning	42

3.4.2 Microbiological assessment of collagen supports treated with CSS's.

Based on standardizated methods [11-14] for determination of collagen supports resistance at fungi (fig. 13,14) and bacteria (table 3, [15]) we could conclude that improved microbiological resistances could be obtained by using SNP materials designed for leather and furskins use.

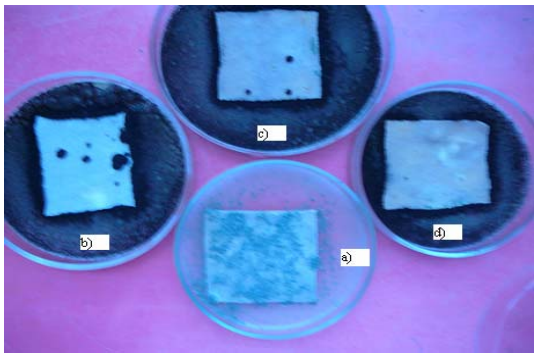


Fig. 13. Fungitoxic effect on leather support expressed by mould growth, for treatment by immersion with: a) witness, b) 32 ppm Ag CSS with 10 g/l TiO_2 sol. c) Ag/ TiO_2 CSS with 10 g/l TiO_2, d) Ag/ TiO_2 CSS with 50 g/l TiO_2, after 7 days.

Fig.14. Fungitoxic effect on furskins after 3 days 01 mark on fur and 0 mark on derma.

Table 3. Antibacterial action of leather treated with CSS's by immersion.

Leather sample (Ag conc)	*Staphylococcus aureus*	*Acinetobacter baumannii*	*Stenotrophomonas maltophilia*
1 (490 ppm)	+	+	+
2 (4110 ppm)	+	+	+
3 (3450 ppm)	+	+	+

"+" – with antibacterian activity

4. Conclusions

❖CSS, with 5nm main diameters of SNP's, by electrochemical and chemical approaches were synthesized;

❖Collagen triple helix structure modification in interaction with SNPs with different additives was analyzed by using circular dycroism spectrometry on gel solution which represent a model for leather and furskin supports;

❖ In order to use these CSS like antifungal and antibacterial agents for collagen treatment, different mixes were realized;

❖The concentration of silver nanoparticle in collagen supports are highly influenced by CSS type and technology of application. The improvement of fungitoxic and antibacterian activity of collagen supports treated with CSS was obtained;

❖Even all CSS presents a very good fungitoxic effect, at treated leather this effect is evidenced only in the case of immersion treatment, and is more pronounced in the presence of 50g/l TiO_2;on furskins the effect is diminishing;

❖Leather treated by immersion presents a good antibacterial action, even at lower Ag concentration;

❖These results are promising and offer information for further investigations in realizing biological resistant medical leather and furskins.

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