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COLLAGEN FLUORESCENCE MEASUREMENTS ON NANOSILVER TREATED LEATHER

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Abstract Silver ions are a strong antiseptic with a broad spectrum. Treatment of textiles and leather with nanosilver particles is a new idea for real clean wear. The nanometer-size particles have a large specific surface resulting in the release of silver ions in antiseptic concentration. One way to investigate nanosilver treated leather is the intrinsic fluorescence of organic material. This fluorescence is provided mainly by collagen content. Fluorescence spectrum shows an interaction between silver and collagen.

Key words: nanoparticles, silver, collagen, fluorescence, leather.

1. INTRODUCTION

In the last years, there has been increased interest in reducing the availability of commercial textile containing antibacterial agents due to environmental pollution. Silver appears as an interesting material to be used in different kind of textile fibers, because is a good antibacterial agent and non-toxic and natural inorganic metal [1–6].

Silver nanoparticles are used in many different areas especially for biomedical products [7-10]. In general, silver has germicidal effects and kills many lower organisms effectively without harm to higher animals. Silver is capable of rendering stored drinking water potable for a long period of time (several months). Water tanks on ships and airplanes are often "silvered".

Silver nanoparticles with high specific surface and nanometer sizes (<20 nm), as colloidal solutions or inserted in solid, organic or inorganic matrices are more and more intensively researched for development of new antibacterial applications for protein structures of collagen and keratin such as leathers and fur skins.

The treatment of natural leathers and fur skins with materials based on nanomaterials in view of their functionalization against microorganisms specific to hospital environment, direct contact with ill or healthy patient represents an innovative step, an ecological alternative to macro-concentration use of chemical and pollutant materials [11].

The study of natural leathers treated with different nanosilver colloidal solutions was performed by fluorescence spectrometry and reflection spectroscopy. The target of these studies was the identification of collagen structure modifications under the interaction with diverse types of nanosilver colloidal solutions in view of improving syntheses or application technologies.

The base of this experiment was to study the effects of nanosilver solutions on leather used for making shoes. One way to investigate these effects is intrinsic fluorescence of organic material. The main component of the tissue and which provides the fluorescence of material is collagen. Collagen has evolved to provide strength. It is found in connective tissue such as tendons, cartilage, the organic matrix of bone, and the cornea of the eye. Collagen molecules consist on three amino acid chains convoluted in a rod shaped, triple helix, and this tertiary structure of collagen is of microcrystalline triclinic nature. The fluorescence of collagen in UV and Visible spectral regions has been investigated in many papers and several attempts have been made to clarify its nature and origin. The existence of at least four chromophores absorbing and emitting throughout the UVA and visible spectral regions were observed. Some collagen chromophores were isolated and identified as pentosidine and pyridinoline. Both these chromophores are suggested to be products of cross-linked, pentosidine is age related. There are also various “endogenous” chromophores in collagen found in the amino acid chains like tyrosine and phenylalanine [12].

Collagen occurs in many places throughout the body. The 29 types of collagen have so far been identified and described in literature. Over 90% of the collagen in the body, however, is of type I, II, III, and IV:

– Collagen I: skin, tendon, vascular, ligature, organs, bone (main component of bone);
– Collagen II: cartilage (main component of cartilage);
– Collagen III: reticulate (main component of reticular fibers), commonly found alongside type I;
– Collagen IV: forms bases of cell basement membrane.

2. METHODOLOGY

2.1. SAMPLES

In this work we analyzed leather samples, treated with different silver nanoparticles solutions.

Materials:

• Ecologically tanned sheepskins, without chromium salts, treated by immersion with different nanosilver colloidal solutions;

• Colloidal silver nanoparticles solutions prepared by electrochemical synthesis method (SC-Ag) and deposited on TiO_2 (Ag/TiO_2);

• Colloidal silver nanoparticles solutions prepared by chemical synthesis (P-Ag and, respectively, G-Ag).

2.2. METHODS

• Silver concentration measurements in leather samples by using atomic absorption spectroscopy with flame and oven atomization (AAS).

• Reflectance measurements for leather treated with silver nanoparticles by using UV/VIS Jasco V-550 spectrophotometer connected to Jasco ISV-469 integrating sphere, in the wavelength range of 220-859 nm, with a spectral band width of 5 nm, integrating sphere diameter of 60 nm, photomultiplier detector.

• Fluorescence measurements of leather samples treated with silver nanoparticles performed with SkinSkan.

We studied the emission of fluorescence of every treated sample, genuine leather, but also for nanosilver solutions used in treating leather. Emission spectra were recorded in two different areas of same leather sample. During the experiment, the emission spectra were collected for 360 nm wavelength excitation. This excitation wavelength, that we have found to be good, corresponds to cross linked collagen [13].

In order to study collagen fluorescence we used the SkinSkan spectrofluorometer. SkinSkan is the only in-vivo, fiber-optic spectrofluorometer designed specifically for skin-fluorescence measurements. It is also ideal for many remote sensing steady-state fluorescence applications. A quartz fiber bundle selectively delivers UV-NIR radiation to the subject, and then collects the resulting fluorescence. There's no need for sample preparation, cuvettes, or adaptation. Double monochromator designs isolate the fluorescence signal from strong scattered backgrounds. Fast wavelength scanning decreases the time of measurement and exposure to excitation light. Compact and transportable to take the instrument to where the action is. Some of its applications are: acquire direct fluorescence from the skin, evaluate topical cosmetics and pharmaceuticals, differentiate normal, aged or diseased skin, validate UVA protection of sunscreens, determine epidermal proliferation, asses hair damage from sun or chemicals, identify or quantify trace contaminants for environmental testing.

3. DATA

Colloidal solutions of silver nanoparticles show concentrations in the range of 17 ppm Ag (electrochemically obtained solutions) and 61-140 ppm (chemically obtained solutions) determined by UV-VIS absorption spectrophotometry at $\lambda = 360$ and 424 nm.

Table 1

Ag concentration of leather samples treated with different nanosilver colloidal solutions

No.	Notation	Sample description	Ag concentration (AAS) ppm
1	G-Ag	Leathers treated with G solution, chemical synthesis	4420
2	P-Ag	Leathers treated with P solution, chemical synthesis	4010
3	Ag/TiO_2	Leathers treated with Ag/TiO_2 solution 10g/l	790
4	SC Ag	Leathers treated with SC Ag solution 16.7ppm	1970
5	Control	Chromium-free tanned leathers, untreated with nanoAg	-

Treatment of complex protein media such as skins or natural fur and efficiency of these nanosilver colloidal solutions are closely related to the possibility of interaction with the collagen macromolecule.

Ag concentrations in the leather samples treated with nanosilver colloidal solutions are presented in Table 1.

The obtained reflection data were transformed in absorption spectra to show a correlation between Ag concentration in the leather samples and reduction of reflectance properties of the samples, except the sample of nanosilver colloidal solution deposited on TiO_2 base, which presents a higher reflectance than the untreated control, due to known properties of titanium dioxide (Fig. 1).

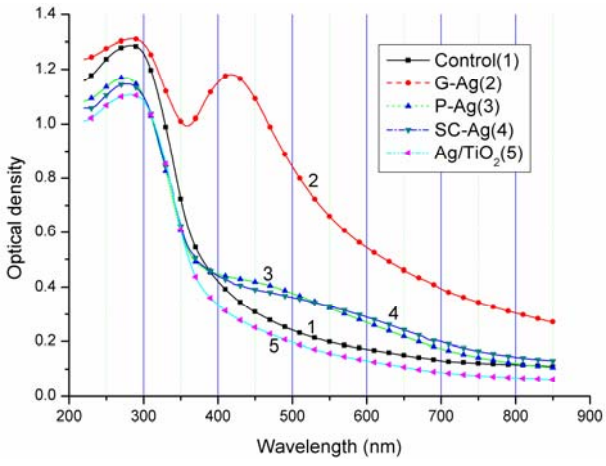

Fig. 1 – Absorption spectra of leather samples obtained by diffuse reflection measurements.

4. RESULTS AND DISCUSSIONS

We first measured the emission of fluorescence of pure collagen, in two different states: as a gel and as a powder. Fig. 2 shows the comparison between the two spectra.

It is not usual to measure maximum of collagen fluorescence at more than 400 nm like in our case. There are situations when some reagents (hypericin), age of skin or UV exposure can shift the fluorescence maximum from 395 nm to 430 nm [14]. The fluorescence spectrum of skin in visible range is difficult to understand, because it depends of the biochemical composition. Elastin and collagen are both responsible for emission in this range. Complex fluorescent molecules such as elastin and collagen have more than one fluorophore. Any change in fluorescence spectrum is due to chemical changes in structure of these molecules [15].

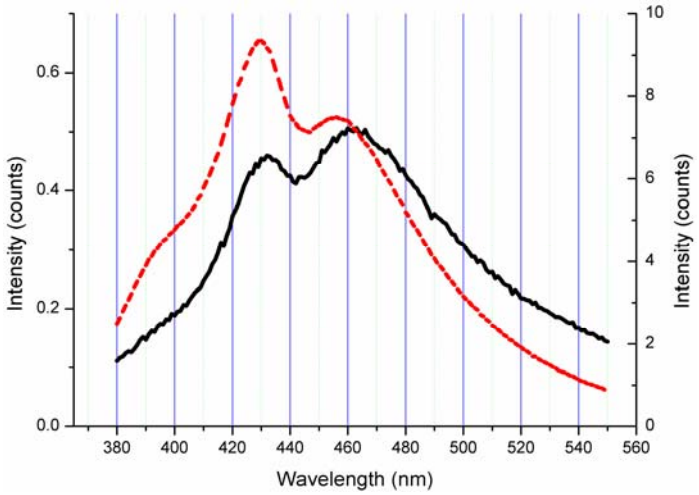

Fig. 2 – Spectra of fluorescence emission of two different states of collagen: collagen gel (solid line, left axis) and collagen hydrolyzed powder (dash-dot line, right axis).

It is noticeable that the state of collagen influences the emission of fluorescence spectrum. We notice the same form of the spectrum, with two high intensity maxima, at approximately 430 nm (I_{430}) and 460 nm (I_{460}), but the intensity ratio for each spectrum is modified: $R_1 = 0.908$ for collagen gel and $R_2 = 1.247$ for collagen hydrolyzed powder. Intensity ratio is equal to ratio between the intensity for the first maximum of the spectrum (430 nm) and the intensity of second maximum (460 nm):

$$R = \frac{I_{430}}{I_{460}}. \tag{1}$$

Then we have measured genuine leather emission (Fig. 3) to compare its emission of fluorescence spectrum with the spectrum of pure collagen. Genuine leather spectrum has the same general form as the collagen hydrolyzed powder spectrum, as we can see from intensity ratio: $R = 1.015$, which is over the unit. It must also be mentioned that the leather we used in this study was sheep dressed leather, which means that the external layer of the skin (epidermis) was removed. The percentage of collagen increases during sheepskin processing. An example is given in [16] where was found a variation of collagen content from 13.75% in clean, sheared skin to 29.13% in dry skin. In case of fibrous collagen, other than collagen solution, denaturation of the triple helix is an irreversible rate process. The denaturation rate, which is corresponding to the denaturation temperature, and the intrinsic stability of the helix, depends on hydroxyproline content, pH, addition of hydrotropic agents and salts [17]. Hydroxyproline is a major component of the protein collagen and plays key role in collagen stability.

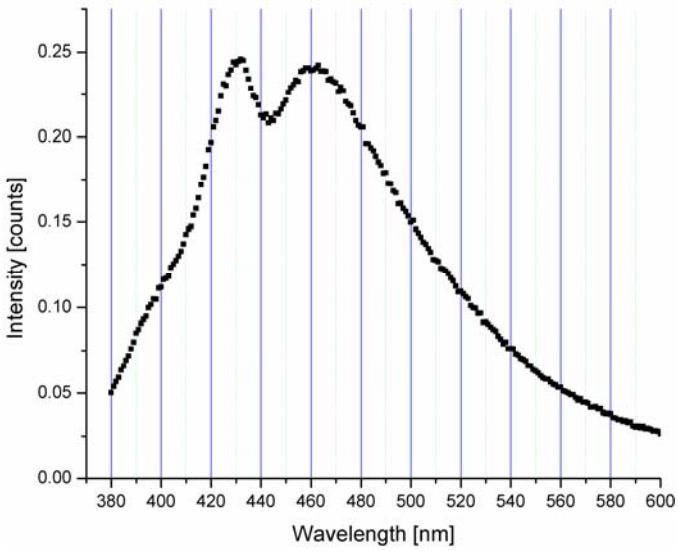

Fig. 3 – Fluorescence emission spectrum of genuine leather sample.

The intensity of measured signal is lower than in case of pure collagen (wet or dry). It is not only a consequence of lower collagen concentration. The emission is not strong because if no dyes or pigments are added, leather will reflect most of the radiation. This means that an un-dyed crust is a material with very good solar reflectance. It can be assumed that this reflectance is due to the structure of the papillary layer which will scatter and reflect the radiation.

Next, we were interested by the spectra of nanosilver solutions used in treatment of the leather. These spectra were recorded for solutions in glass cell. Quartz cell was not necessary because we used only 360 nm like excitation

radiation, which we have found to be the best excitation wavelength for both blue emission bands.

The intensity of emission was very low with high spreading as expected. Only the Ag-TiO_2 solution presents a very low emission with a maximum at approximately 430 nm. We believe this behavior is due to Ti^{4+} ion presence. Glasses doped with TiO_2 present a large excitation band between 300 and 400 nm, which corresponds to the charge transfer from O^{2-} to Ti^{4+}, and one emission band centered at 425 nm [18]. For the solutions with spectra which do not have general form similar to collagen spectrum, we can say that will not have a noticeable influence in treated leather spectra. It means the total emission spectrum is not the addition of two spectra. Comparing emission spectrum of solution Ag/TiO_2 with emission spectrum of collagen we suppose there could be a small influence which could be neglected.

But leather was not treated with pure nanosilver solutions. Before treating the leather, solutions were dissolved in collagen gel solutions. Fig. 4 shows the differences in emission spectra between two different solutions of nanosilver particles mixed with collagen gel and one pure collagen gel. We note here that nanosilver solutions have influence over the intensity of fluorescence emission.

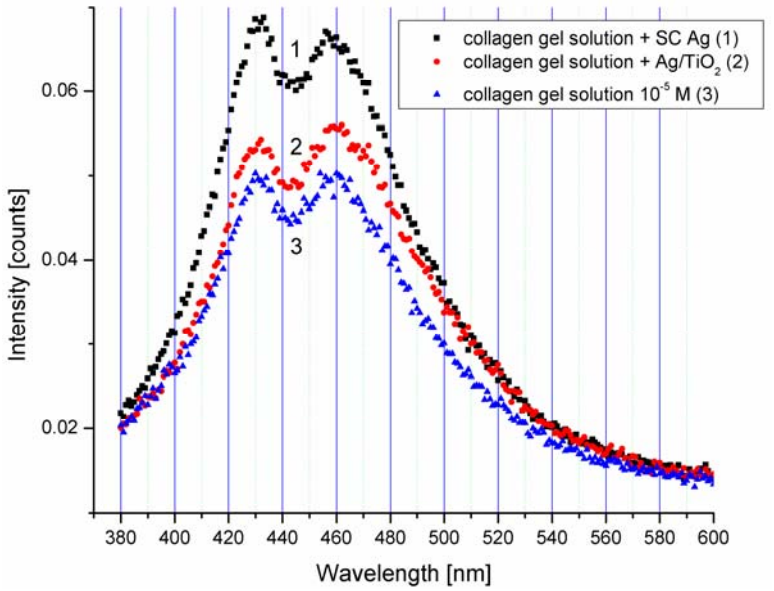

Fig. 4 – Spectrum of fluorescence emission of different collagen solutions used in treated leather.

The nanosilver solutions are those which change the form of the emission of fluorescence spectra not the band positions. Pure silver solution increases the ratio *R* over unit. Titanium ions have influence on 430 nm maximum intensity.

Then we have tested the differences between genuine leather and silver treated leather (Fig. 5). For the treated leather, three different methods of treatment were used: treatment with electrochemical colloidal nanosilver in collagen solution, treatment with electrochemical colloidal nanosilver on titanium dioxide in collagen solution and treatment with chemical colloidal nanosilver in collagen solution. The silver presence induces spectrum changes as follow:

– The global fluorescence emission increases;
– The global fluorescence emission could decrease as a result of increased absorption (Fig. 1) if silver is higher concentrated;
– The intensities for the two maxima changes for each method of treatment of the leather (Fig. 5), the ration between two maxima increases. More silver concentration results in increased ratio.

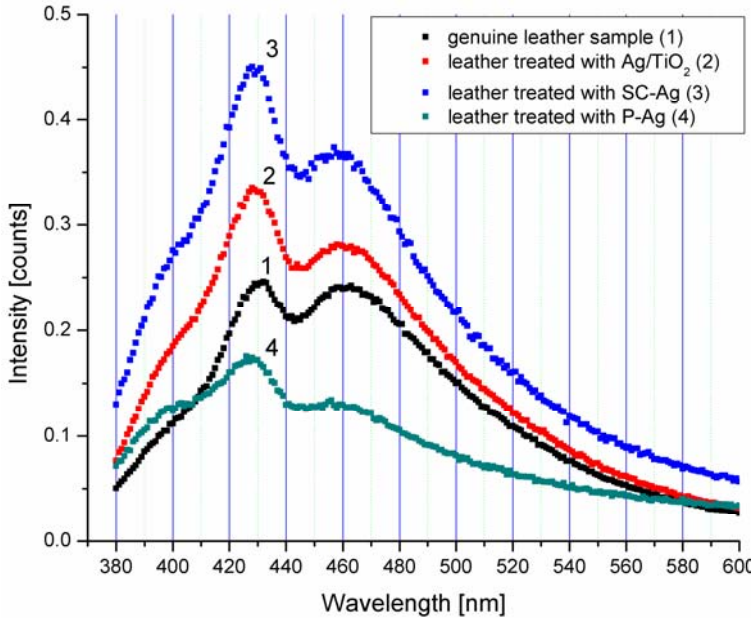

Fig. 5 – Differences between spectra of fluorescence emission of genuine leather sample and treated leather samples.

Another observation during the experiment is about the homogeneity of the samples. It is probably that leather was not equal covers with nanosilver solutions, because we registered a difference of intensity for two separate points of the same sample area but maxima ratio was the same.

The table below illustrates both differences of intensity for each maximum and intensity ratio for each spectrum, for representative samples that were studied.

Table 2

Intensity ratio of emission bands

Sample	First maximum intensity [a.u.]	Second maximum intensity [a.u.]	Intensity ratio
Collagen gel	0.4598	0.50636	0.908
Collagen hydrolyzed powder	0.93393	0.7498	1.247
Genuine leather	0.24586	0.24214	1.015
Ag/Ti 10g/l solution	0.01856	0.01858	0.998
Collagen gel solution + SC Ag 16.7ppm	0.06878	0.0671	1.025
Collagen gel solution + Cr + SC Ag 16.7ppm	0.02454	0.02708	0.906
Leather sample treated with SC Ag 16.7ppm	0.45042	0.37344	1.206
Leather sample treated with P solution, chemical synthesis, 141ppm	0.17392	0.13392	1.298

* SC= colloidal solution

5. CONCLUSIONS

We used spectra of fluorescence emission to highlight the way different solutions affect leather used in shoes industry.

Using collagen as a base solution in treating leather with silver nanoparticles, we noticed that silver nanoparticles do not have a negative impact on leather samples. They do change intensity of the emission of fluorescence, but they don't alter the leather.

From the fluorescence spectra, we can see that, treated in different ways, the leather changes its properties, fact reflected in changing the proportion between intensity of first and second maximum, as shown in Table 2. In conclusion silver reacts with collagen, this process thus indicates the formation of a silver bridge linking two peptide chains. From this position silver ions could act as antibacterial agent long time.

This study demonstrated the possibility of use synthesized silver nanoparticles and their incorporation in leather materials, providing them sterile properties.

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