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Study of collagen and leather functionalization by using metallic nanoparticles

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The paper presents the study of different kinds of silver nanoparticle interaction with collagen model solutions in view of optimizing the processing technology of collagen based materials. The collagen model solutions were interacted with classical crosslinking agents and silver nanoparticles synthesized by chemical, electrochemical methods or by deposition on TiO_2. The same nanosilver colloidal solutions and dispersion were interacted with leather. The main possibilities of nanosilver interaction with collagen macromolecule were assessed by FT-IR, atomic absorption and fluorescence spectroscopy. The study has revealed the IR spectral modification of amide I band in the case of interaction of chemically synthesized nanosilver with collagen in solution and in the case of interaction of chemically and electrochemically synthesized nanosilver with leather. This result proved that nanosilver reacts with collagen macromolecule inducing modification at secondary structure level through hydrogen bonds in carbonyl groups. The sensible modification of the amide band III in the case of interaction of nanosilver with leather collagen and the fluorescent emissions at 360 nm excitation have proved binding in the collagen chain and the influence of silver concentration. The analysis of amide I and amide II band positions of collagen in different conditions of interaction with chromium or polyurethane crosslinking agents and nanosilver allowed assessing the influence of nanosilver on macromolecule denaturation.

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Keywords: Collagen, Silver Nanoparticle, FT-IR Spectroscopy, Fluorescence Spectroscopy.

1. Introduction

Collagen is among the widespread natural polymers with various utilization possibilities [1,2]. The interaction of collagen with nanometals is a recently-developed research field, with the prospect of creating new materials with biocide activity or self-cleaning properties applicable in medicine [3] or in the case of consumer goods [4,5].

The method of synthesis of metal nanoparticles and that of processing collagen with these play an important role in modifying its structure. IR spectroscopy and fluorescence are two frequently used methods to understand discrete interaction mechanisms of various types of chemical materials with the collagen macromolecule. The most frequent hypotheses regarding the reactivity of silver nanoparticles start from the fact that silver, in atomic form, has nucleophilic character due to the plasmon state and interacts with the non-participating electrons from the atoms of nitrogen or carbonyl oxygen from polypeptide [6] or polyurethane [7] structures. Thus, by IR analysis, arrangement effects of the collagen macromolecule structure have been identified in the conditions of interaction with silver nanoparticles obtained in situ, by chemical synthesis methods [8,9]. The study of interaction of TiO_2 nanoparticles with collagen, by IR spectroscopy, allowed identification of modifications at the level of the polypeptide chain by substitution of C=O groups with water molecules adsorbed on the surface of TiO_2 nanoparticles [10,11]. Other studies have indicated the fact that through the interaction of collagen with nano TiO_2 weak conformational changes of the macromolecule are recorded, preserving the secondary structure, which supports the bioactive character of collagen in medical applications [12].

Analysis by fluorescence spectroscopy is another investigation technique which allowed identification of possible interactions between collagen and various materials with collagen reticulation ability. The influence of crosslinking agent concentration on the fluorescence signal has been reported in the literature in the case of various types of collagen-based materials [13,14].

In our study, model collagen solutions interacted with chemical auxiliary materials specific to natural leather processing and nanomaterials based on silver and nanosilver deposited on TiO_2 have been studied by IR spectroscopy in view of understanding the way of interaction and optimization of processing technologies. The study was extended in the case of leathers processed and interacted with the same nanomaterials, using IR spectroscopy and fluorescence analyses.

2. Experimental

2.1. Materials

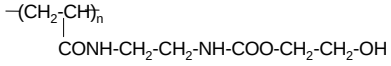
Silver nanoparticles (Table 1) with particle size of 5-10 nm which were synthesized according to methods described in the literature and turned into colloidal solutions or dispersions [15].

Table 1. Silver nanoparticles in the form of solutions or dispersion

No.	Description	Notation	Synthesis method	Characteristics
1	colloidal solution of silver nanoparticles	G	chemical	61 ppm Ag concentation, average diameter 10 nm
2	colloidal solution of silver nanoparticles	P	chemical	141 ppm Ag concentation, average diameter 10 nm
3	dispersion of silver nanoparticles deposited on TiO_2	Ag/Ti	electro-chemical	10g/l TiO_2, concentation, average diameter 5 nm
4	colloidal solution of silver nanoparticles	SC	electro-chemical	16.7 ppm Ag, concentation, average diameter 5 nm

Model collagen solutions of 10^{-5}M concentration which have been obtained by dissolving in water a collagen gel with molecular mass of 300000 Da, obtained by hydrolysis of bovine gelatin hide [16].

Chemical auxiliary materials specific to leather processing: basic chromium sulphate (Chromosal B, BASF), polyhydroxyurethane (PHU) in aqueous solution [7], with the following structure:



Sheepskins for medical use processed in pilot station at ICPI.

2.2. Methods of preparing samples and analyses

Model collagen solutions have been interacted with silver nanoparticles and chemical materials specific to leather processing in various combinations, according to Table 2, by stirring at room temperature for 30 minutes.

Tanned leathers were first washed to remove soluble substances, and their treatment with nanoparticle solutions was carried out by three successive static immersions for 24 hours, with intermediary drying.

IR spectrometry: model solutions and treated leather samples have been spectrally analyzed in the infrared with a FT-IR 620, Jasco device, from Japan, in the range 4000 – 400 cm^{-1}, using the KBr pelleting technique and/or the film deposited for solutions.

Fluorescence spectra were done directly on leathers treated with metal nanoparticles, by excitation in the 260 - 360 nm range with a FP-6500 Jasco device.

Concentrations of Ag and Cr have been determined by atomic absorption spectroscopy with the AAS-Vario 6, Analytik Jena Spectrometer.

3. Results and discussion

3.1. Study of structural modifications of collagen by IR spectroscopy

Collagen solutions treated with silver nanoparticles and various chemical materials specific to leather processing are presented in Table 2.

Table 2: Model collagen solutions treated with specific chemical materials and silver nanoparticles

No.	Description	Ag, ppm	Cr, ppm
1	collagen solution + basic Cr sulphate	-	70
2	collagen solution + neutral environment + PHU	-	-
3	collagen solution + basic Cr sulphate + neutral environment + PHU	-	74
4	collagen gel solution + basic Cr sulphate + neutral environment + P	20	54
5	collagen solution + neutral environment + PHU + P	970	-
6	collagen solution + neutral environment + P + PHU	1230	-
7	collagen solution + basic Cr sulphate + neutral environment + PHU + G	1030	71
8	collagen solution + neutral environment + PHU + G	1020	-
9	collagen solution + SC	480	-
10	collagen solution + basic Cr sulphate + SC	575	73
11	collagen solution + SC + basic Cr sulphate	550	66
12	collagen solution + Ag/Ti	310	-
13	collagen solution + Ag/Ti + basic Cr sulphate	320	63
14	collagen solution + basic Cr sulphate + Ag/Ti	350	73
15	collagen solution	-	-

3.1.1. IR characterization of treated collagen solutions (Table 3) was carried out on sample groups according to added materials, characteristics being established based on the main bands specific to functional groups involved, namely:

- $\nu_{NH\text{-}CH}$ – amide III at 1230 – 1240 cm^{-1};

- ν_{OH+NH} – at 3450-3300 cm^{-1};
- $\nu_{C=O}$ – amide I at 1650-1660 cm^{-1};
- δ_{NH} – amide II at 1555-1545 cm^{-1}.

Knowing the position of bands attributed to amide I and amide II structures from the peptide chain, $\Delta\nu$ ($\Delta\nu=\nu_I - \nu_{II}$) was calculated for the purpose of highlighting a potential degradation process (chain deformation), resulting from the interaction with various specific chemical auxiliary materials and/or with silver nanoparticles in various synthesis variants.

Table 3. Spectral characteristics of treated collagen solutions

No.	ν_{OH+NH} cm^{-1}	A_I cm^{-1}	A_{II} cm^{-1}	$\Delta\nu$ cm^{-1}	A_{III} cm^{-1}
1.	3325	1655	1550	105	1237
2.	3323	1650	1552	98	1239
3.	3319	1656	1547	109	1237
4.	3438	1623	(u)	=	(u)
5.	3429	1628	1552	76	1230
6.	3450	1646	1552	94	1234/1264
7.	3429	1650 (1632)	1552	98	1239
8.	3427	1650 (1633)	1544	106	1237
9.	3426	1648	1543	105	1237
10.	3326	1656	1550	106	1237
11.	3322	1658	1550	108	1236
12.	3327	1647	1552	95	1237
13.	3327	1648	1551	97	1236
14.	3321	1657	1550	107	1236
15.	3427	1651	1544	107	1230

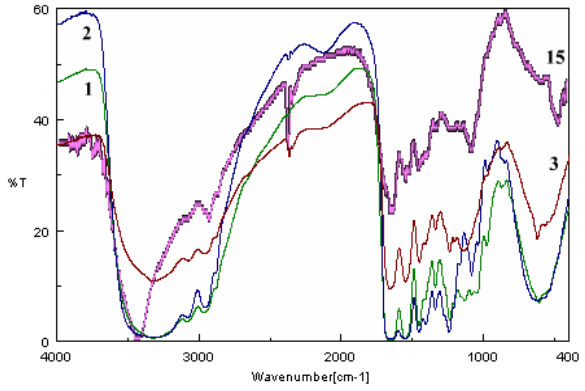


Fig. 1: IR spectra of untreated (15) collagen solutions and treated with specific chemical auxiliary materials (1, 2, 3).

In Fig. 1 an important modification of the collagen macromolecule is found in samples 1 and 3 compared to sample 2 and to the uninteracted model collagen solution, sample 15. $\Delta\nu$ allows identification of a larger deformation of the collagen macromolecule in the case of sample 3, compared to samples 1 and 2, due to the reticulation ability of chromium to collagen and of PHU by means of chromium to collagen (Table 3).

In the case of interaction of model collagen solutions with nanoparticle solution P, in various combinations of auxiliary substances (Table 2) it can be noticed from IR spectra (fig.2) that there is a tendency of arrangement of the collagen macromolecule by reducing the value of $\Delta\nu$ ratio, compared to values recorded for samples without nanosilver (fig.1 and table 2, samples 1-3).

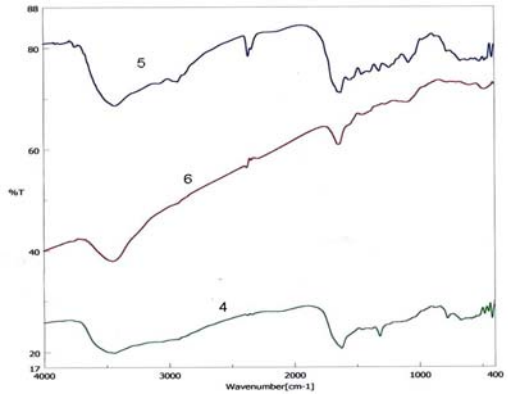


Fig. 2. IR spectra of collagen solutions treated with P solution of silver nanoparticles (4, 5 and 6).

In the case of the interaction between the colloidal nanosilver G solution and the collagen solution that interacted with the basic chromium salt and PHU (sample 7 from Table 2) or PHU (sample 8 from Table 2) a significant arrangement effect can be noticed for the collagen macromolecule interacted with two agents with reticulating ability, while G solution has a significant deformation effect on the collagen treated with PHU (Figure 3).

The amide I group, which exhibits high sensitivity to conformational changes at the level of the secondary structure of collagen [17,18], shows that the peak corresponding to this vibration in the collagen solutions treated with G solution splits exhibiting a second band at 1632 cm^{-1} and 1633 cm^{-1} respectively, which indicates the presence of stronger changes in the helix structure than in the other solutions, implying hydrogen links with carbonyl groups [19].

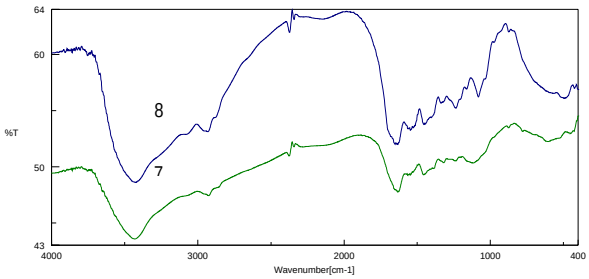


Fig. 3. IR spectra of collagen solutions treated with G solution of silver nanoparticles (7 and 8).

In the case of the SC nanosilver colloidal solution (figure 4) its ability to deform collagen and the slight arrangement effect in the case of the interaction with collagen treated with basic chromium salt can be noticed (sample 9).

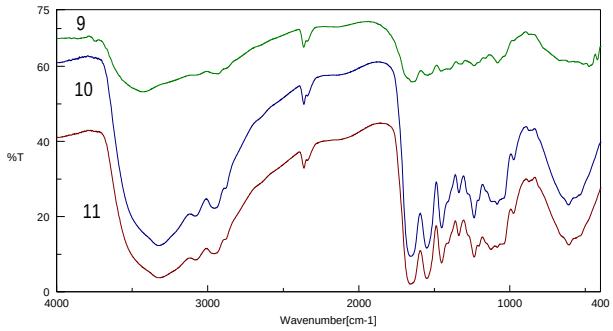


Fig. 4. IR spectra of collagen solutions treated with SC solution of silver nanoparticles (9, 10 and 11).

Silver nanoparticles deposited on TiO_2, (Ag/Ti), interact differently compared to SC nanoparticles (samples 10, 11), in the sense that they do not deform collagen macromolecules during the direct interaction or after the basic chromium salt treatment and does not have any arrangement effects after adding basic chromium salt which significantly deforms collagen (fig. 4). Arrangement effects of the collagen macromolecule are manifested in the case of direct interaction with untreated collagen (sample 13) and is not significantly influenced by subsequent interaction with basic chromium salt (fig.5).

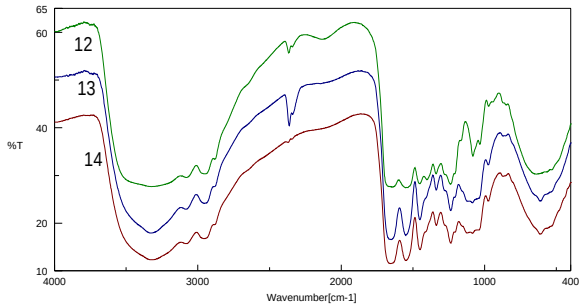


Fig. 5. IR spectra of collagen solutions treated with dispersion of silver nanoparticles deposited on TiO_2 (12, 13 and 14).

The frequency of the ν_{OH+NH} band (Table 3) indicates a higher molecular association degree in the case of combinations of collagen, basic chromium sulphate and PHU (3319 cm^{-1}), collagen, SC and basic chromium sulphate (3322 cm^{-1}) and in the case of interaction between collagen, basic chromium sulphate and nanosilver deposited on titanium dioxide (3321 cm^{-1}).

3.1.2. IR analysis of leather collagen processed and treated with metal nanoparticles

Leather samples processed and treated with the four types of silver nanoparticles in the form of solutions or dispersion are presented in Table 4. The silver content in the treated leathers is influenced by the concentration of solutions/dispersion of silver nanoparticles.

Table 4. Leathers processed and treated with the four types of silver nanoparticles.

No.	Notation	Description	Ag ppm
1	L G	Leather treated with G solution	4420
2	L P	Leather treated with P solution	4010
3	L SC	Leather treated with SC solution	1970
4	L Ag/Ti	Leather treated with Ag/Ti dispersion	790
5	Control (LM)	Processed, untreated leather	-

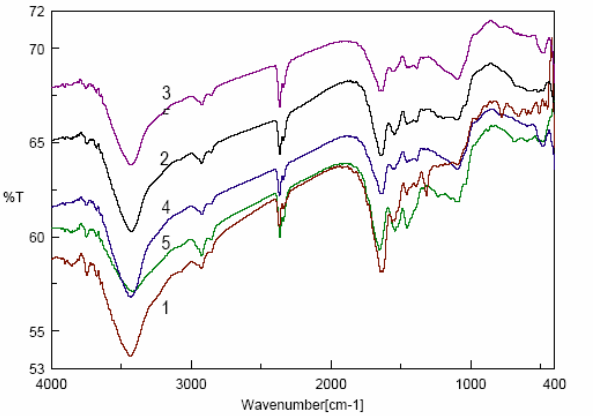


Fig. 6. IR spectra of leathers treated with solutions and dispersion of silver nanoparticles (1-4), as compared to untreated leather (5)

Table 5. Spectral characteristics of treated leathers.

No	ν_{OH+NH}	A_I cm^{-1}	A_{II} cm^{-1}	$\Delta\nu$ cm^{-1}	A_{III} cm^{-1}
LM	3422	1653	1545	108	1230
LG	3441	1650 1628	1554	96 -	it is consumed
LP	3428	1647	1544	103	traces
LSC	3429	1650 1636	1552	98 -	it is consumed
L Ag/Ti	3433	1652 1636	1552	100 -	it is consumed

From the analysis of IR spectra (fig. 6, table 5) of processed leathers, the following have resulted:

- ν_{OH+NH} situated at 3420-3445 cm^{-1} is of comparable intensity in all examined samples;
- Valence vibration of C = O group from – CONH – (amide I) splits when treated with G, SC and Ag/Ti solutions;
- Deformation vibration of NH group from – CONH –, ν_{NH} (amide II) situated at 1555-1540 cm^{-1} shifts to larger wavelength numbers with 7 – 9 cm^{-1} when treated with G, SC and Ag/Ti solutions, indicating partial break of some associations with the participation of the NH group.

In samples LSC, LAg/Ti and LG, the amide I band is split, exhibiting a second band at 1628-1636 cm^{-1}, which indicates the presence of changes in the helix structure and/or traces of water (Fig.6). This second band has the same intensity as that of amide I. The change can be attributed to links at the level of polypeptide chain by substituting C=O with water molecules from the surface of metal nanoparticles [10].

Measuring the distance $\Delta\nu = \nu_I - \nu_{II}$, attributed to a deformation process [20, 21], it is found that LM and LP leathers undergo a slight such process, due to the processing technology. It may be assumed that the other types of treatments lead to collagen structuring and not its deformation, as reported in the literature regarding the interaction of silver with collagen [20 - 22].

In control sample LM the band specific to amide III was highlighted at 1230 cm^{-1}, the band that diminishes increasingly in the case of leathers treated with metal nanoparticles. The amide III band corresponds to C-N elongations and N-H deformations in the amidic links and vibrations given by the CH_2 groups from the glycine existent in the main chain of collagen and the proline in the lateral chains [23]. The disappearance of the amide III band at 1230 cm^{-1} may be attributed to the formation of intermolecular links [24] at the level of the NH catenary group under the influence of metal nanoparticles.

3.2. Fluorescence spectroscopy of the collagen from processed leathers treated with nanoparticles

The fluorescence spectrum of the untreated leather by excitation at 260-280 nm highlights an intense emission band at 306-310 nm given by tyrosine, as well as a low intensity band at 416 nm which may be attributed to segments of reticulated collagen [25].

Excitation at 360 nm highlights an intense band at 440 nm with a shoulder at 502 nm due to reticulations produced in the collagen chain with the participation of tanning material (table 6). The shoulder at 500-510 nm may come from the aromatic structures with extended conjugation from tanning materials.

Excitation at 260 nm of leathers treated with silver nanoparticles (fig. 7) indicates the maintenance of the emission band at 306 nm (similar to that of control) and the presence of a band shifted to larger wavelengths ($\Delta\lambda$ = 24 nm) in the case of samples LSC and LAg/Ti. Samples LP and LG (fig. 8 – overlapped) exhibit two bands of equal intensity at 409 and 468 nm, indicating the presence of reticulated collagen structures with the participation of tanning material, as well as that of treating solutions, also confirmed by their increased intensity compared to the band given by the LSC sample (fig. 8).

Excitation at 360 nm (fig. 9) produces, in the case of samples treated with nanoparticles, bands at 408 – 420 nm, shifted to wavelengths 20 – 30 nm smaller than the LM control, but of much higher intensities.

Assessing the reticulation degree of leather samples based on the intensity of this band, the way in which the treatment solutions have influenced it can be established. Thus, a scale of the reticulation degree can be established: LAg/Ti>LP>LSC>LG.

Table 6 – The main emission bands of analyzed leathers.

Excitation, nm	Emission, nm				
Sample	LP (1)	LG (2)	LSC (3)	LAg/Ti (4)	LM (5)
260	306 409 468	306 409 468	306 440	306 440	306 414
360	420	408 466(u) 508(u)	420	420	440 502 (u)

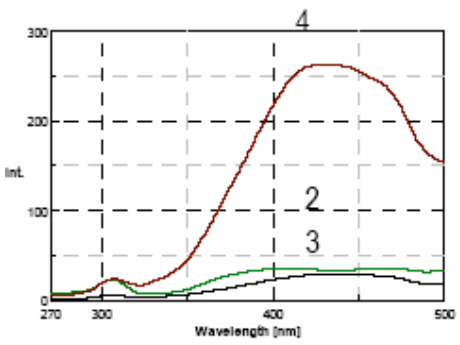


Fig.7 - Emission spectrum (at excitation at 260 nm) for leathers treated with Ag nanoparticles: 4-LAg/Ti, 2-LG and 3- LSC

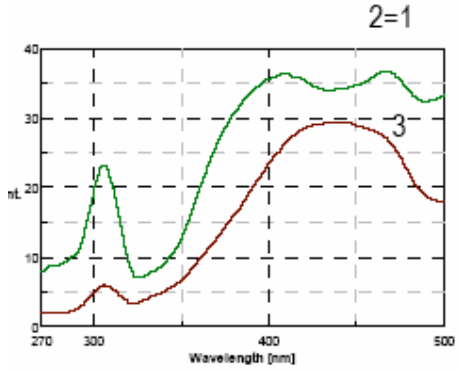


Fig. 8 - Emission spectrum for leathers treated with Ag nanoparticles (detail): 2-LG and 1-LP overlapping and 3-LSC.

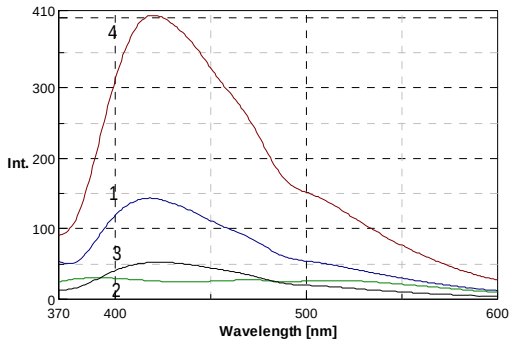


Fig. 9. Emission spectrum (at excitation at 360 nm) for leathers treated with Ag nanoparticles: 4-LAg/Ti, 1-LP, 3-LSC and 2-LG

Data in the literature show that the fluorescence of collagen is diminished due to the presence of tyrosine and phenylalanine in small quantities. The increase of the fluorescence emission may be due to the interaction between collagen and silver nanoparticles through the formation of reticulation links which are dependent on their nature and concentration [14]. The weakest intensities are those corresponding to the leathers with the smallest Ag concentrations, except for the leather treated with silver nanoparticles deposited on TiO_2. TiO_2 influences the intensity of fluorescent emission which is maximum in all cases, even if silver has minimum concentration. Emission recorded at 306nm in the case of excitation at 260 nm corresponds to a possible interaction of silver in all variants with tyrosine, which upon excitation at 266 nm emits at 303 nm [26, 27].

4. Conclusions

The analyses by IR spectroscopy on model collagen solutions in different variants of interaction of silver nanoparticles in the presence of classic links (basic chromium sulphate or polyhydroxyurethane) or not, have highlighted the deformation or arrangement ability of the collagen macromolecule conformation by recording the differences between the frequencies of amides I and II. An arrangement effect of the collagen macromolecule conformation was noticed in the case of the interaction with chemically obtained nanosilver colloidal solutions (P and G), even after reticulation with basic chromium sulphate or basic chromium sulphate and polyhydroxyurethane, which suggests complexing of nanosilver by means of these links. In the case of the interaction with electrochemically obtained nanosilver solutions or dispersions (SC) or deposited on TiO_2, arrangement effects are less obvious, probably because of concentrations poorer in nanosilver of these variants.

The study carried out by IR spectroscopy and fluorescence on more complex collagen matrixes, sheepskins processed and treated with the four types of solutions or dispersions based on nanosilver, allowed the identification of changes at the level of amide III (1230 cm^{-1}), attributed to the interaction at the level of the NH catenary group of collagen. The emission signal by fluorescence at 306 nm suggests possible interactions of silver nanoparticles at the level of the collagen macromolecule and is strongly influenced by the presence TiO_2 and of nanosilver concentrations from treated leathers.

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