Stabilizing derma collagen structure with modified dispersions of montmorillonite

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Abstract. The article reviews structural changes of derma collagen resulting from processing with modified montmorillonite dispersions. The infrared spectroscopic studies of gelatin as a simulated collagen established chemical interaction of chromium modified montmorillonite dispersions with reactive groups of the native protein. The derma collagen structure is stabilized through formation of hydrogen bonds with the surface hydroxyl groups of montmorillonite of Si-O... H-C and Si-O-H...O-C type. The formation of hydrogen bond between the nitrogen atoms of amino groups of collagen and the water proton in the interlayer space of MMT with cations in the exchange complex is possible. The formation of hydrogen bonds of Si-O...H-C type between the functional groups of chromium modified montmorillonite with CH-groups of protein, as well as Si-O...H-N with NH - groups of protein in the low-frequency spectrum range is also very likely. The efficiency of stabilizing collagen with chromium modified montmorillonite dispersions is proven by an increase of the gelatin melting temperature with mineral consumption of 3-4% of protein weight and chromium compounds (III) for the modification of the mineral around 10% of montmorillonite weight.

1. Introduction
The derma collagen is an amphoteric protein. The amphoteric nature of the collagen is caused by carboxyl, amine, peptide, hydroxyl and others groups. Thus, bonds of different nature and location can be formed in the collagen structure [1].

During the processing of animal skins into natural leather, the destruction happens between some of the bonds in the structural elements of the collagen and the formation of new bonds by reaction with chemicals [2].

For effective stabilization of the collagen structure of derma, it is necessary to achieve the structuring of its components at the levels of size. For this, it is advisable to use materials with different functional groups and different sizes of particles.

Mineral and organic tanning agents are used to stabilize the derma collagen structure. The basic chromium sulfate is used as a mineral tanning agent to produce around 80% of the leather in the world. The chromium tanning agents are complex compounds that efficiently bind the structure of collagen in various structural levels as a result of complex creation. They create a chemical bond mainly with the carboxyl groups of the collagen. The stabilization of the derma collagen structure results in the raising of collagen shrinking temperature to 100°C and the hardening of the derma structure against external impact. The main disadvantage of this type of tanning is inefficient consumption of chromium compounds, as 40% of them remains in the waste tanning solution [3, 4].

Organic tanning agents, polymeric compounds and mineral fillers (kaolin, montmorillonite, chalk, etc.) are used to bind the derma structure through other functional groups of derma collagen.

The dispersions of montmorillonite (MMT) are of practical interest because after the modification of the particle of dispersion they may change the surface charge and size. Targeted modification of montmorillonite will allow to effectively stabilizing derma collagen.
The purpose of this work includes a study of the efficiency of structuring the derma collagen with a modified montmorillonite dispersion. In fact, the montmorillonite dispersions are planned to be modified with basic chromium sulphate (Cr-MMT). It is assumed that the use of modified montmorillonite dispersion at the stage of tanning will promote rational use of chromium compounds through the formation of bonds with functional groups of collagen and active centers of montmorillonite [5].

2. Experimental

2.1 Materials and preparation

Gelatin of SER "Lisichansky Gelatin Factory" (Ukraine) was used for the investigation as a model of derma collagen [3]. The properties of gelatin (GOST 11293-89): particle size <5 mm; pH in a soluble state in the amount of 1.0% = 5.6 ± 0.1%, protein content = 83.32 ± 0.03%; ash = 1.38 ± 0.01%; moisture content = 15.3 ± 0.1%.

The basic chromium sulfate (Cr$_2$(SO$_4$)$_3$(OH)$_6$) with basicity 40.0% (Kazakhstan, Aktyubinsk plant of chromium compounds) was used as a chromium tanning agent. The content of chromium oxide (III) Cr$_2$O$_3$: 25.6%.

Bentonite clays of Dashukovsky deposit (Ukraine) was used for investigation. The content of montmorillonite in bentonite rocks constituted 85%.

Montmorillonite dispersion was modified in stages by sodium carbonate and basic chromium sulfate. For that, sodium carbonate in a soluble state in the amount of 6.0%, reported at mineral weight, was introduced into water dispersion with the montmorillonite concentration of 100 g/L. The obtained dispersion was mechanical mixed for 120 minutes. Then, basic chromium sulfate in a soluble state in the amount of 10.0% Cr$_2$O$_3$, reported at mineral weight was introduced to the Na-montmorillonite dispersion.

As the objects for FTIR spectroscopy investigation, we used the film of gelatin (G), of chromium -tanned gelatin (G$_{Cr}$) and Cr-MMT tanned gelatin (G$_{Cr-MMT}$).

The chromium - tanned gelatin (G$_{Cr}$) was prepared as follows: the solution (5%) of eatable gelatin was treated during the 3 hours with chromium (III) salts in the quantity of 3.0% Cr$_2$O$_3$ counting on dry protein at the temperature of 20 ± 2°C. pH was 3.5.

The Cr-MMT added into the gelatin solution in the quantity of 6% reported at dry substance at the temperature of 20 ± 2°C. pH was 3.7. The films with the thickness of 100 μm were formed by pouring on the Teflon disks, with following drying at room temperature for 72 hours, and then in the oven at 30°C till constant weight.

2.2 Characterization techniques

The IR spectroscopic studies were performed on the universal Fourier transform infrared spectrometer TENSOR-37 (BRUKER, Germany) within the 4000-400 cm$^{-1}$. The nature and efficiency of interactions of functional groups of montmorillonite with active groups of gelatin were evaluated by the change in intensity of optical densities of the corresponding characteristic absorption bands. The absorption bands in the infrared spectra of precursors and products of their interaction were interpreted using data of literature [6-10] in accordance with the frequencies of the characteristic groups of atoms. The spectra of the tested samples were used to calculate the peaks areas of relative absorption densities by the Gauss equation [11]. The changes of configuration of absorption bands and their shifts allow drawing a conclusion about the nature of intermolecular interactions in tanning collagen with chromium tanning agents.

The melting point was determined for the galantine of native gelatin (G), the gelatin treated with chromium-modified montmorillonite dispersions (G$_{Cr-MMT}$) at a constant consumption rate of the mineral or chromium oxide. The melting temperature for gelatin was determined by the fusiometric method (GOST 11293-89). The method is driven by determining the flowable temperature of gelatin galantine. The gelatin solution with the concentration of 2.5% was prepared for the research and transferred to a test tube. Then, chromium-modified montmorillonite dispersion
was added to gelatin and the tube was left alone for 24 hours. Upon complete solidification of gelatin, a metal ball was placed on the surface and the test tube was immersed in a glycerin solution. The glycerin solution was gradually heated at the speed of 3-5 °C / min. The temperature at which the ball fell on the bottom of the tube indicated the gelatin melting point.

3. Results and discussion

To evaluate stabilization of the derma collagen structure as a result of chemical interactions between the active groups of collagen and tannins, the information about the characteristic absorption bands of the input substances presented in Fig. 1 and Table 1 was used.

The most characteristic gelatin absorption bands are observed in the frequency range of 3300-3100 cm\(^{-1}\) and 1650-1245 cm\(^{-1}\) (Fig. 1, Table 1).

![Graph](image)

**Table 1. Characteristic bands of gelatin**

| Band (cm\(^{-1}\)) | Group              | Band (cm\(^{-1}\)) | Group              |
|---------------------|--------------------|--------------------|--------------------|
| 3330, 3200 -3500    | - NH\(_{\text{bound}}\), - OH | 1335               | Amid III, -CN + -NH |
| 3071                | - NH\(_{\text{bound}}\) | 1245               | -CN, -NH           |
| 2955                | - CH\(_2\) asym    | 1162               | NH \(^3\)_+        |
| 2834                | - CH\(_2\) sym     | 1082               | - CN               |
| 1654                | Amid I, -C = O     | 1031               | - C-O-C = C        |
| 1543                | Amid II, -NH, -CN  | 972                | -NH\(_{\text{bound}}\) |
| 1454                | CH\(_2\)            | 672                | - NH\(_{\text{bound}}\), Amide V, |
| 1398                | - C = O (COO\(^-\)) | 549                | Amid V I, -NH, -C = O |

The first area corresponds to valence fluctuations of the associated NH\(_2\), NH and OH-groups. The band at 3330 cm\(^{-1}\) is due to the bonded groups of NH (Amide A), that participate in the creation of intramolecular hydrogen bonds. The bands at 2954 cm\(^{-1}\), 2834 cm\(^{-1}\) are associated with the asymmetric and symmetric stretching vibration of CH\(_2\) groups. In the area of 1650 - 1234 cm\(^{-1}\), absorption bands of Amide I, Amide II and Amide III are observed. This area is associated with the absorption band of carbonyl groups C=O and deformation vibrations of NH-groups, including amine, and imine guanosine groups. The intense band of 1650-1660 cm\(^{-1}\) is due to valence fluctuations of associated
carbonyl groups C=O (Amide I). The band 1540-1550 cm\(^{-1}\) characterizes the deformation vibrations of NH-groups (Amide II) and, to a lesser extent, those of CN-groups. The Amide II band is slightly weaker from the carbonyl absorption bands. The interval of 1197-941 cm\(^{-1}\) combines a set of bands. The 1163 cm\(^{-1}\) band indicates the pendulum oscillations of \(\text{NH}_3^+\) groups, 1082 cm\(^{-1}\) and 1031 cm\(^{-1}\) bands are characteristic for valence groups CN, C-O and C=C, and the band at 972 cm\(^{-1}\) corresponds to the bound NH-groups. In the 840-400 cm\(^{-1}\) area, characteristic deformation vibrations of bound NH-groups with peaks of 672 cm\(^{-1}\) (Amide V) and 549 cm\(^{-1}\) (Amide VI). These bands are wide and less intense.

The spectrum of montmorillonite (Fig. 2) is characterized by more intense absorption bands in both high-frequency and low-frequency zones. The strip stretching OH vibrations that are characteristic of structural hydroxyl groups of layered silicates are observed at 3700-3200 cm\(^{-1}\). The weak band with frequency vibrations of 1637 cm\(^{-1}\) probably characterizes the deformation vibrations of the H-O-H groups. Also, the intense band at 1168-1046 cm\(^{-1}\) which is characteristic of silicon oxygen structures, less intense band at 788 cm\(^{-1}\) and strong absorption bands at 529 -461 cm\(^{-1}\) (Fig. 2) are observed. The pronounced wide band (Fig. 2) at 1042 cm\(^{-1}\) corresponds to valence fluctuations of asymmetric and symmetric Si-O-Si tetrahedral of silicon-oxygen frame and indicates presence of surface hydroxyl groups. The band in the range of 788 cm\(^{-1}\) corresponds to fluctuations of \(\text{Al}_2\text{O}_3\) in the tetrahedral rings of (O-Si-O). The band in the area 694 cm\(^{-1}\) is characteristic of structural deformation vibrations of hydroxyl group of the mineral that are associated with octahedral \(\text{Al}^{3+}\) cations. The intensive doublet at 463 and 529 cm\(^{-1}\) is characteristic of dioctahedral minerals of the montmorillonite type and indicates the deformation vibrations of links Me-O and (O-Si-O).

**Figure 2. Characteristic absorption bands of montmorillonite (1) and Cr-montmorillonite (2)**

The modification of montmorillonite with basic chromium sulphate promotes change of the characteristic absorption bands of high and low-frequency range zones of the mineral (Fig. 2, Table 2). For Cr-MMT, the band of bound absorbed water at 3454 cm\(^{-1}\) is shifted to lower frequencies (3428 cm\(^{-1}\)). There is a decrease in its intensity due to OH stretching bands. The major changes in the area of high-frequency spectrum Cr-MMT are associated with the redistribution of water in the MMT interburst space. The deformation fluctuations in H-O-H groups for the range of Cr-MMT are observed at 1642 cm\(^{-1}\) and are characterized by 7 times the intensity compared with the MMT. For Cr-MMT band of 3192 cm\(^{-1}\) shifted to 260 cm\(^{-1}\) in a high-frequency region compared to the band at 2932 cm\(^{-1}\) for the MMT spectrum. Apparently, the phenomenon is due to hydrogen bonds. The chromium complex displaces water from the interlayer space of the MMT to form hydroxocomplex ions, which have a high polarization and adsorption capacity. The presence of the Cr\(^{3+}\) hydroxocomplex ions between the silicate layers of montmorillonite is manifested by the siloxane groups growing around 1046 cm\(^{-1}\) and shrinking OH groups. That also results in almost
doubling the areas under the respective peaks. Such changes may indicate a strengthening MMT structure.

The peaks at 529 and 461 cm\(^{-1}\) that correspond to valence fluctuations of the Cr-O bond in the infrared area have a slightly larger area under the peaks compared with the MMT spectrum, indicating a more orderly crystal structure of Cr\(_2\)O\(_3\) on the MMT surface.

Table 2. Characteristic bands of modified montmorillonite and change of their optical densities

| Band (cm\(^{-1}\)) | Group | Optical density, D/D\(_o\) (for peak areas) |
|-------------------|-------|------------------------------------------|
| 3640-3641         | - OH \(_{\text{free}}\) | 457.8 | 113.0 |
| 3454-3428         | - OH \(_{\text{bound}}\) | 980.0 | 754.9 |
| 2932-3192         | - OH  | 91.3  | 169.1 |
| 2854-2980         | H\(_2\)O - OH   | 6.7   | 295.1 |
| 1637-1642         | - (H-O-H)     | 12.8  | 91.4  |
| 1168-1270         | Si-O       | 207.0 | 413.3 |
| 1131-1189         | Si-O       | 537.5 | 307.6 |
| 1042-1046         | n(Si-O-Si), AlO\(_4\) - - tetrahedral | 1225.5 | 2028.7 |
| 788               | - (O-Si-O), AlO\(_4\) | 36.0  | 76.9  |
| 694               | - (Al-OH)    | 14.6  | 12.0  |
| 529               | - (O-Si-O) Me-O | 205.2 | 357.8 |
| 463-461           | - (O-Si-O) Me-O | 158.2 | 218.4 |

The use of tannins based on basic chromium sulphate, including dispersions of Cr-MMT to structure the derma collagen changes the range of gelatin (Fig. 3, Table 3).

Figure 3. Characteristic absorption bands G(1), G\(_{\text{Cr}}\) (2) and G\(_{\text{Cr-MMT}}\) (3)

Treating of gelatin with basic chromium sulphate (G\(_{\text{Cr}}\)) promotes changes of absorption bands (Fig. 3, Table 3), which are characteristic of absorption of carboxyl, amine, and OH groups. A decrease of the area under the peaks at 3333, 3072, 1082 cm\(^{-1}\) and the corresponding increase of the area under the peaks of 1654, 1545, 1398, 1336, 1246 cm\(^{-1}\) can be observed in the G\(_{\text{Cr}}\) spectrum as compared to
gelatin spectrum. It can be seen the emergence of an intense absorption band at 1122 cm\(^{-1}\). This is evidence of the active interaction of carbonyl and carboxyl groups of collagen with the complex chromium making metal ion. Cross-links that lead to stabilizing of derma collagen structure are formed in the process.

The G\(_{\text{Cr-MMT}}\) spectrum is characterized by absorption bands similar to the G\(_{\text{Cr}}\) spectrum, which indicates the identical mechanism of derma collagen structurization. The main changes are associated with the intensity of the absorption bands and the corresponding peak areas. The G\(_{\text{Cr-MTT}}\) spectrum is characterized by a slight decrease of the area under the peaks of the absorption bands at 3341 cm\(^{-1}\) and a shift of the bands to the high-frequency region compared with the G\(_{\text{Cr}}\) spectrum.

In the range 1246 cm\(^{-1}\) ... 918 cm\(^{-1}\) for the G\(_{\text{Cr-MMT}}\) significant differences are observed in the areas of the peaks corresponding to fluctuations of carbonyl, ammonium, and simple ester sulphate groups of gelatin, as well as free surface hydroxyl groups Si-O - fluctuations. The strip stretching vibrations of Amide I, Amide II and Amide III for G\(_{\text{Cr-MMM}}\) show a special impact of Cr-MMT on the derma collagen structure versus G\(_{\text{Cr}}\). The area under the peaks at 1654 cm\(^{-1}\), 1545 cm\(^{-1}\) and 1336 cm\(^{-1}\) for G\(_{\text{Cr-MTT}}\) is slightly lower compared with the peaks in the G\(_{\text{Cr}}\) spectrum. This corroborates the formation of a hydrogen bond between the nitrogen atoms of amino groups of gelatin and a water proton in the interlayer space of MMT with monovalent cations in the exchange complex. A part of gelatin molecules enters in a specific interaction with exchange cations of the mineral and simultaneously forms hydrogen bonds with the surface atoms of oxygen or adjacent atoms of silicate.

| Band (cm\(^{-1}\)) | Group | Optical density, D/D\(_{0}\) (for peak areas) | G | G\(_{\text{Cr}}\) | G\(_{\text{Cr-MMT}}\) |
|-------------------|-------|------------------------------------------|---|------------|-----------------|
| 3330-3337-3341-3342 | - NH, -OH | 986.7 | 1198.9 | 1139.5 |
| 3072 | NH\(_2\), -NH | 34.7 | 30.9 | 27.2 |
| 2834-2813-2824-2824 | - CH\(_2\) sym., -OH | 78.1 | 89.3 | 81.1 |
| 1654 | - C = O -(H-O-H) | 180.1 | 184.4 | 174 |
| 1545 | - NH \(_2\) | 67.5 | 70.8 | 66.6 |
| 1398 | - C = O (COO\(^{-}\)) | 6.8 | 7.5 | 5.4 |
| 1336 | - CN + -NH | 5.7 | 6.4 | 5.2 |
| 1246 | - CN, -NH | 15.5 | 17.1 | 24.9 |
| 1162-1160-1145-1150 | NH\(^{3+}\) | 1.0 | 2.2 | 17.9 |
| 1122 | S = O, -CN, C=O, SO\(_4^{2-}\) | - | 12.7 | - |
| 1082-1081-1100-1103 | C=O, -CN, SO\(_4^{2-}\) | 16.7 | 10.8 | 4.7 |
| 1021-1034-1036 | C=O, n(Si-O Si), AlO\(_4\) tetrahedrons | 2.9 | 3.1 | 88.5 |
| 972-973 | - CH, NH bound | 0.4 | 0.3 | - |
| 918 | Si-O, OH | - | - | 2.1 |
| 873 | CO\(_3^{2-}\), AlO\(_4\) tetrahedral | - | - | 1.2 |
| 786 | - (O-Si-O), AlO\(_4\) | - | - | 5.9 |
| 672-685-680-691 | NH, Amide V, (Si-O \(_4\) . Cr\(^{3+}\), Al\(^{3+}\) | 57.9 | 43.4 | 39.1 |
| 604 | Amide V, -NH, Cr\(^{3+}\) | - | 22.8 | - |
| 549-537-529-561 | Cr-O, Amide V, -NH | 23.1 | 42.9 | 105.9 |
| 524 | - (O-Si-O) Me-O | - | - | 14.3 |
| 466 | - (O-Si-O) Me-O | - | - | 14.2 |

There is also a decrease of the area of maximum absorption peaks at 1398 cm\(^{-1}\) for G\(_{\text{Cr-MMM}}\). The spectral changes of this type are caused by intermolecular interactions where the carboxyl group acts as an electron donor. The lower peak area indicates that during tanning the binding energy goes up, i.e.
it forms a stronger bond with the carboxylic group than the chromium sulfate complex. Also, the interaction of the carboxyl group of gelatin with cations of lateral sides of the mineral surface is possible. Based on the comparative analysis of the relative densities of absorption spectra for the G\textsubscript{Cr-MMT} at 2824 cm\textsuperscript{-1} and 680-466 cm\textsuperscript{-1}, we conclude that, most likely, the hydrogen bonds of type Si-O .... H-C type are formed between the functional groups Cr-MMT and CN protein groups, as well as Si-O....H-N with NH protein groups. The increased optical density of absorption bands 529-561 cm\textsuperscript{-1} and the appearance of the peak at 466 cm\textsuperscript{-1} might be the evidence of the formation of such bonds. The analysis of the melting point of a native gelatin galantine before and after treatment with Cr-MMT dispersions showed their efficiency to structure the derma collagen. It was found that the melting temperature and consumption of chromium tanning agents and montmorillonite are interdependent. The output of studies of the impact of consumption of chromium tanning agents (in terms of chromium oxide) and montmorillonite on the melting point of gelatin galantine is shown in Fig. 4.

![Figure 4. Dependence of chromium compound consumption (1) of MMT weight and modified montmorillonite dispersions (2) of protein weight](image)

As shown in Fig. 4, with the increasing consumption of chromium compounds being part of Cr-MMT dispersions, one can observe a steady rise of the gelatin melting point. This results from stabilizing collagen upon its interactions with chromium complexes. In this study (Fig. 4), consumption of montmorillonite was fixed as 4% of the protein weight. In using G dispersions of Cr-MMT for treatment with consumption of 2-5% of the protein weight, the melting point goes up rather slowly. But raising the consumption of chromium oxide up to 10% as part of chromium mineral dispersion of the protein weight promotes a sharp rise in the melting point from 55 to 110°C. The effect of montmorillonite included in Cr-MMT is most evident where the mineral consumption is at 4% of the protein weight. In this study (Fig. 4), consumption of montmorillonite was fixed as 10% of the protein weight. Thus, according to the received output, the optimum consumption rate for efficient stabilization of derma collagen structure is about 3-4% of the protein weight, and 10% of chromium compounds from the mineral weight.

4. Conclusions
1. The article reviews efficiency of derma collagen stabilization with modified montmorillonite dispersions.
2. The infrared spectroscopic studies show that the most characteristic absorption bands of gelatin as a simulated derma collagen correspond to carboxy-, amino-, imino-, hydroxyl groups etc. Modification of montmorillonite with basic chromium sulphate promotes changes in high-frequency and low-frequency ranges of the mineral. The chromium complex displaces water from the montmorillonite interlayer space to form hydroxo-complex ions, which have high polarization and adsorption capacity to support interaction with derma collagen.

3. It is shown that the interaction of the functional groups of native gelatin with active centers of chromium modified montmorillonite is due to the formation of hydrogen bonds O-C-O and CH2 protein groups with the surface hydroxyl groups of montmorillonite of Si-O...H-C and Si-O-H... O-C type. The formation of a hydrogen bond between the nitrogen atoms of gelatin amino groups and water proton in the interlayer space of montmorillonite with cations in the exchange complex is possible. A part of gelatin molecules enters in a specific interaction with mineral exchange cations and simultaneously forms hydrogen bonds with surface oxygen atoms, or neighboring atoms of silicate that efficiently stabilizes the collagen structure. In the low-frequency spectrum range, the formation of hydrogen bonds of Si-O...H- C between the functional groups of chromium modified montmorillonite with CH- groups of protein, as well as Si-O.... H-N with NH - groups of protein is most likely. The presented structural changes are accompanied by changes of the peaks area of the characteristic absorption bands of gelatin and their shift.

4. It was found that the efficiency of stabilizing the derma collagen is manifested in the rise of the melting temperature, which is interdependent on consumption of montmorillonite and basic chromium sulphate for its modification.

5. According to the findings, the optimal consumption rate of the mineral for efficient stabilization of derma collagen is around 3-4% of the protein weight, and that of chromium (III) - 10% of the mineral weight.

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