Досліджено антибактеріальні властивості натуральних шкір, отриманих з використанням композицій природних мінералів на основі цеоліту і монтморилоніту та катіонного поліелектроліту полігексаметиленгуанідін гідрохлориду (ПГМГ-ГХ). Запропоновано використання ПГМГ-ГХ на стадії післядубильних процесів виробництва шкіри. Встановлено, що шкіряні матеріали, модифіковані ПГМГ-ГХ, проявляють виражену бактерицидну дію на бактерії роду Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis

Ключові слова: шкіра, катіонний поліелектроліт, полігексаметиленгуанідін гідрохлорид, цеоліт, монтморилоніт, біоцид антибактеріальні властивості

Исследованы антибактериальные свойства натуральных кож, полученных с использованием композиций природных минералов на основе цеолита и монтмориллонита а также катионного полиэлектролита полигексаметиленгуанидин гидрохлорида (ПГМГ-ГХ). Предложено использование ПГМГ-ГХ на стадии последубильных процессов производства кожи. Установлено, что кожаные материалы, модифицированные ПГМГ-ГХ, проявляют выраженное бактерицидное действие на бактерии рода Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis

Ключевые слова: кожа, катионный полиэлектролит, полигексаметиленгуанидин гидрохлорид, цеолит, монтмориллонит, биоцид антибактериальными свойствами

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FORMATION OF LEATHER BIOSTABILITY WITH THE USE OF CATIONIC POLYELECTROLYTES

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1. Introduction

The problem of bio-damage and biodeterioration implies a wide range of scientific and practical tasks. Biodeterioration can significantly change the properties of materials, impact the lowering of their quality, and, in some cases, lead to the complete destruction of the object [1].

In ordert to prevent bio-damage to genuine leather, biocides are applied at different stages of its treatment. Up to now, the scientific literature [1–8] has described several thousand of chemical compounds that have biocidal properties, however, in practice, due to safety reasons, only hundreds of them are actually used. Every year dozens of biocidal preparations are terminated because of their low antimicrobial action or high toxicity. In addition to the natural resistance of some microorganisms to biocidal preparations, microorganisms quickly adapt to adverse factors, including the effects of antimicrobial preparations.

Improving antimicrobial activity of biocidal preparations leads to the growth in their toxicity. The most relevant areas when creating new biocidal means is to increase the duration of their antimicrobial action to reduce toxicity, allergenicity and environmental safety. Of great importance is also a wide range of biocidal action of preparations in terms of pathogenic organisms, availability of raw materials for manufacturing, manufacturability, shelf life, appropriate physical-chemical, hygiene and consumer properties.

2. Literature review and problem statement

The influence of living organisms on the industrial albuminous raw materials of natural origin, materials and products made of them can significantly change their consumer properties, reduce quality, and in some cases lead to their complete destruction [2–4]. The properties of raw materials may change during storage, operation, manufacturing under the influence of mechanical, physical-chemical and biological factors that cause respective damage.

Article [5] formulated basic requirements for the biocides, including such significant as high activity against harmful biofactors, safety when use, no negative impact on the environment, accessibility, low price. Special attention is paid to the hygienic requirements, in particular low toxicity for animals and humans, not to be accumulated in the environment and not to be allergens.

The highest efficiency among the methods for providing the materials with antibacterial properties is displayed by chemical methods, in the course of which the formation of chemical bonds occurs. Chemical modification and application of protective coatings comprise a group of methods, safe for humans and the environment, which provide anti-bacterial properties for materials. However, chemical methods of modification have considerable technological, economic and environmental shortcomings. This type of modification requires the acquisition, transportation and installation of additional equipment and chemical reagents. This significantly complicates the technology increases complexity of the manufacturing process and leads to higher price of the finished product.

In paper [6], authors presented modified leather, designed for making inside parts of footwear with improved biocidal properties, which includes a collagen base of multilevel structural organization, tanning, greasing compounds and a biocidal additive. The latter contains nanoscale particles of silver and alkyldimethylbenzylammonium chloride. In this case, the content of nanodimensional silver particles is 7×10^{-5}–6.4×10^{-3} % by weight. Technical result is the extension of the range of lining leathers with a reduction in the number of biocidal preparations that are applied to leather and the stabilization of deformation-strength indicators of leather in the course of its usage with enhancing the sustainability of the process of obtaining such leather.

Article [6] presented information about the leather, impregnated with antifungal and antibacterial agent, which is the salts of lanthanum or cesium in amount 0.02–1.25 % by weight of raw material converted to metal oxide. There is also the lining leather that contains tanning, greasing components and, as the antiseptic additive, methyl esters Z-3-benzenesulfonyl or Z-3-(2-naphthalene-sulfonyl) – propenoic acid in the amount of 0.5–1.0 % of weight of the semi-finished product.

A shortcoming of these materials is the high consumption of antimicrobial preparations; adverse effect of the used chemical compounds on the environment and a narrow range of biocidal action.

Strict selection of biocides applied for the treatment of consumer products is regulated by requirements of the Directive of EP and EU Council 98/8/EC as of February 18, 2008, and other legislative acts of the European Union [7]. Careful selection of biocidal preparations used in the production of leather should provide safety of natural leather as a consumer product.

Polymer preparations are widely used in practical medicine and biotechnology mainly as medicines, plasma substituting and detoxifying preparations, as well as in other fields of science and technology. In recent years, there has been increased interest in the application of this group of preparations to fight the microorganisms, that is, to create antibacterial and antiviral polymeric preparations [8].

Among the new preparations that best meet the growing requirements for the biocidal means, polymeric compounds of guanidine or polyalcyleneguanidines (PAGs) play considerable role. This group of biocides by a number of parameters differs significantly from traditional preparations that are manufactured based on quaternary ammonium compounds (QAC), aldehydes, surface-active substances (SAS), derivatives of phenol, chloride-active compounds, etc. Thus, for example, due to their polymeric nature, the biocidal action of PAGs exceeds that of chlorhexidine bigluconate and low-molecular cation SAS, and they are not so toxic [9], and hence safer to work with and will not threaten human health.

Organic compounds containing the guanidine component have excellent bactericidal properties and are used as medicinal preparations and fungicides [10]. Because guanidine contains three active atoms of nitrogen, it becomes possible to introduce into the composition various components and receive a positive charge necessary for biocidal activity. It is because of this fact that its derivatives are not inactivated by proteins and are biologically soluble [11] that they have a wide scope of application as physiologically active preparations (disinfectants, medicines, pesticides, etc.). Moreover, an important indicator of the preparations based on guanidine is its low toxicity for the human organism.

Among the guanidines, which are used in different spheres of human activity to provide antiseptic properties, the one often used is polyhexamethyleneguanidine hydrochloride (PHMG-HC). Its positive effect is well known in the medical and veterinary disinfectants (solutions with concentration 0.1...5 % of active substance), in food industry (at content 0.05...0.5 %), it is used in the systems of ventilation and air conditioning, applied for the purification and disinfection of water, for the disinfection of surfaces. At low concentrations 0.01...0.5 %, it can be used for the treatment of mucous membranes of human organism.

The most important among the biocidal preparations are chlorhexidine (CHD), polyhexamethylenebiguanidine (PHMBG) and polyhexamethyleneguanidine (PHMG). At present, there is no information about the use of these materials in the technologies of manufacturing leather materials with improved antibacterial properties.

In spite of numerous studies on the problems of microbiological resistance of materials, many issues have remained unresolved until now. For the time of conducting the present study, there is a limited number of articles that contain information on the biocidal preparations for leather materials that do not contaminate the environment, capable to resist microorganisms of different systematic groups (bacteria, fungi, etc.), have the long-term protective action, affordable and cheap.

That is why the given work is devoted to examining the potential use of polymeric derivatives of guanidine at the stage of after-tanning processes in the production of leather to provide the finished product with bioresistance.

3. The aim and tasks of the study

The aim of the research we conducted was to determine the features of providing natural leather with anti-bacterial properties by applying the cationic surface-active substance polyhexamethyleneguanidine hydrochloride (PHMG-HC).

To achieve the set aim, the following tasks had to be solved:

– modification of leather materials using natural minerals and PHMG-HC;

– to examine the indicators of leather materials after their treatment with PHMG-HC;

– to explore the bactericidal action of the examined leathers on the cultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis.*

4. Materials and methods of research into biostability of leather materials

4. 1. The examined materials that were used in the experiment

The possibility to use high-molecular cation-active surface-active substances (SASc) as the biocidal means is confirmed by the decision of the Board of the European Communities [12].

PHMG-HC (Fig. 1) is poly-cationic electrolyte that has a unique set of physical-chemical and biocidal properties. It has no color, odor, fire safe, explosion-proof, fully soluble in water and alcohol, does not lose its properties at low temperatures, does not decompose and retains its physical-chemical and biocidal properties up to temperature +120 °C. The chemical structure of PHMG-HC (TU 9392-007-21060124-94) is a linear or branched polymer; molecular weight is typically within 10 kDa [13].

The scope of application of PHMG in various industries is quite wide:

– production of disinfectants;

– preservative, bactericide, antimicrobial reagent;

– biocidal additive;

– medical and veterinary disinfection;

– disinfection in food industry;

– disinfection of systems of ventilation and air conditioning;

– purification and disinfection of water;

– disinfection of surfaces in the premises, equipment and containers for storage, transportation, supply and bottling of drinking water;

– supplement to create biocidal inks;

– provision of biocidal properties to polymers, concrete, wood [14].

PHMG-HC is positioned today as a biocidal agent with wide range of antimicrobial activity against bacteria, viruses, fungi, etc. The antimicrobial effect of PHMG-HC is exerted on the viruses of enteral and parenteral hepatitis, HIV, poliomyelitis, influenza, herpes, etc. It is also bioactive in relation to mushrooms of the Candida type, mold, yeast, candidiasis, dermatophytes. It has a deodorizing effect, provides the surfaces that are treated with a long-lasting bactericidal effect that can be retained, depending on the surface and other external factors, from 3 days to 8 months. All of the above makes this product a unique biocide with "prolonged action".

The mechanism of PAGs action on microorganisms is represented in the following way [12, 16]:

– guanidine polycations are adsorbed at the surface of a negatively charged bacterial cell (Fig. 2), thereby blocking the breathing, nutrition, transport of metabolites through the cell wall of bacteria (this effect depends on the magnitude of the ionic charge of a polycation);

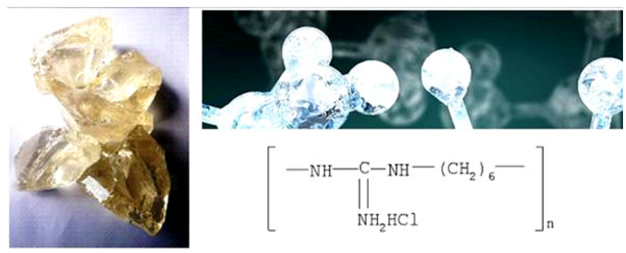


Fig. 1. General view of PHMG-HC and its structural formula [15]

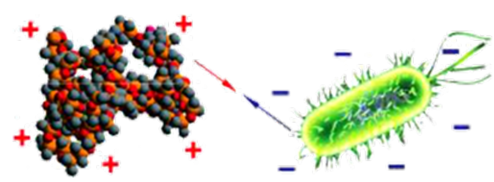


Fig. 2. Schematic of action of PHMG-HC on microorganisms

– the macromolecule of PAG diffuse through the cell wall causing irreversible structural damage at the level of cytoplasmic membrane, nucleotide, cytoplasm (this process depends on the magnitude of surface activity, lipophilicity, solubility in water, molecular volume of the diffusing particle);

– PAGs bind to acidic phospholipids, proteins of cytoplasm membrane, which leads to its tear (this effect depends on the concentration and molecular weight of a biocide);

– the result of the latter is a blockade of glycolytic enzymes of respiratory system of the cell, loss of pathogenic properties and death of microbial cell [16].

Therefore, the use of PHMG-HC in the technology of production of natural leather can simultaneously resolve two tasks: first, to intensify after-tanning processes, second, to provide leather with a certain level of biostability.

In the production of leather, of importance is the extent of formation of the structure of dermis collagen. The employed chemical materials include a variety of mineral and organic compounds. The dermis structure undergoes final formation in the course of after-tanning processes, namely the process of additional tanning-filling. Most often, these processes involve herbal and synthetic tanners, polymeric materials. One of the modern directions in the production of high-quality leather is to use clay compounds based on natural minerals [17].

In the present paper we used, as a filler, a modified dispersion of montmorillonite and peptizied dispersion of natural zeolite. Montmorillonite is a layered clay mineral from the class of silicates, water aluminosilicate. Natural zeolite is a stable aluminosilicate with a frame arrangement of crystal lattice [18].

The modification of montmorillonite was carried out by treating its aqueous dispersion with sodium hexametaphosphate (polyphosphate) sodium (GOST 20291-80) in the amount of 10 % of dry mass, for the purpose of cationic substitution of the ions of exchanging mineral complex with sodium ions.

Sodium hexametaphosphate is a chemical compound with formula $(NaPO_3)n{\cdot}nH_2O$; it is a white, solid, hygroscopic substance. Conducting the cation substitution process contributes to the maximum level of dispersing the aggregates of mineral into its structural formations with dimensions 0.94–1.0 nm [17–19].

Zeolite, in contrast to montmorillonite, is a carcass mineral. In order to obtain finely dispersed (1.0 μm) zeolite fractions, it is possible to use the ultrasonic or mechanical method of grinding. For the peptization of natural zeolite, we also used sodium polyphosphate in the amount of 10 % of dry weight of zeolite.

We use in the present study a chrome semi-finished product, obtained by the procedure of producing the chromium lining leather at PAT "Chinbar" (Kyiv, Ukraine) from raw pork. The chrome semi-finished product is treated with the modified dispersion of montmorillonite (MDM) and the modified dispersion of natural zeolite (MDZ) by developed technologies [20–22].

4. 2. Methods and techniques for conducting the research

In order to determine the content of PHMG-HC in the solution, we use the method of titrating the batch of the examined substance using the solution of indicator prepared in advance. In this case, the titration was performed by the solution of silver nitrate until a sharp transition of yellowish-green coloring of the solution to pink. The share of PHMG-HC was determined by formula:

$$X = \frac{V \times 1{,}7}{m}, \tag{1}$$

where X is the mass fraction of PHMG-HC, %; V is the volume of the $AgNO_3$ solution with concentration 0.1 mol/l, used for the titration, ml; 1.7 is the mass of PHMG-HC, which corresponds to 1 ml of the $AgNO_3$ solution at concentration 0.1 mol/l; m is the mass of the examined solution, g.

In order to prepare the solution of specific indicator, the fluorescent batch in the amount of 0.2 g is poured in a measuring beaker and dissolved in ethanol alcohol, bringing the volume to 100 ml.

The accepted result of the analysis is the arithmetic mean of 3 parallel measurements, whose absolute discrepancy should not exceed the permissible difference equal to 2.0 % for the solid form and 0.1 % for the liquid form.

According to the State standards (DSTU) and accepted techniques, we determined basic indicators of PHMG-HC (Table 1).

According to generalized theoretical data, the concentration of PHMG-HC for the surface treatment of different materials can vary quite widely from 0.05 % (in aerosols) to 14 % (in solution). For the present study, it was decided to choose the solutions at concentrations, %: 0.5; 1.0; 2.5; 5.0; 10.0. We determined main indicators of product quality for both the examined solutions and the starting product (Table 2).

In order to determine the antibacterial properties of the examined leathers, we employed the technique approved by the Order of Ministry of Health of Ukraine No. 167 as of April 5, 2007 "Determining the susceptibility of microorganisms to antibacterial preparations", which sets standard methods for determining the susceptibility of microorganisms to the antibacterial preparations. The research in line with this procedure was carried out at the microbiological laboratory of the Institute of Microbiology and Virology named after D. K. Zabolotny of the Ukrainian Academy of Sciences.

Table 1

Basic properties of PHMG-HC

No. of entry	SASc indicator	Level of indicator	DSTU
1	physical appearance	viscous fluid	–
2	color	clear	–
3	odor	absent	–
4	nature	cationic	ISO 2871-1:2005
5	dry residue, %	53	2207.3-93
6	active washing substance, %	50	2665-94
7	solution pH (1.0 %)	6.9	EN 1262:2007
8	foaminess	низька low	[25]
9	surface tension (1.0 %), din/cm	35	[25]
10	density, g/cm7+^{3}	1.1045	[25]

Table 2

Basic properties of the examined solutions of PHMG-HC

Solution concentration, %		Solution density, g/cm^3	Resistance, in points, to the action of electrolytes		
estimated	determined experimentally		formic acid	ammonium hydroxide	neutral salts
0.5	0.51	1.023	5 (to pH=2)	5 (to pH=12)	5
1.0	1.07	1.033			
2.5	2.52	1.043			
5.0	5.09	1.090			
10.0	10.09	1.079			

In order to examine the biostability, we selected control samples and the samples treated with PHMG-HC at concentrations 0.5 %, 2.5 %, and 5.0 %. Such a choice is based on the maximum degree of processing the working solutions, as well as on curiosity – if the biostability of leather is provided at the minimum consumption of PHMG-HC.

In this paper, we used the method of serial dilutions that is based on the direct determining of the minimum inhibiting (suppressing) concentration (MIC) of a preapration, which suppresses visible growth of the examined microorganism in bouillon culture or in dense nutrient medium.

To determine the magnitude of MIC, the examined skin samples treated at different concentrations of PHMG-HC were taken to the nutrient medium. Next, we carried out the seeding with a culture of the examined microorganism and, upon incubation, assessed the presence or absence of visible growth of a microorganism.

Determining the biostability of samples was performed in the following phases:

1. Preparation of nutrient media.
2. Preparation of the suspension of the examined microorganisms (inoculum) in test tubes (Fig. 3).
3. Introduction of inoculum to the nutrient medium of "bouillon or agar culture" by loop (Fig. 4).
4. Introduction of inoculum to a Petri dish with the examined sample (Fig. 5).

Fig. 3. Preparation of the suspension of the examined microorganisms (inoculum)

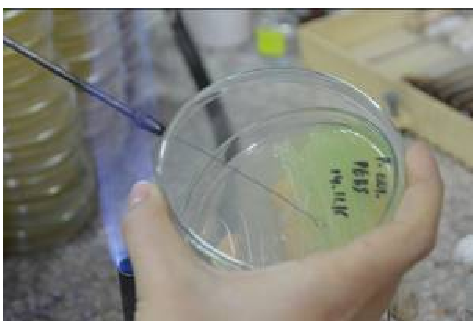


Fig. 4. Introduction of inoculum to the nutrient medium by loop

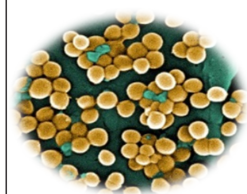
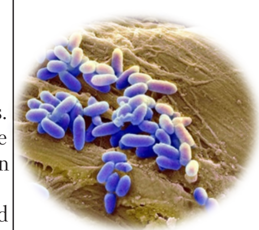
Fig. 5. Taking the examined sample to a Petri dish

In accordance with the procedure, we defined bioresistance of the samples to 4 types of bacteria *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtili*., the description of which is given in Table 3.

The choice of the presented types of bio-damage agents was based on data about the most common biodeterioration of protein raw materials, including genuine leather, by the given types of bacteria.

Table 3

Characteristics of the examined bio-damage agents

General characteristics	*Physical appearance*
ESCHERICHIA COLI (E. COLI)	
Escherichia coli-type is the type of gram negative rod-shaped bacteria, widely spread in the lower part of the intestines of warm-blooded animals. This gram bacteria is the optional anaerob, does not form endospores; very sensitive to the action of a certain kind of biocides (antibiotics), however, it can quickly acquire resistance to their actions	
STAPHYLOCOCCUS AUREUS	
Staphylococcus aureus is the type of globular gram positive bacteria of the genus Staphylococcus. About 25–40 % of the Earth's population are permanent carriers of this bacteria, which can be maintained on skin and mucous membranes of upper respiratory tract. Golden Staphylococcus is the agent of many infections and diseases. It tops the list of bacteria, which often infect in medical institutions	
PSEUDOMONAS AERUGINOSA	
Pseudomonas aeruginosa is the type of gram negative movable rod-shaped bacteria. It lives in water and soil, conditionally pathogenic for humans, the activator of nosocomial infections in humans. Treatment is difficult because of the high resistance to biocides. Thanks to the signal molecules that are formed by cells, they can make common decisions to accommodate to the special features of a particular medium and its own protection. This makes them especially resilient even to large doses of biocides	
BACILLUS SUBTILIS	
Hay Bacillus is the type of gram positive spore-forming aerobic soil bacteria; conditionally pathogenic to humans (according to some data, it is not pathogenic). It is a «model organism». Feeds on dead organic	

5. Results of examining the indicators of leather materials after the treatment with PHMG-HC

5. 1. Application of PHMG-HC for the after-tanning processes in the production of leather

The used filling mineral compositions possess anionic character. In the technology of filling the leather semi-finished product with modified dispersions of natural minerals, in order to fix mineral composition in the structure of collagen, they commonly apply formic acid, which recharges the system, transforming it into the cationic form. Given the fact that PHMG-HC by its nature is a cationic substance, it became possible to replace formic acid with the solution of PHMG-HC. Because of the layered or carcass structure of the examined minerals and their sorption properties, the molecules of cationic PHMG-HC can partly enter the space of molecular lattice of the particles of mineral, holding firmly there.

Therefore, we shall try to replace the fixing treatment in the technology of after-tanning processes with the treatment by the solutions of PHMG-HC with varying concentration. We conduct further research in order to obtain the samples whose technology of treatment is given in Table 4.

Table 4

Parameters for treating the leather semi-finished product with PHMG-HC

Process	Consumption, %	Materials	T, °C	Duration, min.	Note
Filling	100	Water	30		
	4.0	Quebracho		20	Dilution 1:4
	2.0	Tergotan RX		30	Dilution 1:4
	2.0	Sandotan VX		60	Dilution 1:4
	2.5/4.0	**MDM/MDZ**		40	Working fluid discharge
Washing	150	Water	55	15	Working fluid discharge
Greasing	100 3.0 4.0 0.3	Water Provol BA Pellastol 94 S Ammonium hydroxide	55	60	Fat emulsion pH – 7.8–8.0 Working fluid discharge
Fixing treatment	According to plan*	**PHMG-HC**	25–30	60	Working fluid discharge

*Note: * – variants for treating the PHMG-HC 1, 2, 3, 4, 5 are: 0.5 %; 1.0 %; 2.5 %; 5.0 % and 10.0 %, respectively*

In order to conduct the study, we prepared 6 examined batches for each of the used minerals (1 – control, 2–5 – for treating with solutions of PHMG-HC at different concentrations).

When using PHMG-HC in the technology of the after-tanning processes, no any complications occurred. Washing water after a full cycle of additional tanning-filling and greasing contain neither mineral filling compounds (visual analysis) nor fat, introduced in the course of greasing (by results of the conducted Lieberman reaction). Thus, PHMG-HC fulfilled its role of a fixer for the anionic filling and greasing formulations.

At the end of the experiment, we determined the degree of processing the solutions of PHMG-HC, which is summarized in Table 5.

An analysis of Table 5 reveals partial absorption of the examined product by leather. With increasing concentration of PHMG-HC, its absorption by leather increases to 48 % under condition of applying MDM and 52 % under condition of using MDZ. Such a level of indicator is achieved in the solution of PHMG-HC at concentration 5 %. Further increase in the concentration of the preparation is not expedient due to the reduction in its intake from the solution. Most likely, long-chain molecules of PHMG-HC aggregate in the concentrated solution, which slows down its diffusion into the structure of dermis. Interesting is the fact that the absorption of PHMG-HC by the semi-finished product, treated with MDZ, in all examined variants exceeds that of the semi-finished product, treated with MDM. This is obviously due to the structure of the minerals themselves and their arrangement in the dermis.

At the modification of montmorillonite, there occurs the restructuring of layered silicates (Fig. 6), ions are added, removed or regrouped, the aggregates of clay crystallites are dispergated into the aggregates of much smaller size (50–100 nm), or into separate finely dispersed (1–10 nm) clay crystallites.

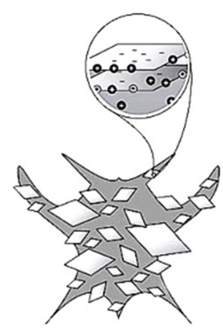


Fig. 6. Schematic of structural changes in MDM

The presence of MDM particles of different size in one system facilitates the simultaneous formation of structure of the dermis collagen at the level of ultra-, micro- and macropores. The ordering of porous structure of the dermis in this case occurs at the level of fibrils and primary fibres [18, 22–24]. Thus, different levels of the dermis structure are filled with finely dispersed mineral, which prevents for high-molecular PHMG-HC to penetrate into the dermis. In spite of this, PHMG-HC itself, due to the high activity of specific surface of MDM (roughly 700–840 m^2/g), allowing for wetting with water or other polar liquids, is fixed by the mineral.

Unique crystalline lattice of zeolite is a kind of molecular sieve (Fig. 7), which has a high absorption capacity.

Table 5

Content of PHMG-HC in the processed working solutions

PHMG-HC solution concentration (acting), %	Filling with MDM: Content of PHMG-HC in the processed working solution, %	MDM: Coefficient of processing the working solution, %	Filling with MDZ: Content of PHMG-HC in the processed working solution, %	MDZ: Coefficient of processing the working solution, %
0.51	0.34	33.3	0.30	41.2
1.07	0.64	40.2	0.60	43.9
2.52	1.49	40.9	1.33	47.2
5.09	2.65	47.9	2.45	51.9
10.09	6.23	38.3	6.03	40.2

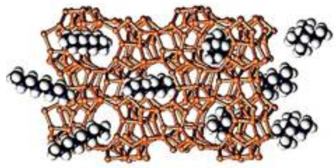


Fig. 7. Schematic of zeolite molecular sieve

Zeolite, according to reported studies [13, 19], does not come in contact with proteins and amino acids but it can sorb the compounds of chromium, calcium and sodium in the technological cycle of genuine leather production. Moreover, the sorbed ions are additional centers to hold PHMG-HC in the dermis structure. Zeolite fills macro- and micropores of the dermis and does not stop the molecules of PHMG-HC from diffusing into it.

5. 2. Determining the bactericidal action of the examined leather

Research results on determining the bactericidal action of the examined leather are given in Table 6 (Fig. 8–10).

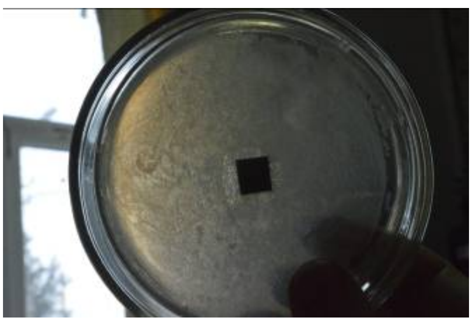


Fig. 8. Results of measuring the microbiological activity of the PHMG-HC solution at concentration 0.5 %

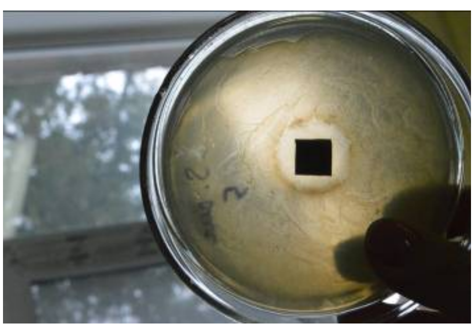


Fig. 9. Results of measuring the microbiological activity of the PHMG-HC solution at concentration 2.5 %

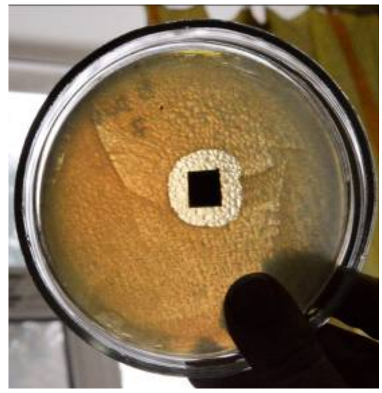


Fig. 10. Results of measuring the microbiological activity of the PHMG-HC solution at concentration 5.0 %

With increasing concentration of the PHMG-HC solution (Fig. 10), its microbiological activity increases that helps increase the radius for eliminating the bacteria.

6. Discussion of results of examining the bactericidal action of leather obtained with the use of cationic polyelectrolyte PHMG-HC

Results of examining the microbiological stability of leather samples (Table 6) indicate that even without being treated with cationic polyelectrolyte PHMG-HC, control leathers, made with the use of dispersions of zeolite, demonstrate a zone of stunted growth of Golden Staphylococcus.

An analysis of the results indicates that the treatment of a leather semi-finished product with the dispersion of zeolite increases resistance of the treated material to the action of bacteria. At the same consumption of PHMG-HC for the treatment (except for the variant with a consumption of the 5 % PHMG-HC), the biological stability of the samples treated with MDZ exceeds that of the samples treated with MDM. An increase in the biostability of leather is explained by the structure of the mineral filler: most bacteria cannot penetrate inside the frame of zeolite because of the dimensions of frame "windows" of the mineral. The outer surface of zeolite is too small to effectively hold colonies of bacteria, but it sorbs well the cationic high-molecular substances, which include PHMG-HC. In addition, zeolite can sorb an unbound chrome tanning agent from a semi-finished product, which is also a biocidal means.

However, as evidenced by results, the examined leathers do not display any bactericidal action towards the aggressive culture of *Pseudomonas aeruginosa*.

Table 6

Results of bactericidal action of the examined leather

PHMG-HC content, %	*E. coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Bacillus subtilis*	
	MDM	MDZ	MDM	MDZ	MDM	MDZ	MDM	MDZ
0 control	–	–	–	+/–*	–	–	–	–
0.5	–	–	+/–*	+/–*	–	–	+2 mm	+2 mm
2.5	–	+1.5 mm	+1.5 mm	+3 mm	–	–	+3 mm	+6 mm
5.0	–	+2.5 mm	+2.5 mm	+6 mm	–	–	+6 mm	+4 mm

Note: +/– – zone of stunted growth (inhibition) of bacteria; + – destruction of bacteria in a certain radius*

7. Conclusions

As a result of the conducted measurements of microbiological activity of the solutions of polyhexamethyleneguanidine hydrochloride at concentration 0.5 %, 2.5 % and 5.0 %, it is possible to formulate the following conclusions:

1. We established special features of the anti-bacterial properties of leather depending on the treatment of a leather semi-finished product with the solutions of different concentration of cationic polyelectrolyte polyhexamethyleneguanidine hydrochloride and the type of mineral filler. It is shown that the experimental samples attain a certain level of biostability when treated in the solution of polyhexamethyleneguanidine hydrochloride at concentration not lower than 2.5 %.

2. It is determined that the structure of natural minerals and their location in the dermis affects the degree of absorption of biocide by a semi-finished product: the examined samples of leathers obtained using the dispersion of zeolite, the absorption of polyelectrolyte is higher than in the samples obtained with the use of dispersion of montmorillonite. The leathers, obtained with the use of dispersion of zeolite, demonstrate a zone of stunted growth of Golden Staphylococcus even without being treated with the examined biocide. It is established that at the same consumption of biocide for the treatment of a leather semi-finished product, biological stability of the examined samples filled with the dispersion of zeolite is higher than the biostability of samples filled with the dispersions of montmorillonite.

3. We examined the bactericidal action of the examined leathers on the cultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis*. The examined leathers do not display any bactericidal action on the aggressive culture of *Pseudomonas aeruginosa*.

Thus, present research demonstrated the possibility to purposefully provide leather materials with biocidal properties by applying, at the stage of filling-additional tanning a leather semi-finished product, the cationic polyelectrolyte polyhexamethyleneguanidine hydrochloride.

The mechanisms of interaction and sorption processes between collagen, a mineral and the biocidal preparation polyhexamethyleneguanidine hydrochloride, which take place in the structure of leather, require further study.

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