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DEVELOPMENT OF POLYMERIC HYDROGEL FORMS FOR USE IN MEDICINE

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Composition and method of obtaining polymeric drug forms were developed - polymer hydrogel dressings and ointments containing trimekain and piromekain. Hydrogel dressings were obtained on the basis of polyvinylpyrrolidone (PVP), agar and polyethylene glycol (PEG). The degree of swelling in various media is determined for the hydrogel dressings obtained.The study found that the resulting polymer matrix provides a high release of the drug. Ointments were obtained on the basis of carbopol (acrylic polymer) with additives of polypropylene glycol and sodium benzoate. Physicochemical, rheological properties and biological parameters of hydrogel dressings and ointments were investigated. It was found that the obtained ointments possess antibacterial properties with respect to Staphylococcus aureus and Escherichia coli.

Keywords: *hydrogel dressing, ointment, trimekain, piromekain, polyvinylpyrrolidone, polyethylene glycol*, a*gar-agar, rheology, dynamic viscosity.*

Тримекаин мен пиромекаин құрайтын гидрогельді таңғыш пен сықпа май – дәрілік полимерлі формаларының құрамы және алу әдістемесі жасалған. Гидрогельді таңғыштар поливинилпирролидон (ПВП), агар-агар және полиэтиленгликоль (ПЭГ) негізінде алынған. Зерттеу барысында алынған полимерлік матрица дәрілік заттың жоғары мөлшерде шығарылуын қамтамасыз ететіндігі анықталды. Карбополь (акрил полимері) негізінде полипропиленгликоль және натрий бензоатының қоспалары бар сықпа майлар алынған. Гидрогельді таңғыштар мен сықпа майлардың физика-химиялық, реологиялық қасиеттері мен биологиялық параметрлері зерттелді.

Тірек сөздер: *гидрогелді таңғыш, сықпа май, тримекаин, пиромекаин, поливинилпирролидон, полиэтиленгликоль, агар-агар, реология, динамикалық тұтқырлық.*

Разработаны состав и метод получения лекарственных полимерных форм – гидрогелевых повязок и мазей, содержащих тримекаин и пиромекаин. Гидрогелевые повязки получены на основе поливинилпирролидона (ПВП), агар-агара и полиэтиленгликоля (ПЭГ). Для полученных гидрогелевых повязок определены степени набухания в различных средах. В ходе исследования установлено, что полученная полимерная матрица обеспечивает высокое высвобождение лекарственного вещества. Мази, получены на основе карбопола (акриловый полимер) с добавками полипропиленгликоля и бензоата натрия. Исследованы физико-химические, реологические свойства и биологические параметры гидрогелевых повязок и мазей. Установлено, что полученные мази обладают антибактериальными свойствами по отношению к Staphylococcus aureus and Escherichia coli.

Ключевые слова: *гидрогелевая повязка, мазь, тримекаин, пиромекаин, поливинилпирролидон, полиэтиленгликоль, агар-агар, реология, динамическая вязкость.*

Introduction. Currently interest in obtaining various forms of anesthetics based on polymers such as hydrogel wound dressing and ointment was increased. Hydrogel dressings have several advantages compared to traditional fat and hydrocarbon framework, in particular, provide active wound cleansing thanks to the draining effect, compatibility with a variety of drugs, to more complete and uniform release, prolonged action, which provides a high therapeutic effect. However, almost all known industrially produced hydrogel dressings do not contain drugs, which often limit the effectiveness of their use. In this regard, the possibility of obtaining hydrogel wound coatings with the inclusion of medicinal substances of various actions, the therapeutic properties of which provide a comprehensive effect on the wound surface, is of absolute interest and relevance. Also polymer-based ointments with the addition of anesthetics are full of interest, because of a number of advantages over ointments on fat or hydrocarbon bases. The latter are characterized by high hydrophobicity, which does not allow absorbing the wound discharge and does not provide exit of therapeutic agents from the composition. Polymer-based ointments differ from their existing hydrophilic (water-soluble) basis. Polymer gels used as carriers of ointments have a number of advantages over other drugs used for this purpose: they dissolve hydrophilic and hydrophobic substances that actively absorb wound exudates well applied to the wound surface. Ointment is evenly distributed, does not interfere with physiological function, well washed off with cold water, which is important in the treatment of wounds. In addition, their fundamental difference is high osmotic activity, providing the necessary dehydrating effect of ointments on the tissue in the focus of inflammation.

The purpose of this study was creation of different polymer forms containing anesthetics in their composition and investigation of their physical, chemical, rheological properties, as well as biological parameters.

Polymer hydrogels, which are used as a polymer matrix in wound healing dressings, were first invented by J. M. Rosiak et al. (1989), and they have many interesting properties: transparency required to observe the healing process of wound; absorption and prevention of fluid loss in the body; creating a barrier against bacteria; good adhesion; ease of application; oxygen permeability; dose control, etc. [1].

Two polymeric forms containing anesthetics were obtained in this work: hydrogel dressings and ointments. Hydrogel dressings were obtained on the basis of PVP, agar-agar and PEG with the addition of anesthetics. Ointments were obtained on the basis of acrylic acid gels known as Carbopol®. The choice of carbopol as the basis for the synthesis of gel with adsorption properties was due to high viscosity at low concentrations, compatibility with many active ingredients, good bioadhesive properties, stability when exposed to heat, excellent organoleptic characteristics and good acceptance by patients [2].

Trimekain and piromekain were used as anesthetics. Piromekain (1-butyl-2,4,6-trimethyl-2-pyrrolidinecarboxamido) is a local anesthetic used for surface anesthesia. Trimekain (α-diethylamino-2,4,6-trimethylacetyl) is an organic compound used as a local anesthetic.

Materials and methods. There are following initial reagents used in this work: PVP (M_w = 10×10^5), PEG (M_w = 2×10^3-10^4) of Merck Chemical Company (Germany), standard agar of SigmaAldrich, carbopol 940 NF (Russia), polypropylene glycol of SigmaAldrich. They were used without further purification. Distilled water is used as a solvent in all experiments. Isotonic solution (0.9 % sodium chloride solution) is obtained by dissolving the salt in distilled water until completely dissolved.

Preparation of polymer forms with anesthetics. Polymer hydrogels were synthesized in plastic containers for the convenience of radiation exposure. The container was filled with 500 mL of 7 % aqueous solution of PVP, agar and polyethylene glycol with the addition of 2 mass.% medicinal substance. The resulting mixture was packed in plastic containers and sent to radiation crosslinking. Irradiation of hydrogel polymer samples was carried out on a linear electron accelerator ELV-4. The total irradiation time was 4 minutes 37 seconds ± 2 seconds, the radiation dose was 20 kGy.

Carbopol ® - based ointments were obtained by gradual dissolution of the required polymer mass in distilled water with moderate stirring at room temperature until a homogeneous consistency was obtained. According to the Lubrizol specification [3], non-neutralized Carbopol® has a pH in the range 2.5-3.5. Neutralization of Carbopol ® was carried out with sodium hydroxide to pH = 6.5-7. Then polypropylene glycol and sodium benzoate were added to the neutralized Carbopol®. Trimekain and piromekain were added to the resulting mass until complete dissolution.

Rheological studies. The study of rheological characteristics was carried out on MCR102 PHYSICA rheometer (Anton-Paar, Austria) using the cylinder-in-cylinder system. S1, S2, cylindrical measuring device was used allowing to measure dynamic viscosity in the range of 1÷100000 PA·s. The measured material was placed in annular gap between two coaxial cylinders.

Calculations of dynamic viscosity were performed using the formula:

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where: η – dynamic viscosity, Pa·s; τ – shear stress, Pa; – shear rate, s^{-1}.

The shear stress were calculated by the following formula:

$$\tau = z \cdot \alpha,$$

where: z – constant of cylinder, Pa/units of the scale; α – instrument reading, units of the scale. The values of the shear rate D and the cylinder constant z were tabular data.

Swelling degree investigation. The degree of swelling was studied by gravimetric method. The degree of swelling was calculated using the following formula:

$$\alpha = (m – m_o)/m_0,$$

where: m – mass of equilibrium swollen polymer hydrogel; m_o – dry weight of the sample. Dry matter mass in gel was determined on the analytical balance Sartorius (Germany) after drying the sample in a vacuum cabinet to a constant weight with accuracy of 0.0001 g.

Study of the kinetics of drug release. To study the kinetics of drug release from the polymer matrix, samples were placed in water and 10 mL isotonic solution at a temperature of 310 K. Drug yield was determined by the calibration graph – dependence of optical density of drug on concentration. Drug content in the solution was determined by the spectrophotometer "UV-2401-PC Shimadzu "(Japan) at the maximum absorption in the UV.

Content of beaker, during the experiment, was constantly mixed with a magnetic mixer and at certain intervals the solution samples were taken, in which the drug content was determined. After

each measurement, the sample was returned to the beaker. The amount of the drug released during t was determined using a calibration line according to the formula:

$$W = \frac{C}{C_0} \cdot 100\%,$$

where: W – amount of released drug, C – drug concentration in the surrounding solution at a time, C_o – drug concentration in the initial solution, and the amount of drug released was found in relation to the initial concentration in the hydrogel dressing composition. The amount of drug that should pass through the membrane when reaching equilibrium was taken for 100 %.

Study of antibacterial activity. Antibacterial activity of ointments was determined by the degree of inhibition of growth of microorganisms of the genus Escherichia coli and Staphylococcus aureus after incubation with the drug in accordance with the European standard for determining the rate of inactivation of microorganisms (European Standard EN 1040, 1997).

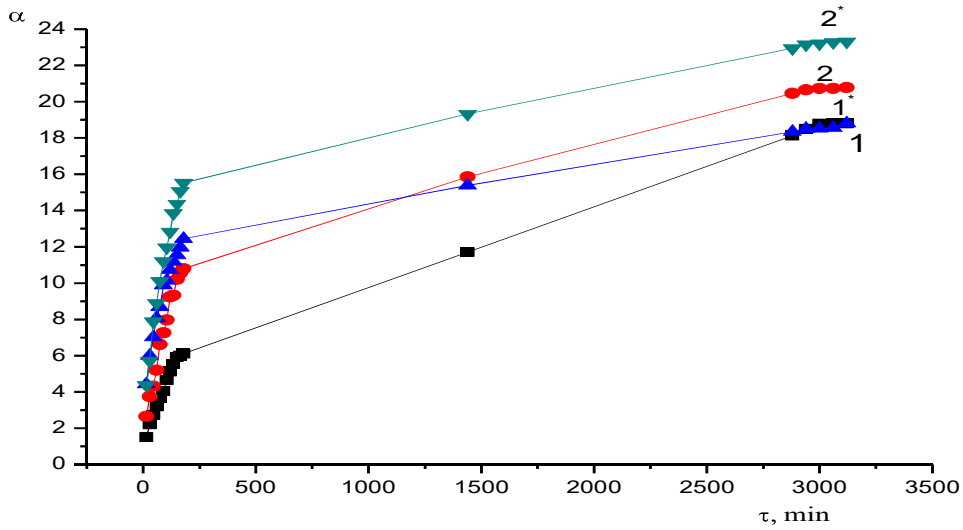
Hydrogel samples under aseptic conditions were supplemented with sterile distilled water and suspension of the daily culture of microorganisms. The total volume of samples of 50 ml with a concentration of 105 CFU/ml mushrooms was placed in flat-bottomed flasks. They were incubated at 37 °C under conditions of mixing on a laboratory shaker with a stirring speed of 100 Rev/min After incubation of the respective flasks seeding was produced by loop into the Saburo medium according to the method of Gold. The number of CFU (colony forming units) of fungi per 1 ml of incubated suspensions were counted in accordance with the criteria of the method of Gold. A drug-free sample was used as a control.

Research result. The swelling of hydrogels plays a significant role in biomedical spheres and is mainly due to the presence of hydrophilic groups (-OH, -COOH, $-CONH_2$, $-SO_3H$) in polymer chains, the degree of cross-linking and the porosity of the polymer. In addition, swelling is a very important property for the practical application of hydrogels, for example, for hydrogels used as wound dressings, rapid capacious absorption of wound exudate is required [4]. For most pharmaceutical applications, it is important to know the swelling kinetics of the wound dressing, as this process has a direct effect on the drug release. In this paper, the kinetics of swelling of wound dressing in water and isotonic solution was studied. In these experiments, an isotonic solution was used to study the behavior of the dressing material under conditions close to the system in vivo. It was found that the rate of swelling of the hydrogel dressing in distilled water was faster than in isotonic solution (figure 1). This is due, firstly, to the formation or rupture of hydrogen bonds between the polymer chains, and secondly, with the activity of water in the equilibrium solution. In PVP hydrogen bonds are formed between two closely spaced functional groups. In addition, preference is given to groups belonging to different polymer chains, as they are more convenient for the formation of such a compound due to the mutual location. Thus, PVP in contact with water destroys some of the hydrogen bonds between groups C = O, which leads to swelling of the polymer. These results correspond to the literature data [5].

As can be seen from figure 1, the obtained hydrogel dressings swelled well enough in water and isotonic solution, which makes it possible to use them as wound dressings, where the release rate and the rate of absorption of exudate are fundamental.

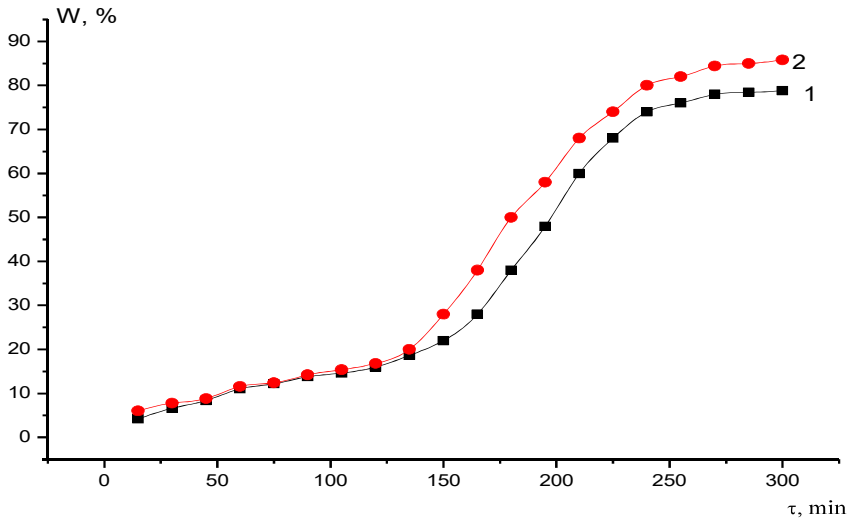
The wound dressings were characterized for drug release properties. Most modern dressings are made from polymers which can serve as vehicles for the release and delivery of drugs to wound sites. The release of drug in isotonic solution is faster than in water, because of the fact that under

the influence of osmotic pressure ionic strength salt in an isotonic solution shows more intense release (Figure 2).



[PVP] = 7 wt. %, [agar-agar] = 2 wt.%, [PEG] = 8 wt.%, radiation dose = 20 kGy

Figure 1 – The kinetics of the equilibrium degree of swelling of wound dressings based on PVP-PEG-agar in water (1) and an isotonic solution (2) with trimecaine (1,2) and pyromecaine (1*,2*)



[PVP] = 7 wt. %, [agar-agar] = 2 wt. %, [PEG] = 8 wt.%, radiation dose = 20 kGy

Figure 2 – The kinetics of the pyromecaine release from the wound dressing in water (1) and an isotonic solution (2)

At first time release of anesthetics of the polymer matrix in water and isotonic solutions occurs at the same rate, after 2.5 hours release of anesthetics in isotonic solution is faster than in water.

In this work, ointments based on Carbopol® containing anesthetics were also obtained. The composition of ointment with anesthetics based on carbopol was determined by Raman spectroscopy (Figure 3). The Raman spectrum shows intense vibrations of C=O groups at 1450–1500 cm^{-1}characteristic for Carbopol units. As well as the absorption band is present at 2850–2950 cm^{-1}, 3100–3250 cm^{-1} and 750–950 cm^{-1} for C-H, NH groups and aromatic compounds respectively.

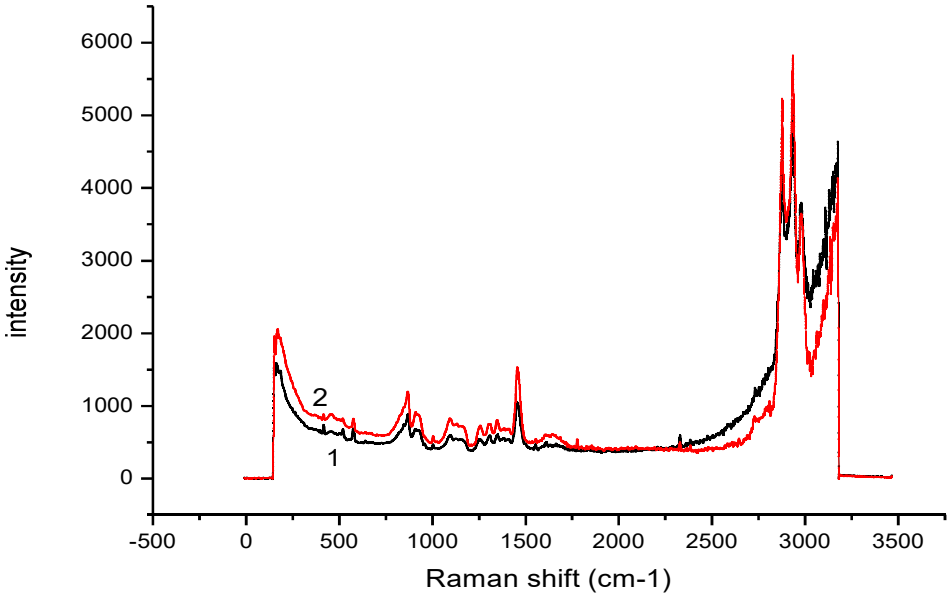


Figure 3 – Raman spectroscopy of ointment with pyromecaine 1 wt.% (1), ointment with trimecaine 1wt. % (2)

For the resulting ointments with different concentrations of medicinal substances rheological studies were conducted. Figure 4 shows the data of rheological studies of ointments with different content of anesthetics, presented in the form of curves of dependence "τ-γ", where the shear stress (τ) is the shear rate (γ), which had minor deformations at initial site in the system. After applying higher shear stress values, a more significant strain was observed. Consequently, the dependence of the shear rate on the shear stress was nonlinear, which indicated the presence in aqueous solutions of the carbopol of a certain structure stabilized, obviously, by hydrogen bonds, as well as physical nodes of engagement. After reaching the critical value of the shear force, the further course of the curves indicated a partial destruction of the solution structure under the influence of mechanical stress and, accordingly, the deformation begun to increase faster than the applied stress. Hysteresis phenomena, namely, the mismatch of curves obtained in the cyclic mode of increase-decrease in voltage, also indicated the processes of formation and destruction of the physical grid in the investigated system of gel with drug.

As can be seen from figure 5, the gels obtained in the work on the basis of carbopol and the drug are not Newtonian liquids, but belong to pseudoplastic structured liquids, for which unlike the Newtonian flow process several elementary processes includes due to the processes of destruction and restoration of the liquid structure, as well as the orientation of macromolecules along the gradient of applied voltage. When the minimum viscosity value is reached, the three-dimensional structure of the hydrogel composition under study is completely destroyed, without having time to recover, and the macromolecules are oriented along the gradient of the applied voltage. It was found that with increase in anesthetic content in gels composition, the maximum dynamic viscosity decreased. But this value is not so significant and noticeable, since the components of drugs are well soluble in water.

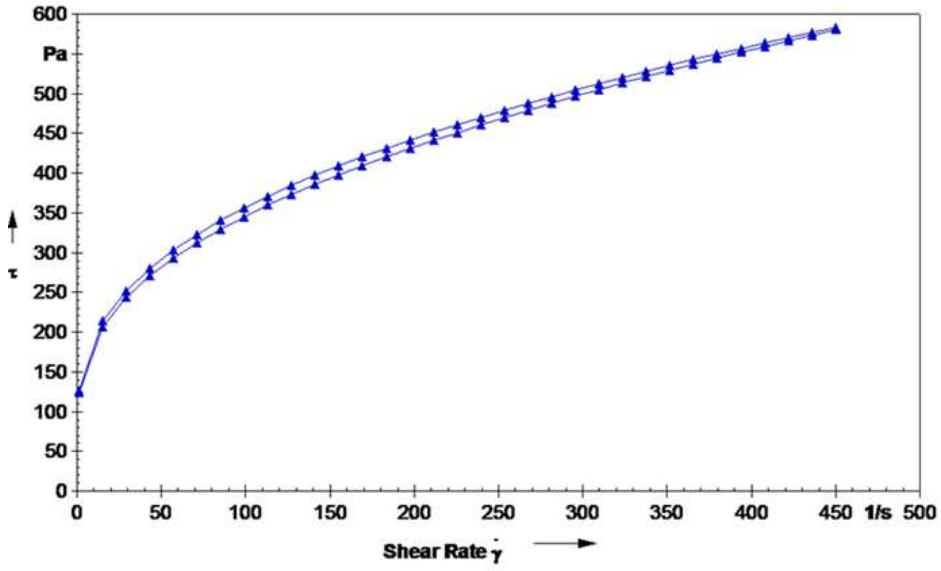


Figure 4 – Dependence of shear stress on the shear rate
for ointments containing 0.5 wt.% trimecaine

The biological activity of ointments against *Escherichia coli* and *Staphylococcus aureus* was investigated. Biological activity was assessed using the diffusion method. The results are presented in table 1.

Table 1 – Antibacterial activity of ointments containing trimecaine

№	The composition of ointment, wt. %	The diameter of the growth inhibition zone test organisms, mm	
		Staphylococcus aureus 3316	*Escherichia coli* *855*
1	*Trimecaine* 1,0	10 be	9 be
2	*Trimecaine* 1,5	12 be	14 be
3	*Trimecaine* 3,0	20 be	25 be
be – bacteriostatic effect			

Samples of ointments containing 1.0 and 1.5 % trimecaine showed low antibacterial activity, mainly bacteriostatic effect. A sample with trimecaine 3.0 % had a bactericidal effect against the test organisms studied-both gram – positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) opportunistic pathogens: the diameter of the growth suppression zone of *S. aureus* was 20 mm, *E. coli* – 25 mm (figure 6). The studied samples of ointments had a more pronounced antibacterial effect against gram-negative bacteria and a less pronounced effect against gram-positive bacteria. It was found that as the drug concentration in ointments increases, the size of the inhibition zone increases. The study found that the ointment based on carbopol had good antibacterial activity.

A B

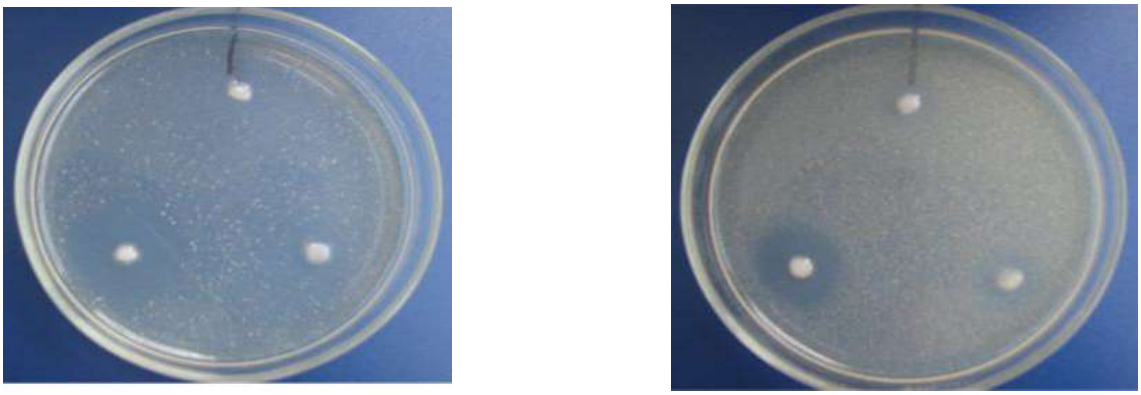


Figure 6 – Antibacterial activity against *Escherichia coli 855* (A) and *Staphylococcus aureus* 3316 (B) (ointments trimecaine samples with a concentration of 1.0, 1.5 and 3 %)

Conclusion. In the present work, polymer hydrogel dressings based on PVP/agar-agar/PEG were obtained for the purpose of studying their potential use as dressings, which have anesthetic activity with improved characteristics of delayed release of drug. In order to expand the use of anesthetics, ointment were prepaired on the basis of polyacrylates with a content of piromekain and trimecaine. Their rheological properties were studied, it was shown that the received ointments show antibacterial activity.

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