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Development of hydrogel ointments based on carbopol with α-lipoic acid

Abstract: This article presents the results of hydrogel ointments development based on carbopol polymer with α-lipoic acid for their application as transdermal therapeutic delivery systems for antibacterial treatment of diabetic foot syndrome. Complex formation of bioavailable α-lipoic acid with hydroxypropyl-β-cyclodextrin was studied using differential scanning calorimetry and scanning electron microscopy. Optimal rheological characteristics of obtained ointments have been estimated. Antimicrobial activity in relation to *Staphylococcus aureus and Escherichia coli* have been observed for ointments containing α-lipoic acid. Transdermal drug delivery properties of obtained hydrogel ointments were investigated using cellulose membrane as a model system and a pig skin.

Key words: hydrogel ointment, alpha-lipoic acid, carbopol, hydroxypropyl-β-cyclodextrin, diabetic foot syndrome.

Introduction

Modern pharmaceutical technology as one of the basic tasks considers the creation of dosage forms based on biopolymers with drugs, with high therapeutic activity, providing controlled release of drugs and their specific delivery to the site of the pathological process. One of the most serious diseases at present is the diabetic foot syndrome [1]. This disease connects with leg problems due to elevated blood sugar levels. During this disease the sensitivity of nerves becomes broken – neuropathy, and the blood flow in the vessels of the legs worsens [2]. As a result, the risk of wounds injury and infection increases. Most of antibacterial ointments presented in pharmacies contain antibiotics that are not desirable to be used often, or are produced on fat bases (animal and vegetable fats, petrolatum, etc.), which create a film that does not allow air to enter the lesion focus. Also, to select the best antibiotic, it is desirable to do bacteriological research of tissues. Often patients with the diabetic foot syndrome have impaired renal function. During diabetic foot syndrome after destruction of the protective layer of the skin, deep tissues undergo bacterial colonization. In such a situation, a standard therapeutic dose of antibiotics can harm a patient's health [3]. Nowadays a method of complications treatment of type 1, 2 diabetes with α-lipoic acid (ALA) which is one of the most powerful antioxidants with antimicrobial properties, is known. Therefore, studies on the development of hydrogel ointments with α-lipoic acid are an actual trend.

ALA decomposes gradually at room temperature and easily polymerizes at temperatures higher than its melting point and insoluble in water [4-5]. Thus, the stabilization of ALA is of great interest for industrial applications. In order to stabilize ALA by complexation, the scientists used cyclodextrins (CD) [6-7]. These cyclic oligosaccharides consist of six (α-CD), seven (β-CD) or eight (γ-CD) α-1,4-linked glucopyranose units, with a hydrophilic hydroxyl group on their outer surface and a hydrophobic cavity at the center [8]. CDs are able to form complexes with various ionic and lipophilic substances, taking the whole molecule or part of it into its cavity. Such formation of a molecular complex affects many physicochemical properties of guest molecules, such as water solubility, stability or bioavailability [9-10]. Thus, a complex of inclusion of ALA and CD, which dissolves in water was obtained in this work. What makes it possible to use it for the preparation of hydrogel ointments based on carbopol.

Carbopol is one of the most common thickeners for aqueous phases. Carbopol polymers are acrylic acid crosslinked with polyalkenyl ethers or divinyl glycol. Carbomer molecules are closely packed spiral molecules. After dispersing in water, the molecules begin to swell and partially "unwind". The most common way for complete "unwinding" of molecules is to convert the acid carbopol to a salt. These polymers are anionic polymers that need to be neutralized for gelling. Organic amines, such as triethylamine or alkali, can be used to neutralize these polymers in liquids [11].

The advantages of using carbopol gels as a therapeutic transdermal agents : good rheological properties, good alternative to oily ointment preparations, anionic hydrogels with good buffering capacity, which helps to maintain the desired pH [12], high viscosity already at low concentrations, a wide range of concentrations and characteristic behavior of the flow, compatibility with many active ingredients, bioadhesive properties, good thermal stability, excellent organoleptic characteristics, good tolerability [13].

Materials and methods

Carbopol (Kadpol 980) was purchased from «Shree Chemicals» (India) and was used as received.α-lipoic acid was purchased from «AXXO GmbH» (Hamburg) and was used without purification. Hydroxypropyl-β-cyclodextrin supplied by «AXXO GmbH» (Hamburg) and was used without purification. Polyethylene glycol (M_n=400) produced by «ChemMed» company (Moscow), was used without purification. Glicerin produced by «ChemMed» company (Moscow) and was used as received. Sodium hydroxide was purchased from «Skat» company (Kazakhstan). Sodium Phosphate and Potassium Phosphate were purchased from «Skat» company (Kazakhstan) and were used without purification. Cellulose membrane (M_n=12-14000 Dalton) was purchased from the «Medicell International Ltd» (London).

Hydrogel ointment preparation.

Hydroxypropyl-β-cyclodextrin was used to dissolve α-lipoic acid. The sample of ALA and HP-β-CD in a molar ratio of 1: 1 was rubbed for 1.5 hours in a mortar. Thus, a water-soluble inclusion complex was obtained. Then, the necessary concentration of the ALA solution in water was prepared at a temperature of 38^0 C in a water bath for 1 hour, the resulting solution was filtered off. To this solution was added a sample of carbopol, made alkaline and thoroughly mixed. At the end, polyethylene glycol and glycerin were added as a humectant and texture improvement of the ointment.

Methods of analysis

Differential Scanning Calorimetry.

The analysis was carried outon the NETZSCH STA 449F3 Jupiter (NETZSCH-Gerätebau GmbH, Germany) in a stream of nitrogen at a heating rate of 10 ^{0} C / min. 1.1-1.9 mg of the test substance (pure α-lipoic acid, hydroxypyr-β-cyclodextrin, ALK-GP-β-CD complex) were placed in the aluminum crucible preliminarily suspended with the lid and pressed. The prepared crucible and the test crucible were placed in a measuring chamber. The thermograms were received in the temperature range 30-100 0C.

Differential-thermal analysis.

The analysis was carried out on the NETZSCH STA 449F3 Jupiter (NETZSCH-Gerätebau GmbH, Germany) in a stream of nitrogen at a heating rate of 100C / min. It were used crucibles from Al2O3. The thermograms of pure gel base on carbopol, α-lipoic acid substance, carbol -based gel with α-lipoic acid were obtained in the temperature range 20-300 ^{0} C.

Scanning electron microscopy.

The analysis was carried out at DGP "National nanotechnology laboratory of open type" KazNU. Al-Farabi on a Quanta 3D 200i Dual system, FEI scanning electron microscope at a voltage of 15 kV with an increase of 2000, 5000 per sample.

Analysis of the rheological parameters of the hydrogel ointments.

The rheological profile of the samples was studied on Anton Paar Modular Compact Rheometer 102 (Anton Paar GmbH, Austria). The measuring geometry of the plate / plate was used. The measurements were carried out at a temperature of 25 ° C. The device made it possible to measure the tangential shear stress at a shear rate of 1.5 to 1312 s-1. When constructing the rheogram, the shear rate was increased from 1 to 450 1 / s (the upper curve), and then decreases from 450 to 1 1 / s (the lower curve).

Drug Diffusion and Skin Penetration Study.

In vitro study of the release of ALA and penetration through the membrane and porcine skin was carried out using the Franz Diffusion Cell. The amount of released ALA was recorded with the help of the UV spectrophotometer Analytyc Jena Specord 200. The spectra of ALA solutions were recorded in the wavelength interval 190-400 nm. The wavelength for α-lipoic acid was 333 nm, which coincided with the reference data according to the state pharmacopeia of the Republic of Kazakhstan. The cellulose membrane and the skin of the porcine ears were held for one hour in a phosphate buffer solution pH 6.86, because the pH of the body cells lies in this range. Then it was placed between the donor and acceptor parts of the Franz Diffusion Cell.

Antimicrobial studies of α-lipoic acid.

The studies were performed on nutrient agar using Gram-positive bacteria – Staphylococcus aureus IMV 3316 and Gram-negative bacteria – Escherichia coli IMV 877 as test microorganisms.

Results and their discussion

The development of medicinal forms, such as hydrogel ointments, which increase bioavailability, the effectiveness of the action of medicinal preparations and cause prolonged action, has acquired in recent decades an undeniable scientific and practical value. In this work, hydrogel ointments based on carbopol with different content of α-lipoic acid were obtained. ALA is a powerful antioxidant, it is used in the treatment of diabetes mellitus, it also has antimicrobial properties, which makes it possible to use it for the production of hydrogel ointments with antimicrobial activity in antibiotic therapy of diabetic foot syndrome. Since ALA does not dissolve in water, the first step was to find the optimal method for increasing the bioavailability of ALA, compatible with the composition of the ointment.

Complex formation of α-lipoic acid with hydroxypropyl-β-cyclodextrin

Complex formation of ALA and HP-β-CD have been assessed by DSC method. The results of the DSC show the disappearance of the peak of active pharmaceutical substances as a result of complex formation. The differential scanning calorimetry method allows analyzing substances in different aggregate states, and provides comprehensive information on the phase transitions of various compounds, which can be used for conformation of complexation because DSC method determines the energy changes in the test substance. DSC thermograms were obtained in a temperature range of 30-100 ° C on a combined TGA-DSC calorimeter NETZSCH STA 449F3 Jupiter in a stream of nitrogen at a heating rate of 10 ° C / min.

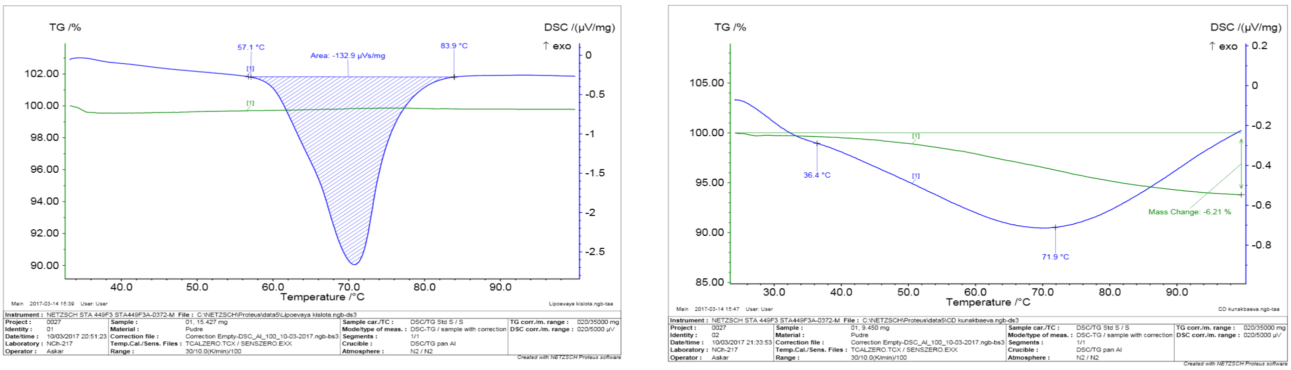


Figure 1 – TGA-DSC pure substance of α-lipoic acid (left) and pure substance of hydroxypropyl-β-cyclodextrin (right)

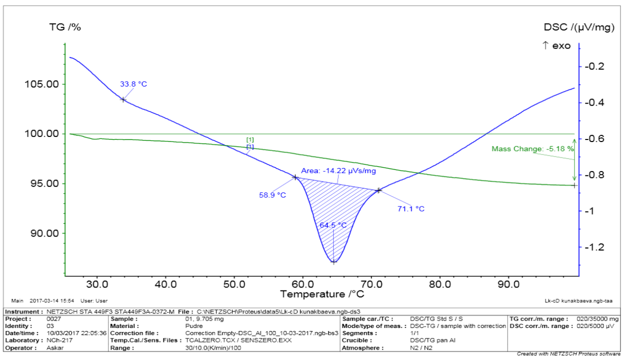


Figure 2 – TGA-DSC analysis of the inclusion complex of GP-β-CD and ALA obtained by grinding

On the ALA thermogram (figure 1, left), a narrow endothermic peak is observed at a temperature of 70.90 ° C, which characterizes the melting point of ALA. On the thermogram HP-β-CD (figure 1, right), a wide endothermic peak is observed with a maximum at 71.90C, which indicates intensive evaporation of the sorbed water inevitably present in hygroscopic substances. On the thermogram of a mixture of ALA and GP-β-CD (figure 2), the melting temperature of ALA is shifted to a region of lower temperatures (on 6.4 ° C), compared to the initial ALA, which is probably due to the interaction of ALA with the surface of GP- CD. The thermogram contains a small wide endothermic peak characterizing the evaporation of water sorbed by GP-β-CD from the environment. The interaction of ALA with the outer surface of GP-β-CD can be explained by the fact that GP-βCD is capable of forming a «cellular» (grid-like) structure due to hydrogen bonds between -OH groups. When the melting temperature of ALA (70.9 ° C) is reached, the ALA melt penetrates into the cells of this grid and, thus, stabilizes. Therefore, we can conclude that the presence of the small endotermic peak (figure 2) is probably due to the fact that it is possible, when the inclusion complex is formed, that unused crystals of GP-β-CD and ALA are preserved.

To confirm the formation of inclusion complex, the scanning electron microscopy method is also have been used. The investigation is based on point scanning of the surface of the investigated sample by a focused electron beam. Photomicrographs obtained with scanning electron microscopy using the Quanta 3D 200i Dual system, FEI at a voltage of 15 kV with an increase of 2000, 5000 for each sample, show that the shape and particles of the resulting inclusion complex of α-lipoic acid and hydroxypropyl-β-cyclodextrin are significantly different. Images of the substance GP-β-CD (Figure 3) show that the particles are large enough, porous, spherical. The ALA particles (Fig. 4) have rather smooth plate-shaped planar surfaces. A micrograph of the obtained ALK-GP-β-CD complex (Fig. 5) shows the change in the shape of the particles as a result of the mechanical treatment of the mixture of starting materials. The particles have an amorphous structure, which confirms the formation of the inclusion complex, i.e. the ALA molecule enteres the GC-β-CD cavity. But unreacted crystals of GP-β-CD are also preserved.

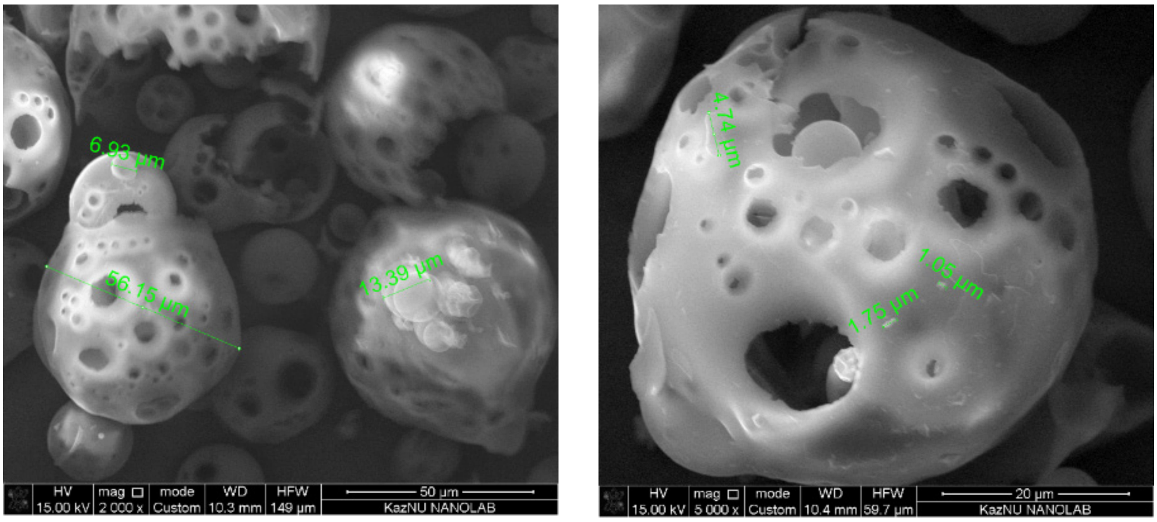


Figure 3 – Scanning electron microscopy of the substance hydroxypropyl-β-cyclodextrin at magnification 2000 (left) and 5000 (right)

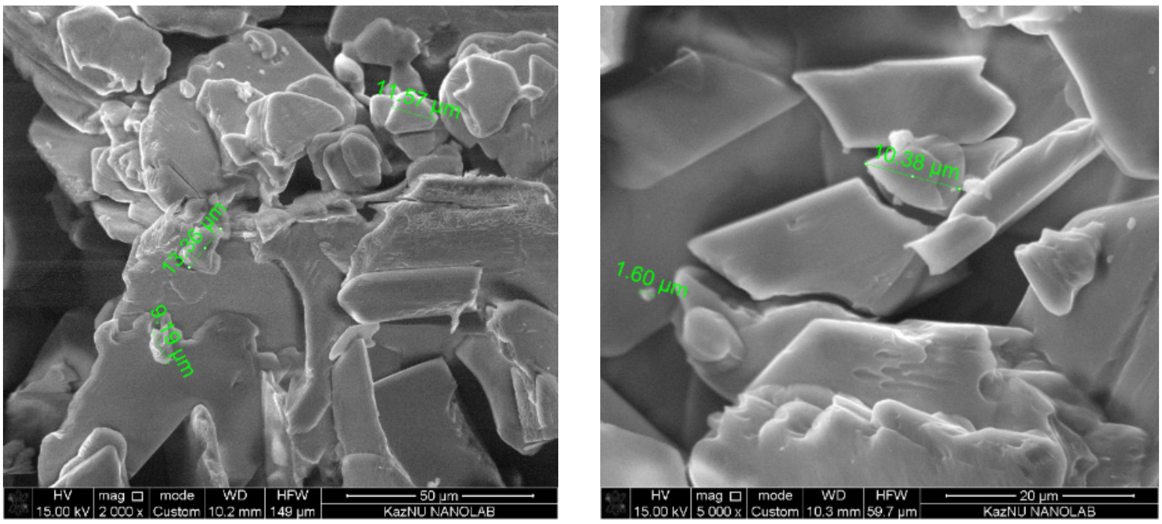


Figure 4 – Raster electron microscopy of α-lipoic acid substance at magnification 2000 (left) and 5000 (right)

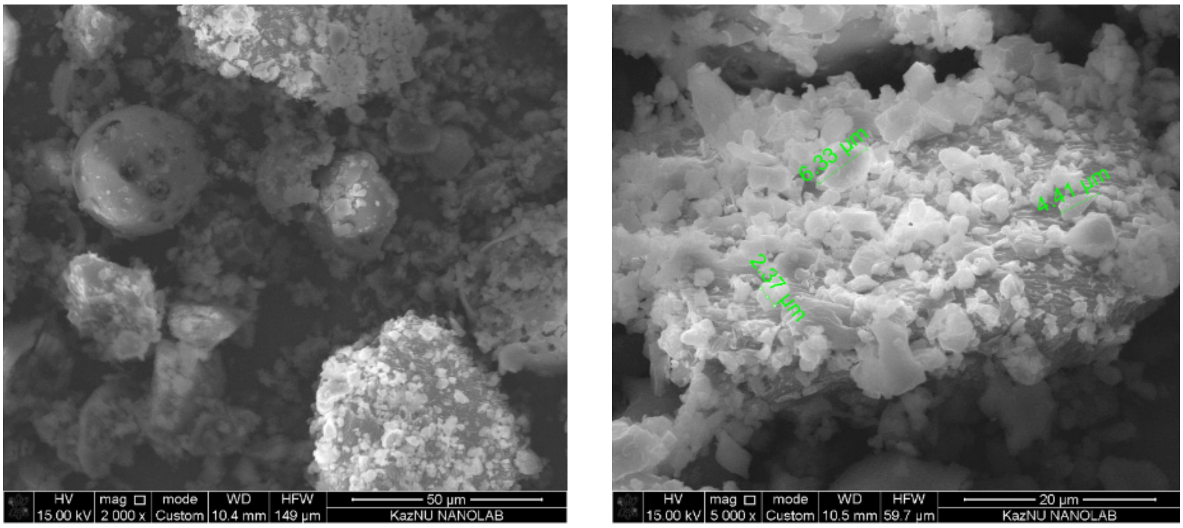


Figure 5 – Raster electron microscopy of the ALA-HP-β-CD complex obtained by grinding at magnification 2000 (left) and 5000 (right)

The solubility test of ALA and HP-β-CD complexes was carried out in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan I, vol. 1, 2.9.3, using a device with a blade. The dissolution medium is water, the volume of the dissolution medium is 500 ml, the temperature of the dissolution medium is 37 ± 0.5 ° C, the blade rotation speed is 100 rpm, the dissolution time is 5, 10, 15, 20, 30, 45 min. The determination was made by the method of absorption spectrophotometry in the ultraviolet and visible regions (SP RK, т. 1, 2.2.25) at a wavelength of 333 nm. The wavelength is determined by scanning a standard solution of α-lipoic acid in the wavelength range from 300 to 365 nm. According to the results obtained, the inclusion complex of ALA and HP-β-CD in a molar ratio of 1: 1 has a maximum solubility of 89% compared to other ratios (Figure 6).

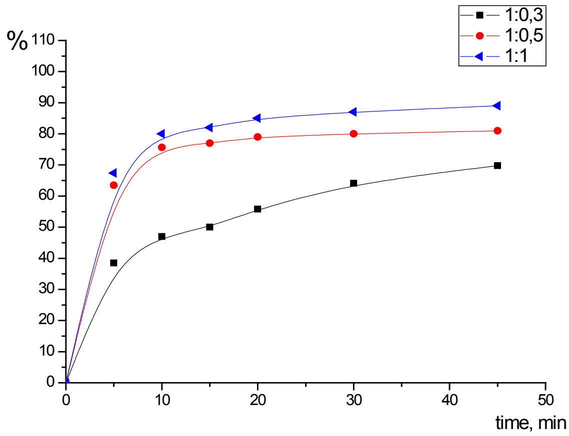


Figure 6 – Dissolution of the inclusion complex of HP-β-CD and ALA in distilled water (in the different molar ratio)

Investigation of the mechanism of interaction of lipoic acid and structurant (polymer) was carried out by differential-thermal analysis. To study the mechanism of interaction of drug substance a in lipoic acid gels based on carbopol, a linear heating of a substance of lipoic acid, pure gel bases and gels from lipoic acid in the temperature range from 20 ° C to 300 ° C in Al2O3 crucibles was carried out. The curves of differential thermal analysis (DTA) of the substance of lipoic acid, hydrogel base and gel of lipoic acid without auxiliary substances are presented at the figure. 7.

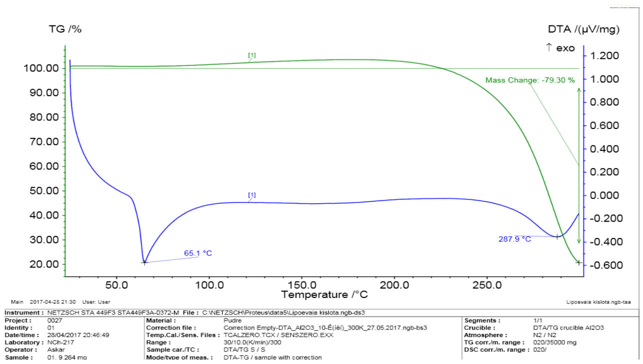


Figure 7 – Differential-thermal analysis curves (DTA) of the substance of lipoic acid

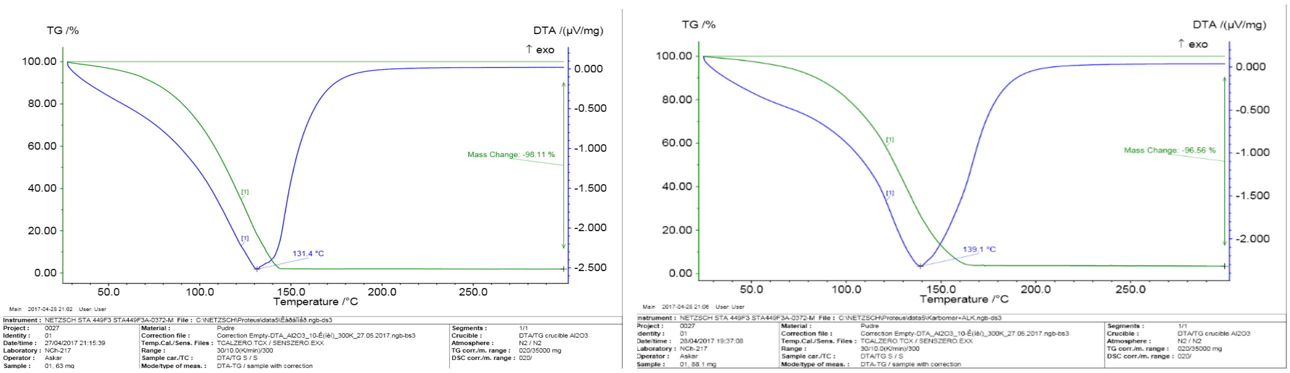


Figure 8 – Differential-thermal analysis curves (DTA) of the carbopol-based gel (left) and the carbopol gel with ALA (right)

According to DTA data presented at the figure 7, an endothermic peak is observed at 65.1 ° C, explained by the melting point of the substance, which agrees with the melting temperature of lipoic acid of 61 ° C. The endothermic peak at a temperature of 287.9 ° C indicates the decomposition of ALA with continuous heating, which is confirmed by the TGA curve dropping from 250 ° C. The 2nd curve of DTA shown at the figure 8 (on the left) shows that when the gel base obtained from carbopol is heated, an endothermic effect with has the maximum at a temperature of 131.40C, which may be related to the melting of the polymer and the desorption of water. The TGA curve of a pure gel base shows that, even at 142 ° C, only 2% of the total mass remains from the gel. The data of curve 3 of DTA (figure 8, right) characterizing the processes of heating the gel with a drug substance, have similarities with the processes of heating the gel base. Thus, endothermic processes are observed in the interval t = 20-190 ° C, which have only one maxima at T= 139.10 ° C, which can also be associated with processes of desorption of water, melting of the polymer. But the endothermic processes in the gel containing ALA shift by 7.7 ° C to the right than in the pure gel base, which is probably due to the action of ALA on the carbopol gel. Also, according to the TGA curve, 4% of the total gel weight remains from the gel at 162 ° C, and only 2% of the total gel weight remains at the pure base at 143 ° C, which is related to the content of ALA in the obtained ointment.

It is known that most of the ointments and gels under the influence of mechanical forces behave like elastic bodies that have reversible deformation. The process of applying the gel ointment and the efforts expended on the distribution of the gel on the skin are similar to the process occurring during the gel shift in the rheometer and the shear stress, which characterizes the material's resistance to shear deformations.

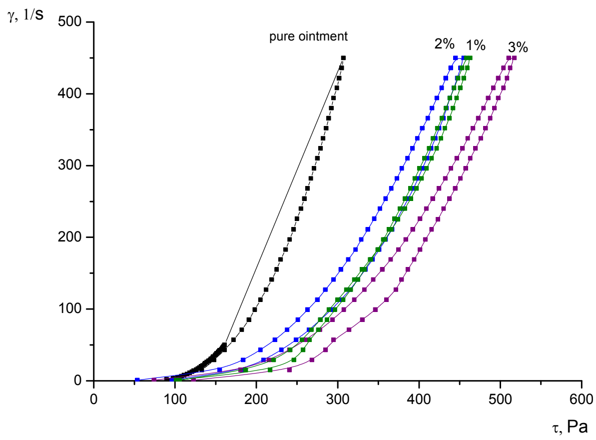


Figure 9 – Rheogram "Hysteresis loop" of hydrogel ointments based on carbopol with different concentrations of α-lipoic acid

To study the thixotropic properties of the studied samples of hydrogel ointments, the curves, obtained as a result of the deformation of these samples in the coordinates «shear rate-shear stress», were constructed. By the thixotropy is meant the property of a disperse system to change its structure under the influence of mechanical influences and to restore the former structure after the cessation of this influence. All hydrophilic ointments have pronounced thixotropic properties, which are expressed in the fact that with mixing the structure breaks down and the consistency changes at a constant temperature, it becomes almost liquid. If you remove the mechanical force, the strength of the structure is restored, but almost never reaches the original level. The obtained curves of the samples of hydrogel ointments (the so-called «flow rheograms») have a nonlinear character and are described by two lines in the «ascending and descending», forming the so-called «hysteresis loop».

The «upward» curve characterizes the destruction of the system and differs from the «descending» curve in that it characterizes the restoration of the system that preserves the residual deformation after a strong weakening of the structure under the influence of the previously applied stress. The presence of upward and downward curves (figure 9), forming hysteresis loops, indicates that the samples under study have thixotropic properties, which means that they are characterized by good spreadability and good extrusion ability from tubes.

The obtained results and the comparative analysis showed that the rheological characteristics shear stress are highest in 3% lipoic acid gel, and exceed both 1 and 2% lipoic acid gel. It was found, that the introduction of lipoic acid into the gel base in an amount of 1% of the weight of the gel, increased the value of the effective viscosity and the processes of structure formation in the system. All investigated samples of gels belong to thixotropic systems and. The rheological characteristics of the obtained ointments lie in the rheological optimum for hydrophilic ointments, which is characterized by a yield strength of 45-160 Pa.

The figure 10 shows the effect of temperature on the dynamic viscosity of hydrogel ointments obtained with ALA. It is seen, that with an increase in temperature from +3 to +260C, the noticeable decrease in viscosity is observed, which become constant at a temperature of 15-26 ° C. Hydrogel ointments on carbopol in the temperature range -4 – + 280^{0}C showed the stability of dynamic viscosity.

Investigation of α-lipoic acid release from hydrogel ointments and their transdermal penetration

The main barrier to penetration into the body of various substances is the skin, which is a multilayered complex system. Transdermal delivery of ALA is carried out by penetrating them through the upper layer of the skin – the dermis. Under the dermis there is a hypoderm consisting of adipose tissue, in which large blood vessels are located, as well as the bases of the hair follicles and sweat glands. On the basis of information on the structure of the skin, it can be concluded that transdermal delivery will be effective provided that the ALA, overcoming the epidermis, will enter into sufficient quantity in the dermis where the capillaries of the blood vessels are located.

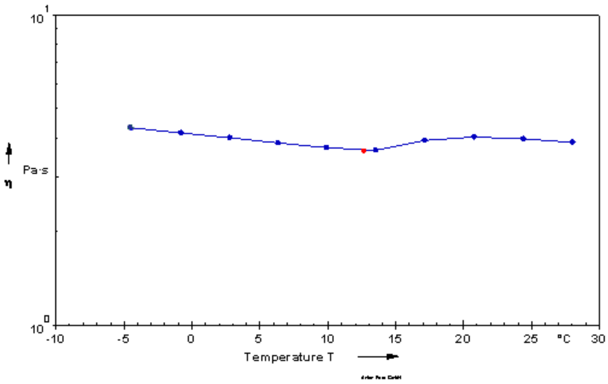
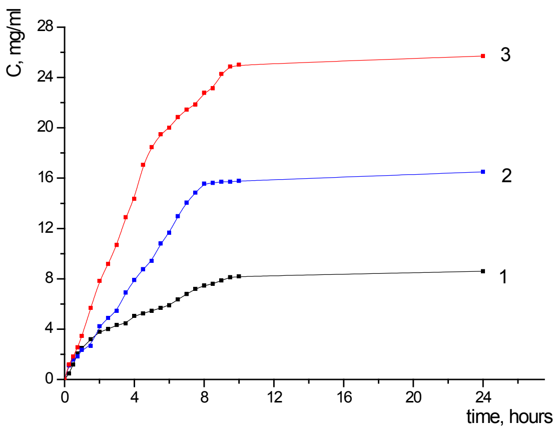
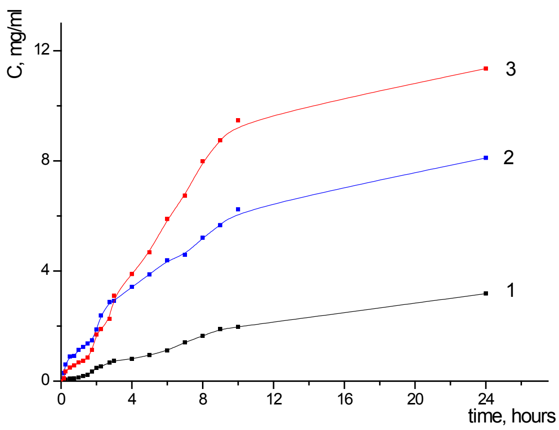


Figure 10 – Effect of temperature on the viscosity of a hydrogel ointment based on carbopol with 1% α-lipoic acid





(1) – 0,9% ointment of ALA, (2) – 1,8% ointment of ALA, (3) – 2,7% ointment of ALA

Figure 11 – Permeability of α-lipoic acid from the hydrogel ointment based on carbopol through a membrane

Figure 12 – Permeability of α-lipoic acid from the hydrogel ointment based on carbopol through pig skin

In vitro study of the release of ALA from the carbopol-based gels obtained with α-lipoic acid was performed using the Franz Diffusion Cell. Prior to operation, the membrane was kept in a phosphate buffer solution at pH 6.86. Pre-thawed pig ears, prepared and also kept in a buffer solution for 1 hour. According to the obtained results , which shown at figure 11-12, the gel base due to carbopol provides a prolonged effect. Thus, during the first 10 hours in all ointments, a prolonged release from the ointment base and penetration of ALA through the membrane are observed (Figure 11). After 10 hours of release of ALA from the ointment, the prolongation slows down and gradually the values reach the plateau. With the increase in the content of ALA in the composition of the ointment, the amount of released ALA increases. The maximum concentration of released ALA in 2.7% of the ALA ointment, which was 25.7 mg / ml. When studying the permeability of the obtained hydrogel ointments with ALA through the pig skin skin (figure 12), with an increase in the concentration of ALA in the composition of the ointment, the increase in the amount of penetrated substance through the skin was also observed. Compared with the membrane, the amount of penetrated ALA is less, since it is related to the structure of the skin. The cells of the skin layers prevent direct penetration of the ALA, which makes it difficult to move. So, 3.18 mg / ml of ALA penetrated from 0.9% of the ointment, which is less by 5.42 mg / ml compared to penetration through the membrane. Despite this, a prolonged effect of the ointment with ALA is observed within 24 hours.

Table 1 – Antimicrobial activity of the samples

Number of a sample	Chemical composition of samples	Diameter of growth inhibition zone of test microorganisms, mm	
		Staphylococcus aureus IMV 3316	*Escherichia coli* IMV 877
№1	A pure gel based on carbopol	0	0
№2	ALA solution	27	23
№3	Ointment based on carbopol with ALA	16	15

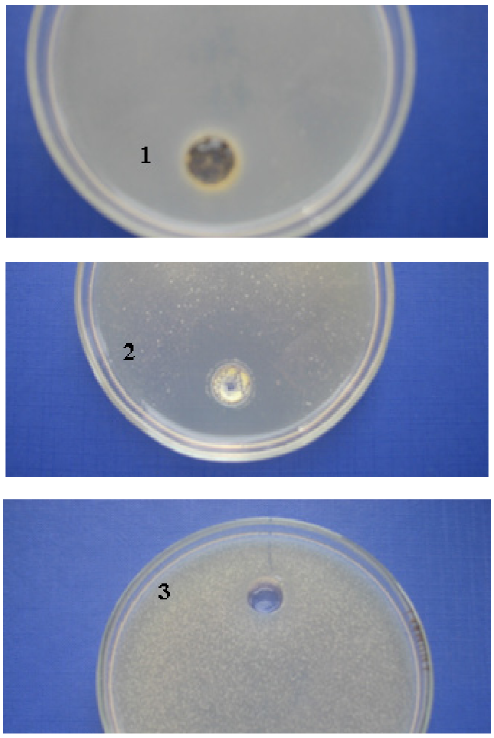


Figure 13 – Antimicrobial activity to Staphylococcus aureus IMV 3316

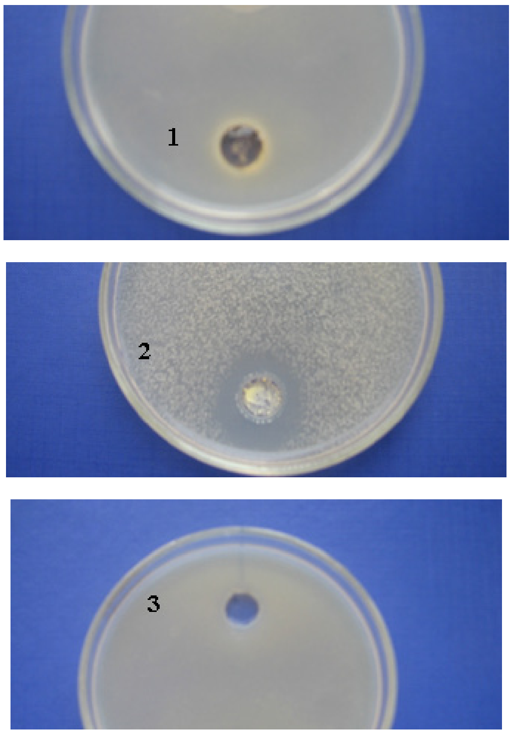


Figure 14 – Antimicribial activity to Escherichia coli IMV 877

The antimicrobial study of obtained hudtogel ointments with ALAwas made with respect to clinical opportunistic gram-positive and gram-negative pathogens of infections. The results are shown in the table 1. The presence of antimicrobial activity in samples № 2-3 with respect to clinical opportunistic gram-positive and gram-negative bacteria (Staphylococcus aureus, Escherichia coli) was established. No antibacterial activity was detected in the sample № 1-gel based on carbopol (figure 13-14).

Conclusion

Thus, the formation of the inclusion complex of α-lipoic acid with hydroxypropyl-β-cyclodextrin was studied, which provides an improvement in the solubility of ALA in water. Hydrogel ointments based on a slightly cross-linked polymer of acrylic acid-carbopol with α-lipoic acid have been developed. The rheological characteristics of the obtained hydrogel forms were studied. According to the obtained rheograms, all hydrogel ointments have pronounced thixotropic properties. The transdermal activity of the obtained hydrogel ointments with a different concentration of α-lipoic acid was studied, which showed that ALA ointments have a prolonged action, which ensures a slow release of the drug substance over a long period of time. The ointments with α-lipoic acid have antimicrobial activity in relation to Staphylococcus aureus and Escherichia coli. The transdermal characteristics were investigated using cellulose membrane and pig skin.

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