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BIOPHARMACEUTICAL RESEARCH AND QUALITY CONTROL PARAMETERS OF A COMBINED OINTMENT FOR THE HERPES TREATMENT

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Introduction. *Considering the etiology and pathogenesis, for the treatment of diseases caused by herpes viruses, it is advisable to use combined drugs that have a comprehensive effect on various links of the pathological process and expand the spectrum of pharmacological action of the drug.For the treatment of mono- and mixed herpesvirus infections, we have proposed a combined drug in the form of an ointment with acyclovir and miramistin.*

Purpose: *to substantiate the relevance of developing a combined ointment with acyclovir and miramistin, to determine the quality profile of the drug, the effect of excipients on rheology, and to evaluate the antiviral activity of the ointment.*

Materials and methods. *The object of the study was an ointment with acyclovir and miramistin. Vaseline oil, Paraffin, Propylene glycol, and Cetostearyl alcohol were used as the base of the combined ointment. The rheological properties of the ointments were studied using a Rheolab QC rotational viscometer (Anton Paar, Austria). The effect of the CSA concentration on the rheological characteristics was investigated by constructing a hysteresis loop and determining the dependence of the effective viscosity on the shear rate. The quantitative content of the active substances acyclovir and miramistin was determined by high-performance liquid chromatography. The in vivo antiviral activity of the ointment was studied in guinea pigs.*

Results. *As a result of the analysis of the pharmaceutical market of Ukraine, it was determined that monopreparations with acyclovir mainly dominate, most of which are in the form of tablets. The target quality profile of the combined ointment containing acyclovir and miramistin was determined, and critical indicators of the quality of the drug were assessed. The study of the rheological parameters of the ointment proved the possibility of regulating the ultimate static shear stress and dynamic viscosity by changing the CSA concentration, which is rational within the concentration range of 1%–3.5%. Studies of antiviral activity in vivo showed that the ointment is more effective than Zovirax cream and allows significantly reducing the duration of the disease caused by the herpes virus type 2.*

Conclusions. *For the treatment of mixed herpesvirus infections, the creation of a combined ointment with acyclovir and miramistin was justified, its antiviral effect was proven. The target quality profile, critical indicators were determined, and a method for quantitative analysis of acyclovir and miramistin was developed. The optimal composition of the ointment base was determined and its rheological properties were studied. In vivo studies showed that the use of the ointment 3 times a day significantly reduced the duration of the disease, and its effectiveness against HSV-II was confirmed in comparison with Zovirax cream.*

Keywords: *herpes, acyclovir, miramistin, ointment, technology, rheology, quality.*

БІОФАРМАЦЕВТИЧНІ ДОСЛІДЖЕННЯ ТА ПАРАМЕТРИ КОНТРОЛЮ ЯКОСТІ КОМБІНОВАНОЇ МАЗІ ДЛЯ ЛІКУВАННЯ ГЕРПЕСУ

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Вступ. *Враховуючи етіологію та патогенез, для лікування захворювань, спричинених вірусами герпесу, доцільно застосовувати комбіновані лікарські засоби, які комплексно впливають на різні ланки патологічного процесу та розширюють спектр фармакологічної дії препарату. Для лікування моно- та змішаних герпесвірусних інфекцій нами запропоновано комбінований препарат у вигляді мазі з ацикловіром та мірамістином.*

Мета: *обґрунтувати актуальність розробки комбінованої мазі з ацикловіром і мірамістином, визначити профіль якості препарату, вплив допоміжних речовин на реологію та оцінити противірусну активність мазі.*

Матеріали і методи. *Об'єктом дослідження була мазь з ацикловіром та мірамістином. У якості основи комбінованої мазі використовували: вазелінову олію, парафін, пропіленгліколь, цетостеариловий спирт. Реологічні властивості мазей досліджували на ротаційному віскозиметрі «Rheolab QC» (Anton Paar, Австрія). Вплив концентрації ЦСС на реологічні характеристики досліджено методами побудови петлі гістерезису та визначення залежності ефективної в'язкості від швидкості зсуву. Кількісний вміст діючих речовин ацикловіру та мірамістину визначали методом високоефективної рідинної хроматографії. Противірусну активність мазі in vivo досліджували на морських свинках.*

Результати: *В результаті аналізу фармацевтичного ринку України визначено, що в основному домінують монопрепарати з ацикловіром, серед яких більшість у формі таблеток. Визначено цільовий профіль якості комбінованої мазі, що містить ацикловір і мірамістин, та оцінено критичні показники якості препарату. Вивчення реологічних параметрів мазі довели можливість регулювати граничну статичну напругу зсуву та динамічну в'язкість за рахунок зміни концентрації CSA, що є раціональним у межах концентрації 1%–3,5%. Дослідження противірусної активності in vivo показали, що мазь більш ефективна за кремом Зовіракс та дозволяє значно скоротити тривалість захворювання, викликаного вірусом герпесу 2 типу.*

Висновки. *Для лікування змішаних герпесвірусних інфекцій обґрунтовано створення комбінованої мазі з ацикловіром та мірамістином, доведено її противірусну дію. Визначено цільовий профіль якості, критичні показники та розроблено метод кількісного аналізу ацикловіру та мірамістину (ВЕРХ). Визначено оптимальний склад мазевої основи та вивчено її реологічні властивості. Дослідження in vivo показали, що застосування мазі 3 рази на добу значно скорочувало тривалість хвороби, підтверджено її ефективність проти ВПГ-II у порівнянні із кремом Зовіракс.*

Ключові слова: *герпес, ацикловір, мірамістин, мазь, технологія, реологія, якість.*

Introduction. Human herpes viruses are widespread and according to the World Health Organization rank second among viral infections after the flu. These infections occur as mono-, mixed- and co-infections and can be asymptomatic, acute, chronic, as well as atypical chronic active infection [1-4].

Herpes, like many other diseases, is aggravated by stressful situations, especially in the context of ongoing martial law, as immunity deteriorates and this leads to the activation of latent herpes. War is often associated with malnutrition and poor hygiene. Psychological stress activates herpes. In war, people may be exposed to more frequent contact with people who are sick, which increases the risk of contracting herpes or spreading an existing virus. Poor living conditions, such as overwork, lack of sleep, and lack of access to clean water and food, can lead to a weakened immune system and, as a result, exacerbation of herpes.

Recently, the importance of mixed infections, in which the infection agents of the disease increase the influence of each other on the patient's body, is increasing. At the same time, suppression of cellular immunity reactions, sensitization to viral antigens and a long chronic disease process are observed [1, 2].

Taking into account the etiology and pathogenesis, for the treatment of diseases caused by herpes viruses, it is advisable to use combined medicinal products that have a comprehensive effect on various links of the pathological process and expand the spectrum of pharmacological action of the drug [1, 5, 8]. For the treatment of herpetic lesions of the skin and mucous membranes, the most effective is the use of soft drugs containing antiviral agents. Acyclovir is a synthetic acyclic analog of deoxyguanosine, a natural component of DNA, and today remains the "gold standard" of antiherpetic treatment [2, 9, 20].

On the pharmaceutical market of Ukraine there are antiviral drugs of the group D06B B Antiviral drugs. According to the ATC classification, antiviral agents are divided into: D06B B03 Acyclovir, D06B B06 Penciclovir, D06B B10 Imiquimod, D06B B11 Docosanol D06B B53 Acyclovir, combinations, D06B B16 Denotivir [10]. Pharmaceutical market of Ukraine invistigations are represented in "Research results" chapter.

With viral infections of the mucous membranes and skin their barrier function is reduced and a secondary infection often joins: bacterial, fungal, or mixed infections [1]. So for the treatment of mono- and mixed herpesvirus infections we proposed a combined drug in the form of an ointment with acyclovir and miramistin [13, 14, 16].

Ionic antiseptic Miramistin is highly effective against bacterial and fungal infections. Miramistin exhibits an antimicrobial effect on gram-positive and gram-negative, aerobic and anaerobic, spore-forming and asporogenic

microflora, including hospital strains with polyresistance to antibiotics and fungal microflora with resistance to chemotherapeutic drugs. This drug has high hyperosmolar activity, as a result of which it stops wound inflammation, absorbs purulent exudate and selectively dehydrates necrotic tissues [15, 16, 20].

The aim of the work was to determine the relevance of conducting research on the development of a combined ointment with acyclovir and miramistin, determine the quality profile of the drug, study the effect of excipients on the rheological parameters of the ointment, and present the results of studying the antiviral activity of a combined ointment with acyclovir and miramistin for the treatment of diseases caused by herpes viruses.

Research planning (methodology). When developing a new medicinal product, it is necessary to justify the stages of planning scientific research.

The first stage of research is information and search. This stage includes determining the feasibility of developing a medicinal product depending on the existing need, searching for literature data on the etiology, pathogenesis, clinical manifestations and methods of treatment of diseases caused by the herpes virus. Based on the received results are selected by active pharmaceutical ingredients, excipients and bases.

The second stage is research. Means for the treatment of herpesvirus diseases should ensure the passage of active substances through a semipermeable membrane to the cell. In view of this, it is necessary to conduct a study to determine the solubility of active pharmaceutical ingredients depending on the type of solvent. According to the results of microbiological studies, the optimal concentrations of active substances are chosen. At this stage, structural and mechanical studies of model samples are also carried out; thermostability and colloidal stability are studied stability of model compositions; osmotic activity was investigated.

They substantiate the scheme of the technological process of manufacturing the product, select the optimal equipment and establish the critical parameters of the process that must be controlled.

The third stage of research is standardization and pharmacological activity determination. This stage includes the study of the antiviral activity of the developed drug, the establishment of the shelf life in the storage process, the standardization of the medicinal product and the validation of quality control methods, as well as the approval of analytical and regulatory documentation.

Materials and methods. The object of the study was an ointment containing the active substances acyclovir (TEVA Pharmaceutical and Chemical (Hang-zhou) Co., Ltd, China) and miramistin (Farmlink Company, Ukraine).

The study was conducted in the period from 2020 to 2024. The pharmaceutical development for the treatment of herpes includes the active substances acyclovir and miramistin as well as auxiliary substances Vaseline oil, Paraffin, Propylene glycol (PG), Cetostearyl alcohol (CSA) [16].

The ointment preparation procedure consists of the following: Paraffin was melted at 65°C, Vaseline oil was added, then CSA was added and stirred for 5 minutes until complete dissolution. The components weighed on the scales in advance were added to the base in the following order: first, acyclovir was added, stirred for 5 min, then a solution of miramistin in propylene glycol was added, also stirred until completely dissolved for 5 min. Then homogenisation was performed. The result was a white ointment of homogeneous consistency.

The rheological (structural-mechanical) properties of the bases were determined with the means of “Rheolab QC” rotary viscometer by (Anton Paar, Austria) with coaxial cylinders CC27/S-SN29766 at the Department of Industrial Pharmacy, now the Department of Industrial Technology of Medicines and Cosmetics National University of Pharmacy, under the leadership of assoc. prof. Kukhtenko H. P.

The rheological parameters were studied at a temperature of 25±0.5ºC. The samples were thermostated using a thermostat MLM U15c.

The batch of sample weighed about 15.0±0.5 g was placed in the container of an external stationary cylinder, the required temperature of the experiment was set, the time of thermostating was 20 min. The device is equipped with RheoPlus 32 V3.62 software. Measurements of the rheological flow curve were performed in 3 stages:

1. Linear increase at the rate of shear velocity from 0.1 s^{-1} to 350 s^{-1} with 105 measurement points and duration of the measurement point is 1 s;
2. Constant shift at a speed of 350 s^{-1} for 1 s of duration;
3. Linear decrease at the rate of shear velocity from 350 s^{-1} to 0.1 s^{-1} with 105 measurement points and duration of the

measurement point for 1 s.

The range of the shear rate gradient 0.1-350 s^{-1} corresponds to the range speed of 0.075-270 revolutions per minute.

The device allows measuring the tangential bias voltage (τ) in the range 0.5-3.0 10^4 Pa, the gradient of the shear rate ($\dot{\gamma}$) from 0.1 to 4000 s^{-1}, the viscosity (η) is from 1 to 10^6 Pa sec.

The area of the hysteresis loop (A, Pa/s), points (limits) of flow (τ0, Pa) and viscosity at infinite shear rate (η∞, Pa·s) were calculated using the RheoPlus 32 V3.62 software.

Identification and quantification of the APIs of acyclovir and miramistin ointment was carried out under the supervision of prof. O. S. Nazarova, Biolik Pharma, Ukraine. The quantitative content of the active substances acyclovir and miramistin was determined by high-performance liquid chromatography (HPLC) using analytical equipment: "Waters 2487" chromatograph (USA), Sartorius BA-210S balance (Germany), class A measuring utensils. The following chromatography conditions were used: Waters Spherisorb CNRP chromatography column (4.6 x 250) mm, filled with a sorbent with a particle size of 5 μm. The mobile phase was a mixture of acetonitrile P - buffer solution pH 5.0 (50:50). The flow rate was 1.0 ml/min and the column temperature was 30°C. Detection at a wavelength of 265 nm. The standard samples of acyclovir and miramistin were used to prepare the reference solution [24].

The content of acyclovir or miramistin in 1 g of the drug, in milligrams, is calculated by the formula:

$$X_i = \frac{S_{1i} * m_{0i} * 50 * P}{S_{0i} * m_{1i} * 250 * 100} = \frac{S_{1i} * m_{0i} * P}{S_{0i} * 25000},$$

Where:

S_{1i} — the average value of the peak areas of acyclovir or miramistin calculated from the chromatograms of the test solution;

S_{0i} — the average value of the peak areas of acyclovir or miramistin calculated from the chromatograms of the reference solution;

m_{0i} — weight of acyclovir or miramistin sample, in milligrams;

m_{1i} — the weight of the drug sample, in grams;

P — the content of acyclovir or miramistin in the C3 of acyclovir or miramistin, %.

Validation of the quantitative determination of active substances was carried out according to the main validation characteristics: specificity, accuracy, precision (convergence), linearity, range of application. The predicted total uncertainty of the analysis results was also calculated [23, 24].

Studies on the validation of the method for the quantitative determination of acyclovir and miramistin were conducted in accordance with the document CPMP/ICH/381/95 (ICH Topic Q 2 (R1)) "Note for Guidance on Validation of Analytical Procedures: Text and Methodology" and the general text 'Validation of Analytical Methods and Tests' of the SPU [6, 22].

The tolerances of the content (B) of acyclovir and miramistin in the finished drug product during storage are ± 5 %, therefore, during the validation, the criteria for evaluating this method were parameters for B = 5.0 %, i.e. the maximum uncertainty of the analysis (Δ_{As}) should not exceed 1.6 %. The standardization of the content of $C_8H_{11}N_5O_3$ (acyclovir) in 1 g of the drug should be from 47.5 mg to 52.5 mg, $C_{26}H_{47}ClN_2O$ (miramistin) in 1 g - from 4.75 mg to 5.25 mg. The requirements for the parameters of the linear dependence of the method for determination of active substances are met.

The study of the antiviral activity of the active substances and ointment was carried out on the base of the laboratory of experimental chemotherapy of viral infections of the National Academy of Medical Sciences of Ukraine, State Institution "L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases" under the direction of prof. S. L. Rybalko.

Method for studying the antiviral activity in vivo of ointment containing acyclovir and miramistin.

Guinea pigs were infected with HSV-II virus (6.0 lgTCA50/ml) by applying virus-containing fluid to a scarified area of skin after anesthesia. The severity of infection was assessed by lesion area, edema, rash, and other symptoms on a 4-point scale for 10 days.

Treatment of animals was started 24 hours after infection with HSV-II. Zovirax 5% cream

(GlaxoSmithKline Pharmaceuticals S.A.) was used as a reference drug.

For the experiment, the animals were divided into 3 groups:

Group 1 - animals infected by HSV-II virus;

Group 2 - animals infected with HSV-II virus and treated by ointment containing acyclovir and miramistin;

Group 3 - animals infected with HSV-II virus and treated with the reference drug - Zovirax 5% cream.

The drug's effectiveness was evaluated at the peak of the pathological process, based on a reduction in the severity of clinical manifestations, a shortened duration of the illness, and the therapeutic action index (TAI) in the experimental groups compared to the control group. Each group consisted of 6 animals.

Statistical Analysis. Statistical processing of the experimental data was carried out in accordance with the requirements of SPU general monograph "Statistical analysis of the results of chemical experiments" by methods of mathematical statistics using Microsoft Office Excel and licensed STATISTICA® for Windows (StatSoft Inc.) [7].

Research results. On the pharmaceutical market of Ukraine by monopreparations containing acyclovir. As a result of the frequency analysis, it was established that on the pharmaceutical market of Ukraine by February 2025, medicinal products with the international non-proprietary name (INN) acyclovir were represented by 42 trade names (TN) in tablet form, powders for solution preparation, ointments and creams (Fig. 1) [11]. Combined products account for an insignificant share: LIPSTER® MINT cream 5% (Farmak JSC, Ukraine) and ZOVIRAX DUO cream, 2 g of cream in a tube (GlaxoSmithKline UK Limited, UK). [12].

Analysis of the pharmaceutical market of Ukraine for INN acyclovir by dosage forms (Fig. 1)

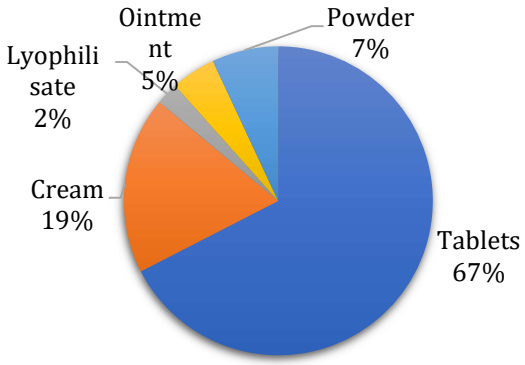

Fig. 1. Ratio of medicines containing acyclovir by dosage form

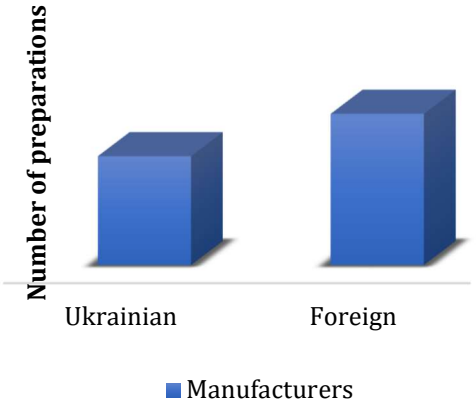

Fig. 2. Ratio of manufacturers on the Ukrainian pharmaceutical market for the medicines containing acyclovir

Ointments belong to dispersed systems with visco-plastic properties that affect production characteristics, therapeutic activity and consumer properties (appearance, application to skin and mucous membranes, extrusion from tubes). The effectiveness, technology, and convenience of using ointments depend on the rational choice of excipients as they affect the speed and completeness of the release of active substances and the therapeutic activity of the drug [17, 18].

Determination of the target quality profile of the medicinal product, which includes a list of the main indicators of the quality of the drug, is a key element in the planning of pharmaceutical development taking into account the process of its production.

First, the target quality profile of the drug under development was defined (Tab. 1) and the critical indicators of the drug quality were evaluated (Tab. 2) [19, 23].

Initially, ointment bases of various compositions, which are often used in modern pharmaceutical practice in the development of soft dosage forms, were investigated. The most common components include Vaseline oil, Paraffin, PG, CSA, which are used to create the basis of the ointment for the treatment of herpesvirus diseases [13].

Previous studies determined the content of Acyclovir (5%), Miramistin (0.5%), PG (4%), Vaseline oil (77%) and Paraffin (13.5%) [13, 14, 16, 21].

The composition of the studied samples is given (Tab. 3).

Determination of the dependence of the shear stress (τ, Pa) of the samples and the base on the gradient of the shear rate (γ , s^{-1}) was carried out according to the above method (Fig. 3).

Table 1

Target quality profile of ointment with acyclovir and miramistin

Determination of the goal by the quality of the product profile	Goal	Justification
Dosage form	Ointment	Corresponds to similar medicinal products registered in Ukraine
The composition of auxiliary substances	Vaseline oil, Paraffin, Propylene glycol, Cetostearyl alcohol	The composition corresponds to similar medicinal products registered in Ukraine
Application	For external use. The ointment should be used only for the treatment of herpes on the lips and face	The method of application is the same as for similar medicinal products registered in Ukraine
Dosage	Acyclovir - 50 mg/g Miramistin - 5 mg/g	The dosage is the same as for similar medicinal products registered in Ukraine
Type of packaging	5 g in a tube. 1 tube in a pack	Necessity to perform proper storage (safety and quality)
Quality characteristics	Description	Meets the requirements of the same or comparable standards as similar medicinal products registered in Ukraine
	Identification	
	Quantitative determination	
	Homogeneity	
	pH	
	Particle size	
	Related substances	
	Weight of package contents	
	Microbiological purity	
Storage conditions	Store in the original packaging at a temperature not higher than 25 °C. Do not freeze	Ensuring the quality of the drug during the shelf life
Stability	2 years	Ensuring the quality of the drug

Table 2

Target quality profile of the ointment with acyclovir and miramistin (description of the criticality of the quality indicators for the composition)

Indicators of the quality of the medicinal product	Goal	Criticality	Justification
Description	The ointment is white or almost white	Yes	A change in the indicator affects the safety of using the drug
Identification acyclovir and miramistin	On the chromatograms of the tested solution obtained in the "Quantitative determination" section, the retention time of the main peaks of acyclovir and miramistin should coincide with the retention time of these peaks on the chromatogram of the comparison solution	Yes	Significant influence on the effectiveness and safety of the drug
Quantitative determination	Acyclovir: ± 5% of the nominal value (at the time of production date and during the shelf life); Miramistin: ± 5% of the nominal value (at the time of production date and during the shelf life)	Yes	Variability in quantitative content affects safety and efficacy for the patient. Therefore, this indicator is defined as critical
Homogeneity	The preparation should be homogeneous	Yes	A change in the indicator affects the safety of using the drug
pH	From 5.0 to 8.5	Yes	When the optimal pH value of the finished product is established, the maximum stability of the medicinal

Indicators of the quality of the medicinal product	Goal	Criticality	Justification
			product during the life cycle of the drug is ensured
Particle size	In 10 fields of view of the microscope, the bulk of the particles must be no more than 50 μm in size; no more than 10 particles with a size of up to 100 microns and no more than 5 particles with a size of more than 100 microns are allowed	Yes	This indicator directly affects safety, so it was defined as critical
Related substances	Standardization in accordance with the requirements of the ISN Q3B guideline	Yes	The limit of decomposition products of the active substance has crucial importance for the safety of the medicinal product. The rationing of degradation products corresponds to the instruction of the ISN Q3B
Weight of package contents	The weight of the contents of each tube must be at least 5.0 g	No	It characterizes the quality of the technological process and does not affect the quality and safety of the drug
Microbiological purity	The total number of aerobic microorganisms (TAMC) is 100 CFU/g Total number of yeast and mold fungi (TYMC) - 10 CFU/g Absence of Staphylococcus aureus in 1 g Absence of Pseudomonas aeruginosa in 1 g	Yes	This indicator directly affects patient safety

Table 3

Composition of ointment samples

Components	The composition of the ointment sample, %						
	F1	F2	F3	**F4**	F5	F6	ointment base
Acyclovir	5,0	5,0	5,0	5,0	5,0	5,0	-
Miramistin	0,5	0,5	0,5	**0,5**	0,5	0,5	-
Vaseline oil	77,0	76,0	75,0	**74,5**	73,5	71,5	87,0
Paraffin	13,0	13,0	13,0	**13,0**	13,0	13,0	13,0
Propylene glycol	4,0	4,0	4,0	**4,0**	4,0	4,0	-
Cetostearyl alcohol	0,5	1,5	2,5	**3,0**	4,0	6,0	-

Figure 3 shows the introduction of SCS in a concentration of 6.0% (F6) is not rational, it leads to an increase in the energy that will be spent to destroy the structure of the ointment (limiting static shear stress) during homogenization, in addition, F6 has signs of delamination, as evidenced by dips in the curve when the shear rate increases. The appearance of the rheograms of F1 and F5 is very similar, although they differ in the values of τ. The obtained results show an almost complete similarity of the values of sample F2 (1.5%) and the base (PG and CSA are absent). The obtained results showed the similarity of the hysteresis curves of F2, F3 and F4.

A decrease in the concentration of CSA to 0.5% (sample F1) leads to a significant decrease in the shear stress necessary to break bonds and may indicate the tendency of the system to flow.

For further research, F4 with a 3% CSA concentration was chosen, the basis for choosing the sample for further research is its highest concentration among those selected, with the possibility of its further reduction if necessary. Fig. 4 shows the dependence of the shear stress of F4 on the shear rate gradient and the dependence of the structural viscosity on the shear rate gradient.

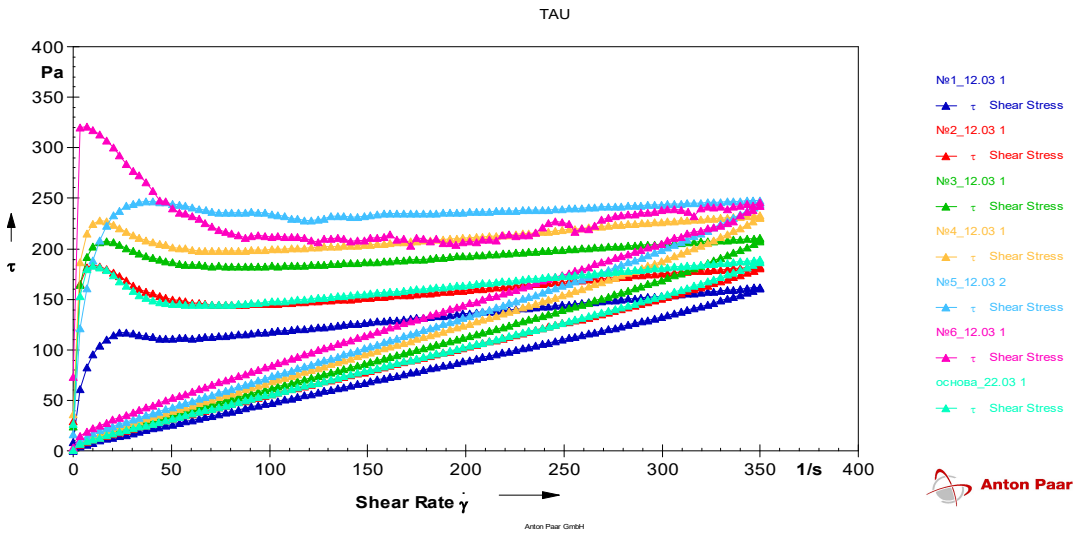

Fig. 3. Dependence of the shear stress (τ, Pa) of the samples on the shear rate gradient (γ, s^{-1})

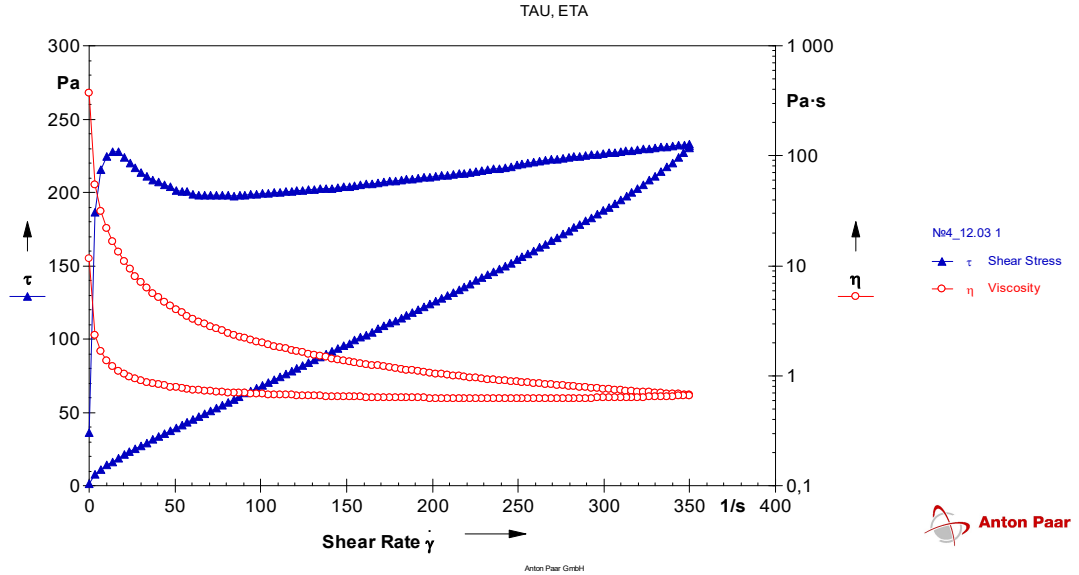

Fig. 4. Dependence of the shear stress (τ, Pa) of F4 on the gradient of the shear rate (γ, s^{-1}) and the dependence of the structural viscosity (η, Pa·s) on the gradient of the shear rate (γ, s^{-1})

The results in Fig. 4 indicate an increase in shear stress (τ = 228 Pa) to the shear rate (10.6 s^{-1}), which indicates the destruction of bonds, the resistance of the system gradually decreases to the value γ = 49,7 s^{-1}. With a further increase in the shear rate, a direct dependence were observed, characteristic of systems with a Newtonian type of flow, which indicates the complete destruction of the structure of the ointment. When the shear stress gradually decreases, the ointment shows an almost linear dependence of the shear stress on the shear rate, which indicates a very slow destruction of the structure. The value of structural viscosity (η) gradually and uniformly decreases with increasing shear rate. When the shear rate decreases, the system gradually recovers, as evidenced by a gradual increase in viscosity.

Developed ointment composition consisting of Acyclovir 5%, Miramistin 0.5%, Vaseline oil 74.5% and Paraffin 13%, Propylene glycol 4.0%, Cetostearyl alcohol 3.0% was used for quantitative determination of content and antiviral activity investigation.

For the standardization of pharmaceutical development, it is important to control the quantitative content of the active ingredients acyclovir and miramistin.

For the identification and quantification of APIs of the semi-solid medicinal forms (SSMF), the HPLC method was used, which allows separation of the components of the combined drug products and their simultaneous quantification and identification with high specificity and accuracy.

Validation studies using the acceptance criteria for a content tolerance of 5% confirmed the specificity, linearity, precision (accuracy) and correctness of the proposed method for the quantitative determination of acyclovir and miramistin.

A specification was developed for the quality control of the medicine [19, 23].

Previous studies conducted by the team of co-authors on the composition of the ointment containing acyclovir and miramistin confirmed the data on the antiviral activity of the proposed composition in comparison with the drug Zovirax [13].

When choosing a comparison drug, the analysis showed that the closest in composition and mechanism of action to the investigational drug is Zovirax cream 5%, 2 g in tubes, containing acyclovir and similar in composition to the ointment base. Zovirax is used to treat infections caused by herpes simplex virus that affect the lips and face [20].

Based on previously conducted studies, it was found that the use of a combined soft dosage form containing acyclovir and miramistin shows the effectiveness of the antiviral ointment on the model of genital herpes compared with the reference drug Zovirax and the control group.

In the control group (group 1) without the use of drugs, the duration of the disease was 10 days, which indicates the natural course of the infection. The severity of symptoms was estimated at 68.0 points, and the TAI is not given, since there is no treatment.

Treatment with the study drug (group 2) led to a significant reduction in the duration of the disease to 1 day, which is statistically significant ($P<0.05$). The severity of symptoms decreased to 4.0 points, and the TAI was 94.2%, indicating a high efficacy of the ointment.

Treatment with Zovirax 5% (group 3) reduced the duration of the disease to 3 days, which is also statistically significant ($P<0.05$). The severity of symptoms was 24.0 points, and the TAI was 64.7%, which is significantly lower compared to the ointment under study.

The use of Zovirax 5% in the study allowed to reduce the severity of symptoms, while the therapeutic effect was 64.7%, which is a statistically significant indicator [13].

Thus, the results of a previously conducted study confirm the significant advantage of the mild dosage form of the combination of acyclovir with miramistin compared to the reference drug Zovirax, providing a more pronounced therapeutic effect and reducing the duration of the disease [13].

Thus, the conducted studies have shown that the combination of acyclovir with miramistin are active inhibitors of HSV-II reproduction, and the drug developed on their basis is an effective drug in the treatment of herpesvirus diseases.

Discussion of research results. On the Ukrainian pharmaceutical market the largest number of dosage forms are solid – tablets 67%; creams 19% and ointments 5%, powders 7% and lyophilisate 2%. Among the manufacturers on the Ukrainian pharmaceutical market, foreign-made drugs dominate, accounting for 58% of the market for INN acyclovir drugs (Fig. 2). This indicates the relevance of developing a combined ointment with acyclovir and miramistin for the treatment of diseases caused by herpes viruses.

Rational selection of excipients affects the speed and completeness of the release of active ingredients and the therapeutic efficacy of the drugs. Obtained results of rheological studies showed it was possible to adjust the ultimate static shear stress and dynamic shear stress due to a change in the concentration of CSA, while the overall appearance of the hysteresis loop almost does not change. When the concentration of CSA decreases less than 3% the resistance of the system to the applied shear stress gradually decreases. Therefore, by introducing CSA into the composition of the ointment, its visco-elastic properties can be adjusted.

Similar studies of the influence of CSA on rheological parameters were conducted by other scientists, some parameters of the study were similar, including temperature, the differences were the use of simpler compositions. The results obtained correlate [25].

In our opinion, the concentrations of CSA of 1.5%, 2.5% and 3% turned out to be optimal in terms of their rheological indicators. Conducting further research will allow to establish the possibility of regulating the rheological parameters of the ointment in a concentration gradient from 1% to 3.5%.

The possibility of regulating the rheological parameters of the ointment in the gradient of CSA concentrations from 1.5% to 3.0% while keeping the general shape of the hysteresis loop curve is proven.

Quantitative joint determination of acyclovir and miramistin is proposed to be performed by liquid chromatography (SPU 2.2.29), which is highly selective and allows for their simultaneous determination, which is economically feasible [6].

For the quantitative determination of miramistin, acidimetry in a non-aqueous medium, biphasic titration with sodium lauryl sulfate and mercurimetry are used. Miramistin can be determined by bromothymol blue extraction photometry. However, these methods have some limitations in the analysis of SSMF. Based on the above, HPLC was used for the identification and quantification of the API of SSMF.

The use of the studied ointment allows to significantly reduce the duration of the disease to 1 day, which significantly exceeds the effectiveness of Zovirax (3 days) and the control group (10 days). It has been proven that the use of the combined ointment contributes to the reduction of the symptoms of the disease, which corresponds to the therapeutic index of 94.2%.

The TAI of the studied ointment reaches 94.2%, which is almost 30% higher than that of Zovirax (64.7%), which confirms its higher effectiveness.

The results obtained confirm that the studied ointment is a more effective treatment for genital herpes than Zovirax, and can be considered as a promising drug for further study and subsequent introduction into production.

Practical Relevance. The conducted research provides a theoretical basis for the pharmaceutical development of modern ointments, including those with antiviral and antimicrobial APIs. The obtained results can be used in the development of industrial technology of ointment for the treatment of diseases caused by herpes viruses, the selection of equipment, the mode of homogenization, etc.

Research limitations. Rheological studies were limited by the number of samples, the range of excipients and the temperature of 25±0.5ºC. Antiviral activity studies were limited to 18 animals.

Prospects for further research. The conducted research is the basis of further clinical studies with the aim of introducing into production a modern combined ointment containing acyclovir and miramistin.

Conclusions

1. The development of mixed infections necessitates the creation and introduction of combined drugs. For the treatment of herpesvirus mixed skin infections, the creation of a new drug in the form of an ointment with acyclovir and miramistin was theoretically and experimentally substantiated and its antiviral activity was pharmacologically proven.

2. When planning pharmaceutical development, the target quality profile of the drug was determined, its critical indicators were assessed. A method for quantitative determination of acyclovir and miramistin by HPLC was developed and tested.

3. Based on the rheological studies conducted, the composition of the ointment base was proven, consisting of Vaseline oil 74.5% and Paraffin 13%, Propylene glycol 4.0%, Cetostearyl alcohol 3.0%. The feasibility of introducing Cetostearyl alcohol into the composition of the excipient was proven, by changing its concentration from 1.5% to 3%, the viscoelastic properties of the ointment can be regulated.

4. In vivo antiviral activity studies showed that when the ointment was applied to guinea pigs 3 times a day the duration of the disease was significantly reduced, and the Index of therapeutic action was 94.2%. The in vivo antiviral activity against HSV-II of the ointment containing acyclovir 5% and miramistin 0.5% was proven in comparison with Zovirax cream.

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