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STUDY OF SOME PROPERTIES OF HYDROPHILIC OINTMENT BASES DEPENDING ON THEIR COMPOSITION

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The aim. *To study the properties of multicomponent hydrophilic ointment bases.*
Materials and methods. *The study focused on hydrophilic bases with varying formulations and ointments containing ofloxacin. The rheological properties of the bases were studied using rotational viscometry, and water absorption and ofloxacin release were investigated using diffusion through a semipermeable membrane. The water content was determined by the semi-micro method, and the ofloxacin content was determined by liquid chromatography. Four spin probes were utilized in the experiment, and the EPR spectra of these spin probes in a mixed solvent PG – M400 and bases were obtained. The type and parameters of the EPR spectra were evaluated. Surface tension was measured using the maximum bubble pressure method, and antibacterial activity was assessed by the agar diffusion method.*
Results. *The rheological parameters of hydrophilic bases are contingent on the ratio between macrogol 1500 (M1500) and poloxamer P338 (P338), as well as between macrogol 400 (M400) and propylene glycol (PG), water content, temperature, and shear stress. It was demonstrated that P338 increases the surface-active properties of bases. The water absorption capacity of the base containing solely a mixture of macrogols is approximately 1.2 times higher than that of the base, which also contains P338 and PG. The release rate of ofloxacin is shown to increase with an increase in PG content, but it is unaffected by the replacement of Proxanol 268 with P338. The incorporation of macrogol 20 cetostearyl ether (M20CSE) and cetostearyl alcohol (CSA) markedly retards water absorption and the release of ofloxacin, and also increases the rheological parameters of the bases. It was demonstrated by the spin probe method that, within a non-aqueous medium, no aggregates are formed from molecules of P338, as well as surfactant and CSA molecules. The PG content affects the growth inhibition zones of P. aeruginosa. The antibacterial efficacy of ointments containing fluoroquinolones against resistant clinical bacterial strains was found to be enhanced by hydrophilic bases.*
Conclusions. *The rheological parameters of hydrophilic bases can be controlled by modifying the ratio between consistency factors and dispersion medium components, by varying water content, temperature, and shear stress, as well as by adding surfactants and CSA to their formulations. Hydrophilic bases are able to absorb water and they promote the release of ofloxacin. Surfactant and CSA have a significant impact on these processes, reducing their rates. The formation of aggregates from P338 molecules, as well as molecules of surface-active substance and CSA, was not observed in the hydrophilic bases. PG, when incorporated into hydrophilic ointments containing ofloxacin, enhances their antibacterial efficacy*

Keywords: *hydrophilic base; ointment; rheological parameter; absorption; release; EPR spectrum parameter, growth inhibition zone*

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1. Introduction

Semi-solid preparations for topical application are of paramount importance in dermatology, surgery, combustiology, oncology, and other fields of medicine [1, 2]. They are intended for application to the skin in order to deliver active substances, thereby providing either local or systemic action, or a softening or protective effect [3, 4].

Ointments are defined as semi-solid preparations for topical application, consisting of a single-phase base in which solid or liquid substances can be dispersed. Ointments can be classified as hydrophobic, water-emulsifiable, or hydrophilic [3–5]. Hydrophilic ointments are preparations consisting of a base that mixes with water and typically contains a mixture of liquid and solid polyethylene glycols (PEG) (macrogols [3]). These ointments may contain some amount of water [3, 4, 6, 7].

Hydrophilic ointments based on macrogols 400 and 1500 (M400 and M1500) are most commonly used for the local treatment of purulent wounds in the inflammatory phase [8]. Specifically, this is the medicinal product Levomekol ointment [9].

Research is being conducted worldwide on the development of PEG-based ointments. For instance, a study of a combined preparation in the form of a PEG-based ointment containing 0.1% isohydrafural and 4% methyluracil, as well as 76.72% PEG 400 and 19.18% PEG 1500, was conducted, as outlined in the paper [10].

PEG-based ointments should be used in the inflammatory phase, as such bases absorb purulent exudate due to their high osmotic activity. However, during the repair phase, macrogols have been observed to exert a detrimental effect on the granulation process. Therefore, the application of ointments comprising alternative substances with considerably diminished osmotic activity becomes necessary. Consequently, the addition of methyluracil, as a stimulant of repair processes, to PEG-based ointments for local treatment of wounds in the inflammatory phase is not advisable [8].

The development of formulations comprising a combination of norfloxacin and metronidazole, with PEG base and varying gel bases, is outlined in [11]. This combination of active substances has been demonstrated to broaden the spectrum of the antibacterial action of the medicinal product [8].

In vitro experiments have demonstrated that macrogol-based ointments exhibit a superior water absorption capacity in comparison to emulsion-based ointments. The modification of base type is essential during local treatment of bedsores (pressure ulcers) [12]. The incorporation of cetostearyl alcohol (CSA) and colloidal surfactants (SAS) into the composition of hydrophilic bases is a pivotal factor for the modification of rheological parameters of hydrophilic bases. Furthermore, the incorporation of these excipients has been shown to influence the kinetics of propylene glycol (PG) and M400 release from these bases, as well as water absorption in *in vitro* experiments [13]. It has been established that in *in vivo* experiments on wound models in mice, PEG bases, compared to emulsion bases, contribute to the manifestation of therapeutic effects. This phenomenon can be attributed to two primary factors: firstly, the absorption of water, and secondly, the formation of extracellular matrices between the hyaluronan and macrogols [14]. The effect of ointment bases (including PEG bases) on skin wound healing in streptozotocin-induced diabetic rats has been studied. It was concluded that using a PEG base is important, as it promotes wound drying [15]. It has also been demonstrated that PEG-based ointment significantly accelerates the healing of skin wounds in rats compared to hydrophobic ointment and aqueous gel. The number of fibroblasts and the level of epidermal growth factor in skin treated with PEG-based ointment were significantly higher than in untreated skin [16]. It has been demonstrated that ozonated macrogol ointment exhibits pharmacological effects without cytotoxicity at low concentrations. This ointment, in a dose of 0.5 ppm, significantly elevated Saos-2 cell proliferation, type 1 collagen production, and ALP activity [17].

The antimicrobial activity of *Cymbopogon martini* essential oil was most evident in ointments based on a mixture of macrogols [18]. A PEG base was found to be best suited to the development of ointments containing the natural immunomodulator *Alnus sibirica* extract [19]. The development of a photostable ointment containing 0.13% silver chloride in a PEG base, and the subsequent evaluation of its antimicrobial activity, are outlined in the paper [20]. An ointment containing *Tridax procumbens* extract on a PEG basis provided a positive effect on wound healing in rats, as demonstrated in *in vivo* experiments [21]. PEG ointment could improve the imiquimod-induced psoriasis-like inflammation by down-regulating the functions of T helper 17 cells and myeloid-derived suppressor cells [22].

As demonstrated in [23], the use of PEG-based ointments, which exhibit both antimicrobial properties and high osmotic activity, is necessary for local wound treatment.

The research team proposed the manufacture of PEG-based ointments using hot-melt extrusion technology [24].

Research on hydrophilic ointment bases is mainly focused on the use of PEG bases for the development of various medicinal products, with a particular emphasis on the preparations for the topical treatment of wounds. Additionally, research is directed towards the comparison of the properties of PEG bases with those of other types of bases. It is important to acknowledge that, in addition to its advantageous properties, the PEG base exhibits certain disadvantages. In particular, these are: low bioadhesion, dehydration of granulations, and the absence of penetration enhancers with low molecular weight, which would facilitate the penetration of active substances into biological objects.

Poloxamers, which are block copolymers of ethylene oxide and propylene oxide, are considered to be promising excipients for hydrophilic ointments [25]. Poloxamers are non-ionic surfactants that exert a positive influence on the bioadhesive characteristics of hydrophilic ointment bases [26].

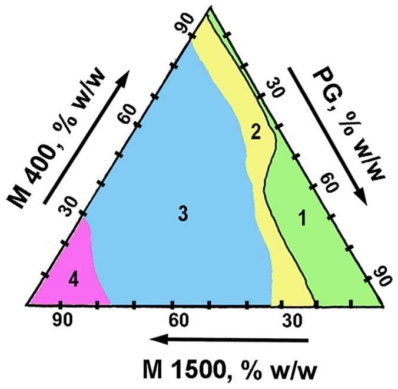
Research has been conducted in Ukraine on the development of hydrophilic ointment bases containing poloxamer 268, M400, and PG [27] (Table 1, Fig. 1).

Table 1

Surface areas for systems presented in triangular diagrams (Fig. 1)

Constituents	Surface area, %			
	1	2	3	4
M1500 – M400 – PG	18.7	14.5	59.6	7.2
Poloxamer 268 – M400 – PG	3.3	14.8	31.0	50.9

The consistency of the resultant systems is dependent on the composition of excipients in the M1500 – M400 – PG and Poloxamer 268 – M400 – PG systems. Incorporating PG into the composition of PEG bases allows for a considerable degree of variation in their formulation, as demonstrated by the substantial surface area exhibited by the hydrophilic ointment base (No. 3 in diagram *a*). Using poloxamer 268, it is also possible to obtain ointment bases containing a mixture of M400 and PG. Surface area for hydrophilic ointment base (No. 3 in diagram *b*) is 31.0% [27]. The release of M400 and PG from mixed solvents and hydrophilic bases occurs in different ways [28, 29], which may allow the release of active substances to be controlled.



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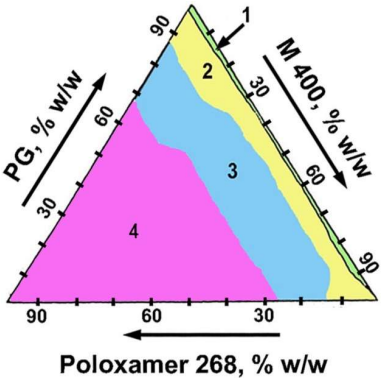

b

Fig. 1. Triangular diagrams of the studied systems at a temperature of 25°C: *a* – M1500 – M400 – PG system; *b* – Poloxamer 268 – M400 – PG system; 1 – transparent liquid; 2 – turbid unstable gel; 3 – hydrophilic ointment base; 4 – solid

A range of hydrophilic-based ointments containing poloxamer 268, M400, and PG have been developed for the topical treatment of purulent wounds in the inflammatory phase, in particular, Oflocain-Darnitsa® ointment, Miramistin-Darnitsa® ointment, etc. [9]. However, Poloxamer 268 was withdrawn from the Ukrainian market, which resulted in the necessity to replace it in formulations with one of the pharmacopoeial poloxamers. In terms of hydrophilic-lipophilic properties and molecular weight, P338 is the most similar to poloxamer 268 [3]. Of interest was the use of a mixture of M1500 and P338, which are both consistency factors, as well as M400 (M_r ~400) and PG (M_r 76.1), which together form the dispersion medium of these bases.

This study **aimed** to examine the properties of multicomponent hydrophilic ointment bases.

2. Planning (methodology) of the research

The study aimed to investigate the rheological properties of hydrophilic ointment bases by rotational viscometry. The rheological properties were studied depending on the following factors:

– the mass ratio between the content of P338 and M1500, with the constant content of M400 and PG;

– the mass ratio between M400 and PG, with the constant content of P338 and M1500;

– temperature ranging from 25°C to 45°C;

– water content in the hydrophilic bases;

– shear stress and mixing time.

The study was designed to ascertain the structure of hydrophilic bases through the utilization of rotational viscometry and spin probes.

The investigation focused on the water absorption and surface-active properties of M1500 and P338 solutions, along with the osmotic properties of ointment bases, particularly their capacity to absorb water and release ofloxacin (assessed using an *in vitro release test* – IVRT) [30, 31]. Additionally, the study examined the release of ofloxacin in a scenario where poloxamer 268 was substituted with P338.

The diameters of growth inhibition zones of the standard strain of *P. aeruginosa* by ofloxacin ointments on hydrophilic bases were studied depending on the mass ratio between M400 and PG. Furthermore, the effect of the hydrophilic base on the antibacterial efficacy of levofloxacin ointment and moxifloxacin ointment against clinically resistant bacterial strains was also investigated.

3. Materials and methods

The following excipients were used in the experiments:

– macrogol 1500 (Pluracare® E 1500 Flakes) – M1500;

– poloxamer 338 (Kolliphor® P 338) – P338;

– propylene glycol (Kollisolv® PG) – PG;

– macrogol 400 (Kollisolv® PEG 400) – M400;

– macrogol cetostearyl ether 20 (Kolliphor® CS 20) – M20CSE;

– cetostearyl alcohol (Kolliwax CSA 50) – CSA, all of these materials were produced by BASF.

Purified water (hereinafter referred to as water) was also used. Pharmacopoeial grade excipients (Ph. Eur.) were used for the experiments [3].

The compositions of ointment bases under study are presented in Tables 2–4.

Table 2

Compositions of ointment bases with different contents of non-aqueous solvents PG and M400, with constant total content of P338 and M1500

Constituents	Content, % m/m:					
	No. 1.1	No. 1.2	No. 1.3	No. 1.4	No. 1.5	No. 1.6
P338	5.0	5.0	5.0	5.0	5.0	5.0
M1500	20.0	20.0	20.0	20.0	20.0	20.0
PG	75.0	60.0	45.0	30.0	15.0	–
M400	–	15.0	30.0	45.0	60.0	75.0

Table 3

Compositions of ointment bases with different contents P338 and M1500, with constant total content of mixed non-aqueous solvent *PG* – *M*400

Constituents	Content, % m/m:					
	No. 2.1	No. 2.2	No. 2.3	No. 2.4	No. 2.5	No. 2.6
P338	–	5.0	10.0	15.0	20.0	25.0
M1500	25.0	20.0	15.0	10.0	5.0	–
PG	45.0	45.0	45.0	45.0	45.0	45.0
M400	30.0	30.0	30.0	30.0	30.0	30.0

Table 4

Compositions of ointment bases with different contents of water

Constituents	Content, % m/m:				
	No. 3.1	No. 3.2	No. 3.3	No. 3.4	No. 3.5
P338	5.0	5.0	5.0	5.0	5.0
M1500	20.0	20.0	20.0	20.0	20.0
PG	45.0	43.5	42.0	40.5	39.0
M400	30.0	29.0	28.0	27.0	26.0
Water	0	2.5	5.0	7.5	10.0

The following parameters were subjected to variation in the test samples: the mass ratio between PG and M400 at a constant content of P338 and M1500 (Table 2), the mass ratio of P338 and M1500 at the same composition of the mixed non-aqueous solvent *PG – M400* (Table 3), as well as the water content, which was increased by reducing the total content of PG and M400 (Table 4).

Reference base No. 1.7 was composed of 20 % m/m M1500 and 80 % m/m M400. The aqueous solutions of P338 and M1500, as well as the mixed solvents *PG – M400*, the composition of which is delineated in the text, were utilized for the study.

The ointment base No. 2.7, which contained 5.0% P338, 20.0% M1500, 42.0% PG, 28.0% M400, 1.5% M20CSE, and 3.5% CSA, was also examined. The rheological properties, water absorption, and the release of the studied drug substance (ofloxacin) could be impacted by the presence of surfactants and CSA in the composition of this base [13]. Surfactants and CSA in the composition of this base could potentially impact its rheological properties, water absorption, and the release of the studied drug substance (ofloxacin) [13].

The ointment bases were obtained by melting the components at a temperature of 65–70°C with stirring/homogenization, degassing the molten base, and cooling it while stirring to a temperature of ~25°C.

In the course of *in vitro* experiments, the release of ofloxacin was studied using experimental ointments containing 0.1% ofloxacin [3] in bases No. 1.3 and No. 1.5, in base No. 2.7, as well as two samples of the drug Oflocain-Darnitsa® ointment: one sample contained Proxanol 268, and the other contained P338.

The rheological properties of the experimental samples were examined at different temperatures using a rotational viscometer «Rheolab QC» with coaxial cylinders CC-27 («Anton Paar GmbH»; RHEOPLUS software, version 2.66). The flow behaviour, the apparent viscosity (η), the yield stress (τ_0), and the hysteresis loop area (A_N) were determined from the rheograms. The following equation was utilized to determine the thixotropy index (λ_T):

$$\lambda_T = \eta_2/\eta_1, \quad (1)$$

where η_1 is the initial apparent viscosity at 25°C; η_2 is the apparent viscosity at 25°C after stirring the ointment base at a rotation speed of 10 rpm for 30 minutes and then holding without agitation for 24 h.

The dynamic viscosity (η) of the mixed non-aqueous solvent *PG – M400* (6:4) was calculated using the formula [3, 4]:

$$\eta = v \times \rho, \quad (2)$$

where v is kinematic viscosity; ρ is density.

The kinematic viscosity (v) of the mixed solvent was determined using a Ubbelohde viscometer. Using a DMA 500 densitometer (Anton Paar GmbH; software version V1.003), the density (ρ) of the solvents was measured to calculate the dynamic viscosity and interfacial tension (γ).

The absorption of water by solutions and bases, as well as the release of ofloxacin from ointments, were determined in *in vitro* experiments. These experiments employed dialysis through a semi-permeable cellulose membrane at (32.0±0.5)°C, utilizing a vertical diffusion cell system HDT 1000 (Copley Scientific, UK) [30, 31]. A quantity of 0.5 g of solution or ointment base or ointment was placed in the donor chamber, resulting in a contact area with the membrane of 1.00 cm^2. The volume of receptor medium (water) was 6.6 ml, and the stirring frequency was set at 600 rpm.

The water content absorbed by the solution or ointment base was determined at specific intervals per unit of membrane area (1 cm^2). The water content in the donor chamber was determined by the semi-micro method [3] using an automatic titrator type 870 KF (Metrohm AG; software Firmware 58700025).

The quantity of ofloxacin (μg) released into water per unit of membrane area (1 cm^2) was determined. The volume of the dialysate sample was 0.3 ml. The quantification of ofloxacin in the dialysate was conducted by liquid chromatography, employing the analytical procedure described below that has been previously validated.

The rate of water absorption or ofloxacin release, linearity (R), coefficient of determination (R^2), cumulative content (A) (after 6 hours), and level of water absorption or recovery for ofloxacin (after 6 hours) were calculated. USP acceptability criteria were used to assess the equivalence of ofloxacin release from two different ointments in *in vitro* experiments [32].

Analytical procedure for quantitative determination of ofloxacin in receptor medium.

Buffer solution pH 2.5. Dissolve 2.06 g of *lithium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust the pH of the solution to (2.5±0.05) with *phosphoric acid R*, then dilute to 1000 mL with *water for chromatography R*.

Test solution. Sample of dialysate from the receptor chamber.

Reference solution. Dissolve 25.0 mg of ofloxacin (*Ofloxacin BP CRS, cat. No.* 1288) in *water R* and dilute to 1000 mL with the same solvent (25 μg/mL).

The analytical instrument employed was a Shimadzu Prominence-i LC-2030C 3D liquid chromatograph with a diode array detector (Shimadzu; software: LabSolutions Lite version 5.82).

Chromatographic conditions were as follows:

1. Column: LiChrosper RP select B (Supelco, cat. No. 150981);

– size: 4.0 mm × 125 mm;

– stationary phase: *silica gel for chromatography, octylsilyl R* (5 μm);

2. Mobile phase: *buffer solution pH 2.5 – methanol R* (70:30, v/v);

3. Flow rate: 1.0 ml/min;

4. Detection: at 290 nm;

5. Injection: 20 μl;

6. Run time: 5 min;

7. Temperature: 40°C.

The validity of this analytical procedure was demonstrated by the results of validation studies, which indicate its compliance with the requirements of the State Pharmacopoeia of Ukraine [4]. The limit of quantitative determination of ofloxacin in dialysate, as determined by the results of the linearity investigation, is 0.72 μg/ml.

Electron paramagnetic resonance (EPR) spectroscopy was employed to analyze the microstructure of the systems [33]. The following spin probes were utilized:

– **probe 1**: 4-hydroxy-TEMPO (M_r 172.24; CAS: [2226-96-2]);

– **probe 2**: 4-Palmitamido-2,2,6,6-tetramethylpiperidine-1-oxyl (M_r 409.67; CAS [22977-65-7]);

– **probe 3**: 5-Doxyl Stearic acid, ammonium salt (M_r 401.61; CAS: [2315262-05-4]) (5-DSA, NH_4 salt);

– **probe 4**: 16-Doxyl Stearic acid (M_r 384.57; CAS [53034-38-1]) (16-DSA).

The spin probes were added to the studied systems at a concentration of 10^{-4} mol/l. The EPR spectra were recorded at 25°C, 32°C and 37°C using the ESR Spectrometer CMS8400 («Adani»; software EPRCMD).

EPR spectra were used to determine the peak heights at the low-field (h_{+1}), central (h_0) and high-field (h_{-1}) components, as well as the linewidth at the low-field (ΔH_{+1}) and central (ΔH_0) components. The rotational correlation times of the spin probes (τ_{-1}, $\tau_{\pm1}$) and the anisotropy parameter (ε) were calculated using the following equations:

$$\tau_{-1} = \left(\sqrt{\frac{h_0}{h_{-1}}} - 1\right) \cdot \Delta H_0 \big/ 3.6 \cdot 10^9; \quad (3)$$

$$\tau_{\pm1} = \left(\sqrt{\frac{h_{+1}}{h_{-1}}} - 1\right) \cdot \Delta H_{+1} \cdot 6.65 \cdot 10^{-10}; \quad (4)$$

$$\varepsilon = \frac{\sqrt{h_0/h_{+1}} - 1}{\sqrt{h_0/h_{-1}} - 1}. \quad (5)$$

According to Stokes' equation, the rotational correlation time (τ) is directly proportional to the viscosity (η) of the local microenvironment of the spin probe and inversely proportional to the absolute temperature (T)

$$\tau = \left(4 \cdot \pi \cdot R^3 \cdot \eta\right) \big/ \left(3 \cdot k \cdot T\right). \quad (6)$$

The A_N constant, which characterizes the polarity of the radical's environment, in the case of triplet spectra of the spin probes 1, 2 and 4, was determined as the distance (in mT) between the central and high-field components. In the case of the EPR spectra for probe 3, the order parameter (S) was calculated after determining the hyperfine splitting constants $A_{\parallel}$ and $A_{\perp}$ according to the following equation

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}} \times 1.66. \quad (7)$$

The surface-active characteristics of 5% aqueous solutions of M1500 and P338 were studied. The surface tension (σ) at the liquid/air interface was determined by the maximum bubble pressure method, and the interfacial tension (γ) at the liquid/liquid interface was determined using a stalagmometer based on the mass and volume of the drop at a temperature of 32°C. Liquid paraffin was utilized as a hydrophobic liquid in the estimation of γ.

The work of cohesion (W_c) was calculated using the equation

$$W_c = 2 \cdot \sigma. \quad (8)$$

The work of adhesion was calculated using the Dupré equation

$$W_a = \sigma_{2,1} + \sigma_{3,1} - \gamma, \quad (9)$$

where 1 means air, 2 – an aqueous solution, and 3 – liquid paraffin.

The contact angle (θ) was calculated using Young's equation:

$$\cos\theta = (\sigma_{3,1} - \gamma) / \sigma_{2,1}. \quad (10)$$

Harkins` spreading coefficient was calculated using the equation:

$$f = W_a - W_c. \quad (11)$$

A circulating thermostat Julabo F12-ED («Julabo Labortechnik GmbH») was used to maintain the necessary temperature.

Agar diffusion test (*agar well method*) was used to study the antibacterial efficacy of ointments containing ofloxacin, levofloxacin, or moxifloxacin [34]. After 24 hours of incubation, the diameter of the zones of inhibited growth was measured. The standard strain *Pseudomonas aeruginosa* ATCC 27853 and clinical bacterial strains of extensively resistant phenotype were used for the test [35]. The average values from three replicates were used to determine the final result. The efficacy of the tested ointments and the susceptibility of the bacterial strains to the ointments were evaluated using the following criteria:

– no zone of growth inhibition: no efficacy and no susceptibility;

– inhibition zone diameter up to 15 mm: low efficacy and low susceptibility;

– inhibition zone diameter from 15 mm to 25 mm: effective and susceptible;

– inhibition zone diameter more than 25 mm: high efficacy and high susceptibility.

The statistical analysis of initial data was carried out using Microsoft Excel spreadsheet ($P \leq 0.05$) [36].

4. Research results

Study by rotational viscometry.

The rheological parameters of ointment bases were contingent on the mass ratio between PG and M400 (Fig. 2, Table 5) at a constant content of P338 and M1500 as consistency factors. It was demonstrated that an increase in the mass fraction of M400 resulted in a significant increase in all rheological parameters of the bases. The ointment bases exhibited a plastic flow type with thixotropic properties, as evidenced by the hysteresis loop on the rheograms.

The rheological parameters of the ointment bases depended on the mass ratio between P338 and M1500 (Fig. 3, Table 6) at a constant content of PG and M400. With an increase in the mass fraction of P338, the rheological parameters of the bases increased several times. Commencing with base No. 2.3, at elevated shear rate gradients, zones of pseudoplastic flow are present on the rheograms.

In the event of a gradual replacement of part of the mixed solvent *PG – M400* in ointment base No. 1.3 with water, a marked decrease in rheological parameters was observed (Fig. 4, Table 7). At a water concentration of 7.5%, the base exhibited a liquid consistency, and at a water content of 10%, it transformed into a Newtonian liquid.

At temperatures in excess of 25°C, hydrophilic bases melted, resulting in a decrease in their rheological parameters (Fig. 5, 6). Within the temperature range of 32.5–35.0°C, the bases acquired a liquid consistency.

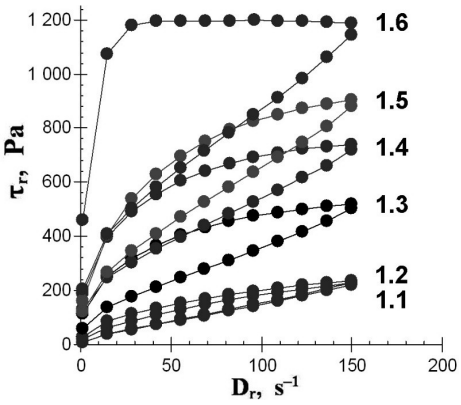

Fig. 2. Reograms of ointment bases with different contents of PG and M400

The rheograms of hydrophilic bases demonstrate the presence of hysteresis loops, which are indicative of potential thixotropic properties. Applying shear stress for a certain period of time to the base, in which the coagulation structure was formed, resulted in a decrease in its apparent viscosity (η) (Fig. 7). In the absence of agitation for a period of 24 hours, there was a possibility of an increase in apparent viscosity. In the case of hydrophilic bases, however, the initial values of the parameter η were not achieved. Therefore, for base No. 1.3 (Fig. 7), the apparent viscosity decreased from 21.7 to 8.4 Pa·s, and after 24 hours it increased by only 1.8 Pa·s (thixotropy index $\lambda_T = 0.47$).

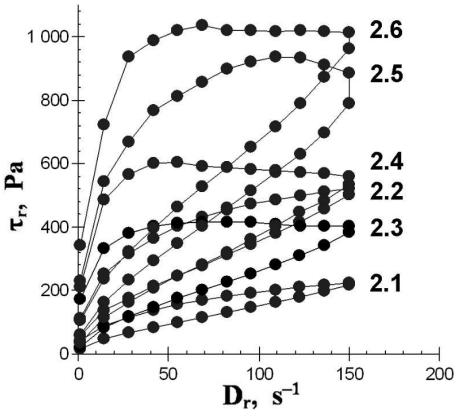

Fig. 3. Reograms of ointment bases with different contents of P338 and M1500

Table 5

Reological parameters of ointment bases with different contents of PG and M400 (compositions see in Table 2)

Base number	*C*, % m/m		τ_0, Pa	η, (Pa·s) at D_r:			A_N, Pa/s
	M400	PG		14.6 s^{-1}	41.6 s^{-1}	82.3 s^{-1}	
No. 1.1	0%	75%	17.9	4.3	2.6	2.0	4454
No. 1.2	15%	60%	27.9	5.9	3.3	2.2	6670
No. 1.3	30%	45%	112.2	17.3	8.8	5.5	17265
No. 1.4	45%	30%	189.9	27.3	13.3	8.1	22307
No. 1.5	60%	15%	161.1	27.9	15.1	9.6	24291
No. 1.6	75%	0%	461.1	73.7	28.6	14.5	61184
Base No. 1.7 that consists of M400 and M1500			136.1	21.9	7.8	4.0	14583

Table 6

Reological parameters of ointment bases with different contents of M1500 and P338 (compositions see in Table 3)

Base number	*C*, % m/m		τ_0, Pa	η, (Pa·s) at D_r:			A_N, Pa/s
	M1500	P338		14.6 s^{-1}	41.6 s^{-1}	82.3 s^{-1}	
No. 2.1	25%	0%	42.4	6.1	3.3	2.2	6184
No. 2.2	20%	5%	112.2	17.3	8.8	6.3	17265
No. 2.3	15%	10%	172.8	22.8	9.7	5.5	26251
No. 2.4	10%	15%	211.8	33.5	14.4	7.2	38088
No. 2.5	5%	20%	230.5	37.3	18.4	10.9	55074
No. 2.6	0%	25%	342.5	49.4	23.7	12.4	59299
Base No. 2.7 additionally containing M20CSE and CSA			466.7	44.0	22.7	5.9	47989

Base No. 2.7, which additionally contained 1.5% M20SSE and 3.5% CSA, had a significantly higher appar-

ent viscosity compared to base No. 1.3. Applying shear stress resulted in a decrease in the apparent viscosity of base No. 2.7 from 54.3 to 16.3 Pa·s. Following 24 hours, an increase of 8.6 Pa·s was observed (λ_T = 0.46).

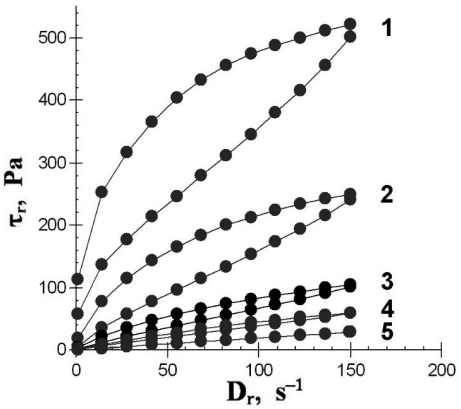

Fig. 4. Rheograms of ointment bases with different content of water: 1 – 0%; 2 – 2.5%; 3 – 5.0%; 4 – 7.5%; 5 – 10.0%

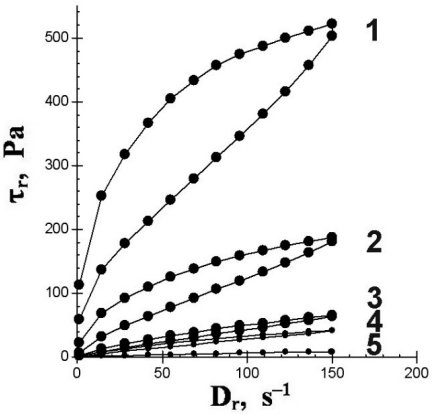

Fig. 5. Rheograms of ointment base No. 1.3 at: 1 – 25°C; 2 – 30°C; 3 – 35°C; 4 – 40°C; 5 – 45°C

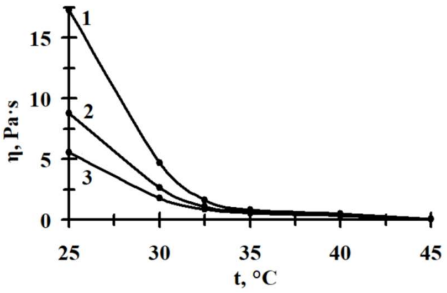

Fig. 6. Apparent viscosity (η) of ointment base No. 1.3 (at D_r: 1 – 14.6 s^{-1}, 2 – 41.6 s^{-1}, 3 – 82.3 s^{-1}) *vs* temperature (*t*)

Table 7

Rheological parameters of ointment bases with different content (*C*) of water

C, % m/m	τ_0, Pa	η, (Pa·s) at D_r:			A_N, Pa/s
		14.6 s^{-1}	41.6 s^{-1}	82.3 s^{-1}	
0	112.2	17.3	8.8	5.5	17 265
2.5	18.2	5.3	3.4	2.4	7 561
5.0	3.1	1.5	1.1	0.9	2 079
7.5	1.1	0.6	0.5	0.4	650
10.0	0	0.2			0

The plots illustrating the change in apparent viscosity under the influence of shear stress for base No. 1.7 and base No. 1.3 are almost identical (Fig. 7). The apparent viscosity of base No. 1.7 was observed to decrease from 25.4 to 9.3 Pa·s following a 30-minute mixing period. However, a subsequent increase of 8.9 Pa·s was recorded after 24 hours (λ_T = 0.72).

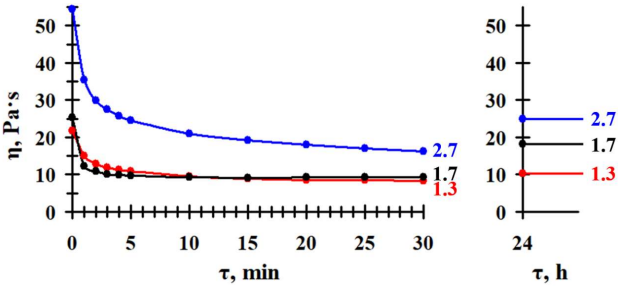

Fig. 7. Apparent viscosity (η) of bases, at 25°C, *vs* time (τ), in the context of shear stress application: after a 30-minute period of shear stress induction, the system was left without agitation for 24 h

Study of water absorption.

The parameters of the water absorption by the solutions of P338 or M1500 are illustrated in Fig. 8 and outlined in Table 8. Due to their high molecular weight, these excipients do not penetrate the semipermeable membrane into the chamber with water.

The parameters of water absorption after a period of six hours demonstrate that the values were approximately 1.6 times higher in the case of the 20% aqueous solution of M1500 than for the 20% solution of P338 (Fig. 8 and Table 8). During 24 hours, the M1500 solution absorbed 221.2±11.2% of water, while the P338 solution absorbed 162.8±6.9% of water, which is 1.36 times less.

The absorption of water through a semipermeable membrane was the subject of study for four hydrophilic bases: No. 1.7, No. 1.3, No. 2.7, and No. 1.2 (the compositions are in Tables 2, 3).

Ointment bases No. 1.2 and No. 1.3 were statistically indistinguishable in terms of water absorption parameters (Fig. 9, Table 9). With regard to water absorption capacity, base No. 1.7 exceeded the absorption parameters of bases No. 1.2 and No. 1.3 by a mere ~1.2 times.

The water absorption parameters for base No. 2.7 were found to be lower than those for base No. 1.3, with the absorption rate being 2.32 times lower and the cumulative content and level of absorption being 2.15 times lower.

Table 8

Parameters of water absorption by 20% M1500 solution and 20% P338 solution (*n* = 5)

Parameter	20% M1500 solution	20% P338 solution
Rate, μg/cm²/h$^{-1/2}$	250.99 ± 6.03, SD: 4.85	154.61 ± 12.07, SD: 9.72
Cumulative amount (*A*) (at the time point 6 h), μg/cm²	484 ± 25.75, SD: 20.74	302 ± 13.60, SD: 10.95
Absorption level (at the time point 6 h), %	96.8 ± 5.15, SD: 4.15	60.4 ± 2.72, SD: 20.19

Table 9

Parameters of water absorption by ointment bases (n = 5)

Parameter	Value for ointment base:			
	No. 1.7	No. 1.3	No. 2.7	No. 1.2
Rate, μg/cm²/h$^{-1/2}$	669.52 ± 8.81, SD: 7.09	573.35 ± 7.26, SD: 5.85	247.09 ± 6.92, SD: 5.57	550.41 ± 13.15, SD: 10.59
Cumulative amount (A) (at the time point 6 h), μg/cm²	1332 ± 69.91, SD: 56.30	1132 ± 41.55, SD: 33.47	526 ± 58.63, SD: 47.22	1128 ± 37.66, SD: 30.33
Absorption level (at the time point 6 h), %	266.4 ± 13.98, SD: 11.26	226.4 ± 8,31, SD: 6.69	105.2 ± 11.73, SD: 9.44	225.6 ± 7.53, SD: 6.07

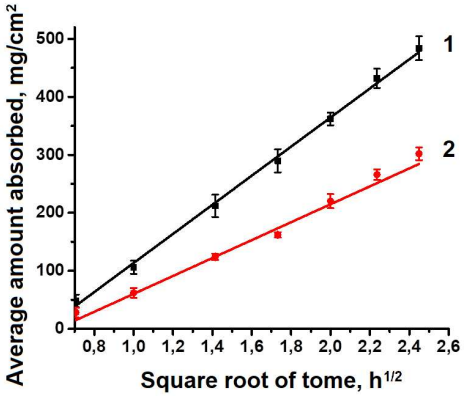

Fig. 8. Amount of water absorbed *vs* square root of time: 1 – 20% M1500 solution; 2 – 20% P338 solution

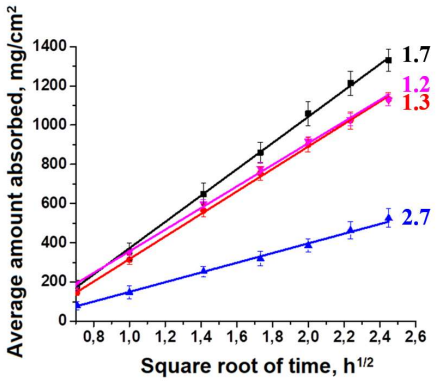

Fig. 9. Amount of water absorbed by ointment bases *vs* the square root of time

Study of ofloxacin release (*in vitro release testing – IVRT*).

The chromatograms of the reference solution of ofloxacin and the representative chromatograms of dialysates obtained during IVRT are presented in Fig. 10, 11. The findings of the study of the ofloxacin release from hydrophilic bases No. 1.3, No. 1.5, and No. 2.7 are illustrated in Fig. 12 and outlined in Table 10.

The release rate plots of ofloxacin (i.e., the amount of released ofloxacin per unit membrane area *vs.* the square root of time) exhibited linearity for all ointments under study (Fig. 12, 13, and Tables 10, 11). Furthermore, the coefficients of determination obtained exceeded the acceptability criterion of 0.90 [30, 31].

Table 10

Ofloxacin release parameters (n = 5)

Parameter	Value for ointment base:		
	No. 1.3	No. 1.5	No. 2.7
Release rate, μg/cm²/h$^{-1/2}$	129.10 ± 13.61, SD: 5.48	100.47 ± 9.74, SD: 3.92	21.63 ± 3.29, SD: 1.32
Correlation coefficient (R)	0.99552	0.99661	0.99075
Coefficient of determination (R^2)	0.99106	0.99323	0.98159
Cumulative amount (A) (at the time point 6 h), μg/cm²	228.54 ± 10,10, SD: 4.06	193.30 ± 12.01, SD: 4.83	43.69 ± 5.70, SD: 2.29
Recovery (at the time point 6 h), %	45.71 ± 2.02, SD: 0.81	38.66 ± 2.40, SD: 0.97	8.74 ± 1.14, SD: 0.46

Base No. 1.3 contained 45% PG and 30% M400, while base No. 1.5 contained 15% PG and 60% M400. The investigation sought to ascertain whether there was a discrepancy in the parameters of ofloxacin release from these two bases. The rate of ofloxacin release from base No. 1.3 was found to be approximately 1.29 times higher, and the cumulative amount and recovery were 1.18 times higher (Table 10).

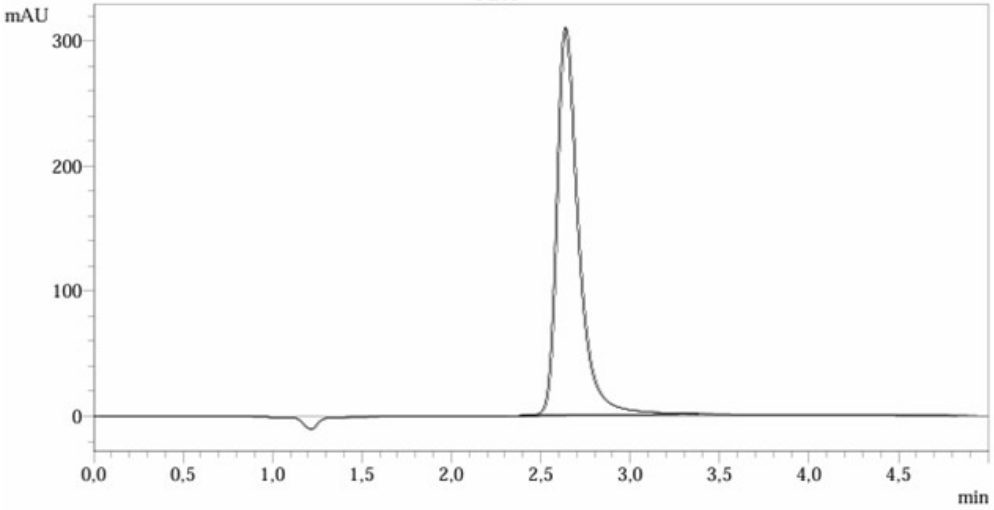

Fig. 10. Chromatogram of the reference solution with ofloxacin content 26.50 μg/ml. Peak with Rt ≈ 2.639 min is due to ofloxacin

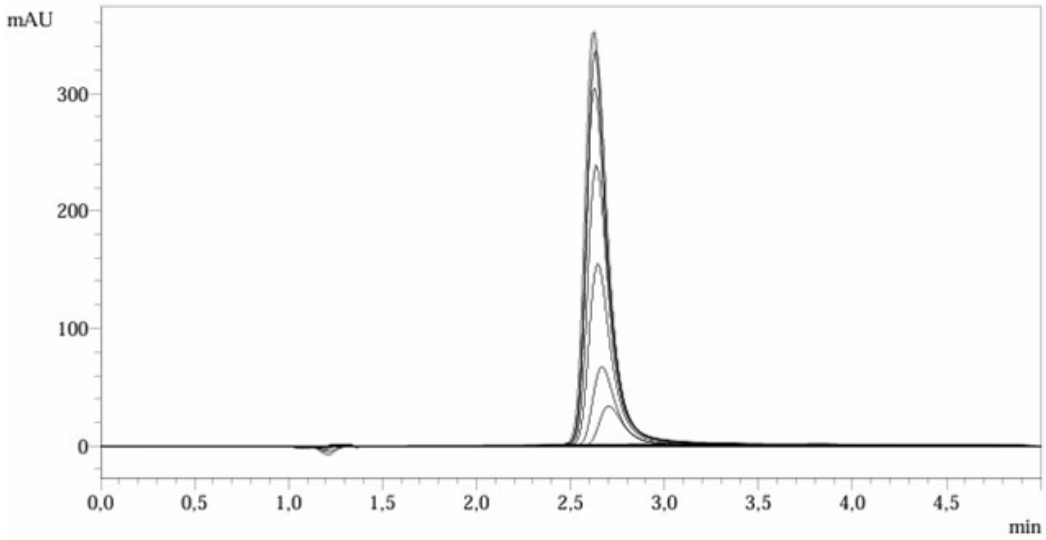

Fig. 11. Representative chromatograms of dialysate samples obtained during *the in vitro* release test for ofloxacin. Peaks with *Rt* ≈ 2.649 min are due to ofloxacin

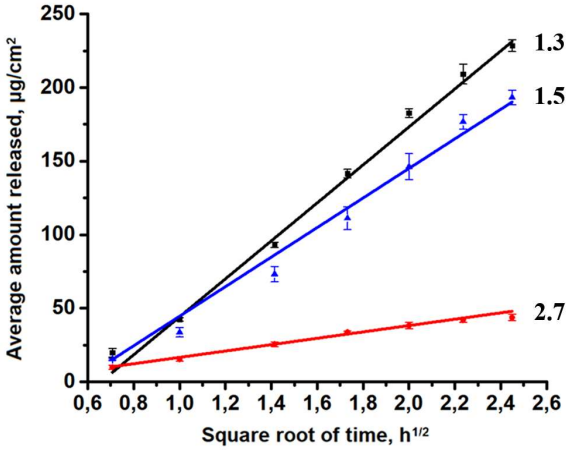

Fig. 12. Ofloxacin release rate plots

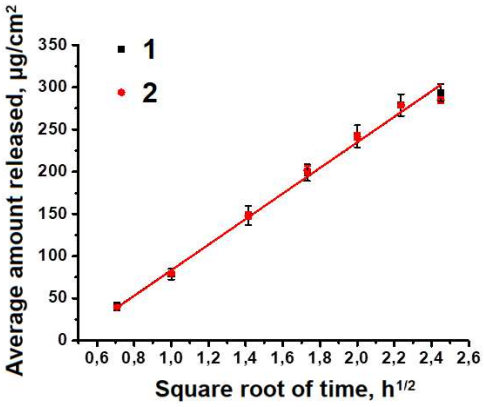

Fig. 13. Ofloxacin release rate plots in the case of: 1 – ointment with Proxanol 268; 2 – ointment with P338

The rate of ofloxacin release from base No. 2.7, as well as the cumulative amount and recovery, were significantly lower than in the case of base No. 1.3 (Table 10).

When Proxanol 268 was substituted for P338 in Oflocain-Darnitsa® ointment, the variation in the release rate of ofloxacin was 0.22%, and the discrepancy in cumulative amount and recovery was 3.05% (Table 11). With regard to the release of ofloxacin during *in vitro* testing, ointments containing Proxanol 268 and P338 are equivalent according to USP criteria [32].

Table 11

Ofloxacin release parameters (n = 5)

Parameter	Value for ointment with:	
	Proxanol 268	P338
Release rate, μg/cm²/h$^{-1/2}$	151.31 ± 4.24, SD: 3.42	151.64 ± 5.42, SD: 4.37
Correlation coefficient (R)	0.99873	0.99793
Coefficient of determination (R^2)	0.99746	0.99586
Cumulative amount (A) (at the time point 6 h), μg/cm²	293.74 ± 12.4, SD: 10.02	284.79 ± 4.45, SD: 3.58
Recovery (at the time point 6 h), %	58.75 ± 2.49, SD: 2.00	56.96 ± 0.89, SD: 0.72

Research using spin probes.

The EPR spectra of four spin probes in a mixed solvent *PG – M400* (6:4) and two ointment bases No. 1.3 and No. 2.7 are illustrated in Fig. 14. The parameters of these EPR spectra at 25°C, 32°C, and 37°C are presented in Tables 12–15.

At a temperature of 25°C, the dynamic viscosity of the mixed solvent *PG – M400* (6:4) was measured to be 0.052 Pa·s. This value is several orders of magnitude lower than the apparent viscosity of base No. 1.3 (Table 5) and base No. 2.7 (Table 6). However, the shape of the EPR spectra of each of the spin probes (probe 1, probe 2, probe 3, and probe 4) was the same in both the mixed solvent and two ointment bases (Fig. 14). The values of isotropic constant (A_N) of the EPR spectra of each of the spin probes (probe 1, probe 2 and probe 4) were the same in the mixed solvent and in the ointment bases (Tables 12, 13, 15), which indicates the same polarity of the microenvironment of free radicals. The rotational correlation times (τ_{-1}, τ_{+1}) of hydrophilic spin probe No. 1 (TEMPOL) in the mixed solvent and two ointment bases were practically the same (Table 12). At 25°C, the EPR spectra parameters of spin

probes 2, 3 and 4, which contain alkyl chains in their molecules, exhibited slightly higher values in ointment bases than in mixed solvent (Tables 13–15). Nevertheless, these disparities are rendered negligible when juxtaposed with the discrepancy in the apparent viscosity of the ointment bases and the dynamic viscosity of the mixed solvent. In the case of EPR spectra of probes 2 and 4, the values of the anisotropy parameter (ε) were practically the same in the mixed solvent and in the two ointment bases (Tables 13 and 15).

This finding suggests that the molecules of the spin probes were dissolved in a mixed solvent *PG – M400,* and that the parameters of the EPR spectra of these probes were not significantly affected by such excipients as P388, M1500, and emulsifiers.

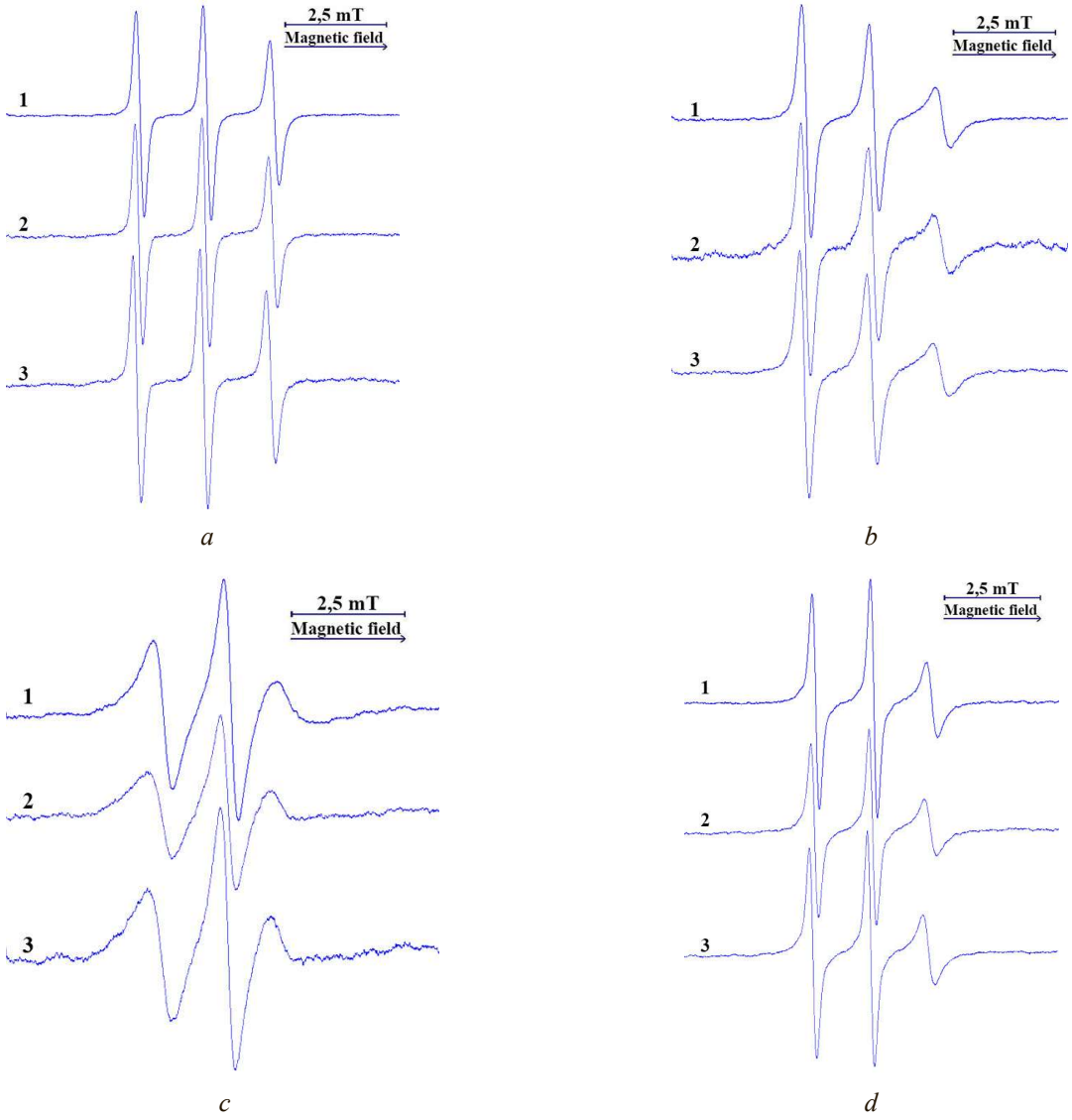

Fig. 14. EPR spectra at 25°C of: *a* – probe 1; *b* – probe 2; *c* – probe 3; *d* – probe 4 – in mixed solvent *PG – M400* (6:4) (1), base No. 1.3 (2) and base No. 2.7 (3)

Table 12

EPR spectra of probe 1 (TEMPOL) in a mixed non-aqueous solvent *PG – M*400 (6:4), base No. 1.3 and base No. 2.7 at different temperatures (*t*)

Test sample	Composition		*t*, °C	Parameter			Spectrum shape
	Consistency factor	Solvent		τ_{-1}, ns	τ_{+1}, ns	A_N, mT	
–	–	*PG – M400* (6:4)	25	0.13	0.27	1.62	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	25	0.13	0.27	1.62	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5 %	*PG – M400* (6:4)	25	0.13	0.27	1.62	triplet
–	–	*PG – M400* (6:4)	32	0.07	0.13	1.62	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	32	0.08	0.14	1.62	triplet
Base No. 2.7	P338 5%, M1500 20 %, M20CSE/CSA 5%	*PG – M400* (6:4)	32	0.06	0.14	1.62	triplet
–	–	*PG – M400* (6:4)	37	0.05	0.09	1.62	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	37	0.05	0.11	1.62	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	37	0.05	0.11	1.62	triplet

Table 13

EPR spectra of probe 2 in a mixed non-aqueous solvent *PG – M400* (6:4), base No. 1.3 and base No. 2.7 at different temperatures (*t*)

Test sample	Composition		*t*, °C	Parameter				Spectrum shape
	Consistency factor	Solvent		τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	A_N, mT	
–	–	*PG – M400* (6:4)	25	0.51	1.53	–0.14	1.63	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	25	0.60	1.87	–0.16	1.63	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	25	0.68	1.12	–0.14	1.63	triplet
–	–	*PG – M400* (6:4)	32	0.34	1.14	–0.13	1.63	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	32	0.43	1.27	–0.16	1.62	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	32	0.41	1.22	–0.15	1.62	triplet
–	–	*PG – M400* (6:4)	37	0.28	0.88	–0.15	1.62	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	37	0.32	0.91	–0.19	1.62	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	37	0.30	0.88	–0.15	1.62	triplet

Table 14

EPR spectra of probe 3 (5-DSA, NH_4 salt) in a mixed non-aqueous solvent *PG – M400* (6:4), base No. 1.3 and base No. 2.7 at different temperatures (*t*)

Test sample	Composition		*t*, °C	Order parameter *S*	Spectrum shape
	Consistency factor	Solvent			
–	–	*PG – M400* (6:4)	25	0.24	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	25	0.25	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	25	0.27	triplet
–	–	*PG – M400* (6:4)	32	0.18	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	32	0.20	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	32	0.21	triplet
–	–	*PG – M400* (6:4)	37	0.14	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	37	0.16	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	37	0.16	triplet

Table 15

EPR spectra of probe 4 (16-DSA) in a mixed non-aqueous solvent *PG – M400* (6:4), base No. 1.3 and base No. 2.7 at different temperatures (*t*)

Test sample	Composition		*t*, °C	Parameter				Spectrum shape
	Consistency factor	Solvent		τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	A_N, mT	
–	–	*PG – M400* (6:4)	25	0.43	0.94	0.07	1.49	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	25	0.48	1.02	0.07	1.49	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	25	0.50	1.05	0.07	1.49	triplet
–	–	*PG – M400* (6:4)	32	0.27	0.66	0.05	1.49	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	32	0.27	0.68	0.05	1.49	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	32	0.29	0.66	0.04	1.49	triplet
–	–	*PG – M400* (6:4)	37	0.23	0.49	0.06	1.49	triplet
Base No. 1.3	P338 5%, M1500 20%	*PG – M400* (6:4)	37	0.23	0.50	0.05	1.49	triplet
Base No. 2.7	P338 5%, M1500 20%, M20CSE/CSA 5%	*PG – M400* (6:4)	37	0.22	0.53	0.04	1.49	triplet

As the temperature increased from 25°C to 32°C and 37°C, there was an observed decrease in dynamic viscosity of the mixed solvent from 0.052 Pa·s to 0.036 Pa·s and 0.028 Pa·s, respectively. The rotational correlation times (τ_{-1}, $\tau_{\pm1}$) of spin probes 1, 2, and 4, as well as the order parameter S of the EPR spectra for probe 3 in the mixed solvent *PG – M400*, also decreased. Hydrophilic bases melted at temperatures of 32°C and 37°C, acquiring a liquid consistency (Fig. 5, 6). However, analysis of the EPR spectra of the spin probes indicated the localization of their molecules in the mixed solvent *PG – M400* medium (Tables 12–15).

Study of the surface-active properties of aqueous solutions of M1500 and P338.

The surface-active parameters of 5% aqueous solutions of M1500 and P338 at 32°C (skin temperature) are presented in Table 16.

The surface tension of water at 32°C is ~ 70.86 mN/m. P338 was found to possess greater surface activity in comparison to M1500. The surface tension (σ) of 5% P338 aqueous solution and the interfacial tension (γ) between this solution and liquid paraffin, as well as the contact angle (Θ) between this solution and the hydrophobic surface, are all significantly lower than those of 5% M1500 aqueous solu-

tion. Furthermore, the ability to spread out in the case of the 5% P338 aqueous solution is greater, as evidenced by the higher value of the spreading coefficient (φ).

Table 16

Surface-active parameters of 5% aqueous solutions of M1500 and P338 at 32°C

σ, mN/m	γ, mN/m	Θ, °	W_a, mN/m	W_c, mN/m	φ, mN/m
Water					
70.86	48.7	106.2	51.1	141.7	–90.7
5% M1500 aqueous solution					
60.3	27.9	89.1	61.3	120.6	–59.3
5% P338 aqueous solution					
37.8	18.4	73.8	48.3	75.6	–27.3

Note: surface tension (σ) of liquid paraffin is 28.9 mN/m.

Studies of antibacterial activity in in vitro experiments.

As demonstrated in Fig. 15, irrespective of the mass ratio between PG and M400 in hydrophilic bases, the antibacterial activity of all ointments containing 0.1% ofloxacin was highly effective or effective against the standard strain *P. aeruginosa* ATCC 27853.

The diameters of the growth inhibition zones with exposure to 0.1% ofloxacin ointments on hydrophilic bases No. 1.1, No. 1.2, and No. 1.3, containing from 75% to 45% PG, were approximately 30 mm. A decline in PG content was observed to be associated with a decrease in the diameters of the growth inhibition zones of *P. aeruginosa* ATCC 27853. The findings of the studies suggest that the composition of the dispersion medium in ointments on hydrophilic bases can influence the effectiveness of their specific antimicrobial action.

The ointments containing fluoroquinolones were formulated on a multi-component hydrophilic base, comprising P338, M6000, M1500, M400, PG, and *N*-methyl pyrrolidone (6.82%). Levofloxacin and moxifloxacin, in concentrations of 0.1% in these ointments, were found to be effective and highly effective against clinical bacterial strains of extensively resistant phenotype (including those resistant to fluoroquinolones) isolated from infected wounds in patients with combat injuries (Table 17) [35]. The water-soluble ointment base (placebo) did not inhibit bacterial growth in the *in vitro* experiments.

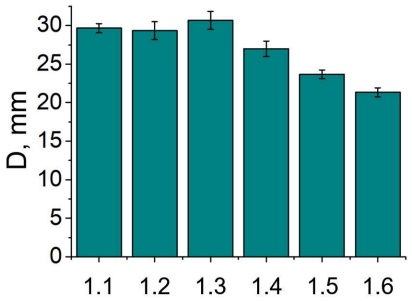

Fig. 15. Diameters (D) of growth inhibition zones of *P. aeruginosa* ATCC 27853 with exposure to 0.1% ofloxacin ointment with hydrophilic bases No. 1.1, No. 1.2, No. 1.3, No. 1.4, No. 1.5, No. 1.6

Table 17

Diameters of the bacterial growth inhibition zones in the case of ointments with exposure to ointments containing fluoroquinolones

Microorganism	Inhibition zone diameter (mm)	
	0.1% levofloxacin	0.1% moxifloxacin
Staphylococcus aureus 70	33.33 ± 3.79	28.67 ± 1.43
Pseudomonas aeruginosa 4791	30.67 ± 2.87	27.33 ± 2.87
Escherichia coli 2093	20.33 ± 1.43	24.00 ± 2.48
Klebsiella pneumoniae 2820	30.00 ± 2.48	23.33 ± 1.43
Citrobacter amalonaticus 2427	35.67 ± 1.43	34.00 ± 2.48
Citrobacter amalonaticus 1574	30.33 ± 1.43	27.33 ± 3.79

5. Discussion of research results

The research results indicate that other excipients, specifically PG and P338, could be added to hydrophilic ointment bases in addition to M400 and M1500. This results in the formation of hydrophilic bases with a plastic flow type (Fig. 2). An increase in the mass fraction of P338 leads to areas of pseudoplastic flow becoming evident on the rheograms at high shear rate gradients (Fig. 3). The rheograms show hysteresis loops: following the application of shear stress, the bases' apparent viscosity decreased and did not immediately recover (Fig. 2–5).

The rheological parameters of these hydrophilic bases can be modified by altering the composition of the excipients (Tables 5–7). It is essential to include low-molecular-weight hydrophilic non-aqueous solvents in the base formulations, as they can function as co-solvents and enhance penetration. For example, PG can be effective in this role. The rheological parameters of hydrophilic bases can be enhanced by the addition of a mixture of colloidal surfactant and CSA in a specific mass ratio (Table 6).

In the experiment, the apparent viscosity of hydrophilic ointment bases decreased with the application of shear stress and increased if the base was left without stirring for 24 hours (Fig. 7). This enabled the calculation of thixotropy indices. A comparison of the area of the hysteresis loop on the rheograms of the studied ointment bases (Tables 5, 6) revealed a lack of correlation with the values of their thixotropy indices. The base No. 2.7 is distinguished by its notably lower thixotropy index of 0.46 and its comparatively larger hysteresis loop area (Table 6). In contrast, base No. 1.7 is characterized by its significantly higher thixotropy index and its comparatively smaller hysteresis loop area (Table 5). The apparent viscosity of base No. 1.3, which had a fairly large hysteresis loop area (Table 5), was almost not restored following the application of shear stress (Fig. 7). However, the thixotropy index of base No. 1.3, which was 0.47, closely matched the thixotropy index of 0.46 calculated for base No. 2.7, which exhibited an apparent viscosity increase of 8.6 Pa·s after 24 hours without stirring. It can be hypothesized that an increase in the PG content in the hydrophilic base resulted in a decrease in the thixotropy index.

There is a risk that, if the structure of the hydrophilic ointment base is destroyed over a long period of time, the apparent viscosity of the base may decrease significantly and irreversibly. Consequently, at the stage of pharmaceuti-

cal development, it is imperative to ascertain the rheological parameters of preparations in the form of ointments with hydrophilic bases, contingent on the parameters of technological processes. Such studies should also be incorporated into the production process validation scheme.

The surface-active properties of ointments are of particular significance as they facilitate the uniform spread of the melted ointment over the affected surface. P338 is characterized by more pronounced surface-active properties in comparison to M1500 (Table 16). The high cohesion value of a 5% aqueous solution of M1500 results in the formation of droplets on the skin surface. The same phenomenon occurs on the affected surface with a PEG base after its melting. In contrast, ointments containing P338 melt on the wound surface and spread across it. Consequently, the incorporation of poloxamers, particularly P338, into the formulation of hydrophilic ointment bases is a rational approach.

The osmotic properties of hydrophilic ointment bases are of particular significance in determining the functional properties of medicinal products in the form of ointments. Diffusion processes through a semipermeable membrane occur due to the difference in osmotic pressure between the receptor medium (water) and the hydrophilic base. These processes occur in two directions: firstly, diffusion of water to the osmotically active ointment, and secondly, diffusion of active substances and low-molecular-weight excipients through the semipermeable membrane from the ointment to the water.

The water absorption capacity of the M1500 aqueous solution is 1.36 times greater than that of the P338 aqueous solution (Fig. 8, Table 8). The findings suggest that the process of water absorption by hydrophilic bases can be regulated by varying the mass ratio between P338 and M1500. However, with regard to water absorption parameters, base No. 1.7 was only ~1.2 times higher than hydrophilic base No. 1.3 (Fig. 9, Table 9). It can be concluded that the substitution of a proportion of M400 with PG, and a proportion of M1500 with P338, did not result in a substantial reduction of the water absorption capacity of the bases. In the case of such a hydrophilic base, a significant factor that reduces water absorption parameters is the introduction of surfactants and CSA into its composition (Fig. 9, Table 9) [13]. This phenomenon may be attributed to the involvement of surfactants and CSA in the process of forming the coagulation structure of ointment base No. 2.7 (Table 6), which results in an increased lipophilicity due to the presence of CSA.

The release of active substances and hydrophilic non-aqueous solvents [13] from the ointment base into the receptor chamber with water is a process opposite to the absorption of water by the hydrophilic base.

It was established that PG (M_r 76.1) in hydrophilic bases promoted the release of ofloxacin more effectively than M400 (M_r ~400) (Fig. 12, Table 10). A significant factor leading to a decrease in the release parameters of ofloxacin from the hydrophilic base was the introduction of a mixture of M20CSE and CSA into the base (Fig. 12, Table 10).

The findings of the research indicate that the incorporation of a combination of colloidal surfactant and CSA in hydrophilic ointments designed for localized wound treatment during the inflammatory phase is not recommended. This results in a substantial reduction in the osmotic activity of the ointment base. However, in other cases, it may be considered rational, for example, when ointments are intended for local treatment of wounds in the repair phase or for the treatment of certain dermatomycoses that are not accompanied by profuse exudation.

The substitution of proxanol 268 for P338 in Oflocain-Darnitsa® ointment had no impact on the parameters of ofloxacin release into the receptor medium (water) (Fig. 13, Table 11). In *in vitro* experiments, ointments containing proxanol 268 or P338 were found to be equivalent in terms of ofloxacin release [32]. The findings of the studies suggest the potential for the use of P338 in ointments with hydrophilic bases, as an alternative to poloxamer 268.

The results obtained from the spin probe method demonstrated that, in the studied hydrophilic bases, the dispersion medium was a mixed non-aqueous solvent *PG – M400* (Fig. 14, Tables 12–15). It was established that in a non-aqueous hydrophilic environment, neither P338 associates nor compatible aggregates of M20CE and CSA molecules were formed. The formation of compatible aggregates of M20CSE and CSA molecules, as well as associates of P388 molecules, was observed if the water structure prevails in the system [13, 37]. This finding is also substantiated by the EPR spectra of spin probes 2, 3, and 4 in a gel containing M20CSE and CSA (Fig. 16), in which the dispersion medium was characterized by the predominance of water structure [38].

The EPR spectra of spin probes 2, 3, and 4 are triplets (Fig. 14); in Fig. 16, the spectrum of probe 2 is a superposition of a singlet and two triplets, the EPR spectra of probes 3 and 4 are anisotropic with order parameters (S) of 0.55 and 0.43, respectively. The value of the order parameter (S) in the case of the EPR spectrum of probe 3 in the ointment base No. 2.7 at 25°C is considerably smaller and amounts to 0.27 (Table 14). The EPR spectrum of probe 3 in a 20% aqueous solution of P338 is anisotropic with an order parameter (S) of 0.45 [37]. A comparison of the EPR spectra indicates that the specified spin probes in hydrophilic base No. 2.7 are dissolved in a non-aqueous dispersion medium, and the aggregates created by M20CSE and CSA are absent in it.

In a medium consisting of non-aqueous solvents at a temperature of 25°C, substances that function as consistency factors for hydrophilic ointment bases become a dispersed phase of suspensions. This process promotes the creation of coagulation structures within the disperse systems, resulting in the formation of viscous-plastic ointment bases [13].

In this regard, the following definition of hydrophilic ointments cannot be considered correct: ‘Ointments are semi-solid preparations for topical application, consisting of a *single-phase base* in which solid or liquid substances can be dispersed’ [3, 4]. Hydrophilic ointment bases are constituted of a solid dispersed phase and a liquid dispersion medium, thus indicating that these bases are *two-phase* systems.

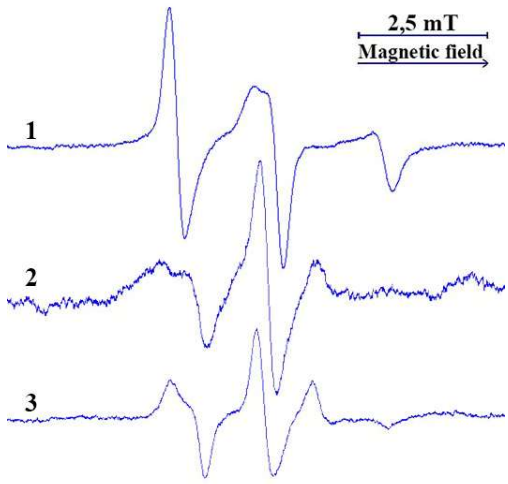

Fig. 16. EPR spectra of probe 2 (1), probe 3 (2), and probe 4 (3) in a gel containing 3.0% M20CE, 7.0% CSA, 9.0% PG, and 81.0% water at 25°C

As hydrophilic bases are dispersed systems with a liquid dispersion medium, the composition of this medium may be significant in medicinal preparations in the form of ointments. It was demonstrated that the parameters of ofloxacin release from hydrophilic ointments were influenced by the composition of the mixed solvent *PG – M400* (Fig. 12 and Table 10). Conversely, the type of poloxamer did not affect the release of ofloxacin (Fig. 13, Table 11). The diameters of the growth inhibition zones of the standard strain *P. aeruginosa* ATCC 27853 with exposure to 0.1% ofloxacin ointments were largest when bases No. 1.1, No. 1.2, and No. 1.3, containing 75%, 60% or 45% PG, respectively, were used for the ointments. A further decrease in PG content and an increase in M400 content resulted in a decrease in the diameters of the growth inhibition zones (Fig. 15).

As demonstrated in Table 17, the components of the water-soluble ointment base contributed to the elimination of resistance and the manifestation of the effective antibacterial action of 0.1% levofloxacin ointment and 0.1% moxifloxacin ointment against clinical strains of bacteria with an extensively resistant phenotype. This was particularly evident in relation to clinical strains of bacteria that were resistant to fluoroquinolones [35].

The findings of the research indicate that by varying the composition of excipients, it is feasible to regulate the properties of multicomponent hydrophilic ointment bases and medicinal products formulated using these bases.

Practical relevance. The findings of the study provide a theoretical foundation for the development of ointments with hydrophilic bases.

Study limitations. The present paper sets out the results for hydrophilic ointment bases containing solely one type of poloxamer (P338) and one type of macrogol (M1500), as well as a mere three solvents: M400, PG, and water.

Prospects for further research. Further research may be related to other hydrophilic bases. In addition, research may be directed towards the improvement of manufacturing processes for ointments with hydrophilic bases.

6. Conclusions

The rheological parameters of hydrophilic bases can be controlled by modifying the ratio between consistency factors and components of dispersion medium, by varying water content, temperature, and shear stress, as well as by adding surfactants and CSA to their formulations. Hydrophilic bases are able to absorb water and they promote the release of ofloxacin. Surfactant and CSA have a significant impact on these processes, reducing their rates. The formation of aggregates from P338 molecules, as well as molecules of surface-active substance and CSA, was not observed in the hydrophilic bases. PG, when incorporated into hydrophilic ointments containing ofloxacin, enhances their antibacterial efficacy.

Conflict of interest

The authors confirm that they have no conflicts of interest related to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm they did not use artificial intelligence technologies when creating the current work.

Authors' contributions

Nikolay Lyapunov and **Olena Bezugla:** Conceptualization, Methodology, Writing, Supervision; **Anna Liapunova:** Investigation (*in vitro* release test), Visualization; **Igor Zinchenko:** Investigation (liquid chromatography), Validation, Visualization; **Oleksii Liapunov:** Investigation (EPR spectroscopy), Visualization; **Oleksii Lysokobylka:** Investigation (rotational viscometry), Visualization; **Svitlana Dzhoraieva:** Investigation (microbiological experiments).

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