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EFFECT OF THE COMPOSITION OF EMULSIFIERS AND THE DISPERSION MEDIUM ON THE PROPERTIES OF BASES FOR SEMI-SOLID PREPARATIONS

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The aim. *To study the effect of cetostearyl alcohol (CSA) on the rheological properties of bases with different dispersion media, the release of propylene glycol (PG) from them, and the ability of these bases to absorb water.*
Materials and methods. *Micelles of a non-ionic surfactant and its aggregates with CSA in a mixed solvent where the structure of water prevails, mixed solvent PG – macrogol 400 (M400) and hydrophilic bases-vehicles with different dispersion media were studied. The research was carried out by the spin probe method using a probe simulating a cationic surfactant and by rotational viscometry. The microstructure of the bases was studied by optical microscopy. The in vitro release test to study the release of PG and M400 from solutions and bases was performed using vertical diffusion chambers. The content of PG and M400 in the dialysate was determined by gas chromatography according to the validated analytical procedures. The absorption of water by solutions and bases was determined by dialysis through the membrane.*
Results. *CSA, which was the part of the bases together with surfactants in certain ratios, was a significant factor in increasing their rheological parameters, reducing the parameters of PG release during in vitro release tests, as well as reducing water absorption. The mechanisms of such influence are different for bases with different structures of the dispersion medium. In the bases, where the structure of water prevailed, lateral phase separation occurred in the supramolecular structures of surfactant and CSA with the formation of liquid domains of surfactant and solid domains of CSA, which contributed to the formation of coagulation structures. In the mixed non-aqueous solvent PG – M400, surfactant micelles and mixed aggregates of surfactant and CSA molecules were not formed; at 25 °C, surfactants and CSA became separate dispersed phases of suspensions, which contributed to the formation of gels. When CSA was added into an aqueous solution of poloxamer 338, PG, M400 and cationic surfactant, the flow behaviour changed, and the rheological parameters increased, which led to a decrease in the release rate and extended for PG and M400 as well as in the ability to absorb water. The rate and extent of PG release from the solution were greater compared to the M400 release.*
Conclusions. *The addition of CSA in combination with surfactants into the bases for semi-solid preparations is a significant factor for modifying their rheological parameters, the kinetics of PG release from them, and water absorption during experiments in vitro. The mechanisms of such an effect are different and depend on the composition and structure of the dispersion medium of the base*
Keywords: *cetostearyl alcohol (CSA), propylene glycol (PG), basis, spin probe, rheological parameters, in vitro release test*

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1. Introduction

Semi-solid preparations (SSPs) are relevant for use in various areas of medicine [1]. However, the development of both novel drugs and generic/hybrid products is quite difficult, and specific problems arise on the way to their authorisation and production [2]. SSPs can represent a variety of dispersed systems with extremely complex structures [3, 4]. SSPs are characterised by more complicated, interdependent relationships in their structure, physicochemical, biopharmaceutical properties and effectiveness as compared to solid dosage forms or parenteral preparations. This leads to an increase in the variability of quality attributes intra-batch, inter-batches, between drugs from different production sites, as well as from different manufacturers [3, 5]. Depending on the specifics of the disease, the SSPs intended for its treatment should meet certain requirements. This necessitates comprehensive and often sophisticated research using various methods.

The development of topical generic/hybrid medicinal products faces specific obstacles compared to other drug products [2, 5] for which bioequivalence can be assessed by traditional pharmacokinetic methods [6]. In general, the establishment of equivalence in the case of SSP is based on comparative clinical trials, which are expensive and often associated with a high degree of

variability and low sensitivity in detecting differences between formulations in terms of efficacy and safety [2].

Recently, different non-clinical, *in vitro*/*in vivo* surrogate methods for assessing the bioequivalence of topical SSPs have been considered acceptable in some cases by European and American regulatory authorities (EMA and FDA) [7]. To support a claim of the equivalence using this approach, it is necessary to demonstrate [8, 9]:

1) Extended pharmaceutical equivalence, in particular:

– qualitative (*Q*1) and quantitative (*Q*2) sameness;

– sameness of the critical quality attributes (*CQA*), microstructure/physical properties (microstructure, pH, density, particle/globule size and rheological parameters), and *in vitro* drug release (within the acceptance criteria) (*Q*3).

2) equivalence concerning efficacy (in the case of complex semi-solid formulations):

– permeation kinetic studies (*in vitro* skin permeation, stratum corneum sampling);

– pharmacodynamic studies (vasoconstriction assay for corticosteroids; *in vitro* test for antimicrobial drug products).

3) equivalence concerning safety and local tolerance (in general, may rely on literature data for the active substance and well-established excipients).

For simple generic/hybrid medicinal products or in the case of some post-approval changes in the dossier, demonstration of equivalence concerning quality i.e., extended pharmaceutical equivalence, may be sufficient [8, 9].

FDA and EMA established different acceptance criteria [9, 10]. The acceptance criteria proposed in the EMA draft guideline are the subject of wide discussions [2, 11]. The acceptance criterion of ±10 % for rheological parameters is the most intensively disputed in the literature and criticised as overly restrictive [2, 12]. In some cases, such a strict requirement is reasonable. For example, analysing the effect of petrolatum from 6 different sources on drug product performance containing petrolatum as the only vehicle, Raghavan L. et al. [7] observed that diverse grades of petrolatum caused significantly different release rates of a topical steroid. This fact can be explained by the different distribution ratios of the hydrocarbon chain lengths and, as a result, different values of rheological parameters. However, from gels with different apparent viscosity values, the *in vitro* release parameters for dimetindene maleate were the same [11]. Significant variability of rheological parameters was observed for diclofenac diethylamine emulgels; the equivalency by *in vitro* release test with the USP acceptance criteria (75133 %) was established only for some batches. However, the products under study were considered bioequivalent based on the *in vivo* bioavailability assay. This finding suggests that different *Q*3 attributes are not prerequisites for different therapeutic efficacy of the studied products [12]. Thus, it is necessary to justify the relevant criteria regarding the correct assessment of the equivalence for locally acting SSPs on a case-by-case basis.

Carbomers are excipients widely used in gels. According to the requirements of the EP monograph «Carbomers», for the carbomers with a nominal apparent viscosity of 20000 mPa·s or greater, the apparent viscosity should be within 70.0–130.0 % of the nominal value; for a product with a nominal apparent viscosity of less than 20000 mPa·s, the apparent viscosity should be in the range from 50.0 % to 150.0 % of the nominal value [13]. In the case of such limits for the viscosity of a gelling substance, the inter-batch variability of the rheological parameters for the same medicinal product may be beyond ±10 %. It was also shown that the gelling agent had almost no effect on the release rate of diclofenac sodium, which depended on the content of isopropyl alcohol [14].

Macrogols [15, 16] and poloxamers [17] are widely used excipients that provide the consistency of SSPs. The dynamic viscosity of macrogol 400 should be 117.5 mPa·s±10.6 %, and dynamic viscosity of 50 % solution of macrogol 1500 should be 42.0 mPa·s±19 % [13]. The average relative molecular mass of poloxamer 407 is 12220±19.5 % [13]. It is obvious that the acceptance criteria for rheological parameters of ±10 % to confirm the pharmaceutical equivalence of SSPs containing these excipients are unreasonably strict and unjustified.

The authors of numerous works on the studies of rheological properties and *in vitro* release parameters have made the following main conclusions: firstly, the criterion ±10 % for rheological parameters is unjustified [18, 19], sometimes it cannot be realised for the same medicinal product both inter-batch and even intra-batch [20]; secondly, to confirm equivalence this criterion should be more than ±10 % [2, 18], and, finally, in order to meet the established criteria, appropriate statistical approaches should be adopted, in particular, the number of examined samples should be justified [21]. However, most of these studies were not aimed at identifying those factors that are significant for the *in vitro* release of active substances.

Viscosity and density can influence *in vitro* release kinetics. However, other significant factors may predetermine viscosity and density as well as the *in vitro* release rate. These factors (regarding ingredients or the medicinal product) should be established and standardised during pharmaceutical development for further routine quality control [14].

In some works, the *in vitro* release of substances, their epidermal delivery and penetration were connected with various structures of solvent [22, 23], in particular, the mixed solvent *propylene glycol (PG) – water* [24, 25]. For example, it was shown that the release rate of acyclovir increased with increasing PG concentration, and PG content also affected the rheological parameters of the system [23]. In this work, soft white paraffin and cetostearyl alcohol (*CSA*) were considered inert excipients that affected only the rheological parameters [23]. Zhang W. et al. found that the penetration of macrogols across the tympanic membrane decreased exponentially with increasing their molecular mass and was lower in the case of the poloxamer-based gel [26]. Laffleur F. et al. determined that surfactants and their content affected the *in vitro* release of dexpanthenol. However, the influence of CSA, which is a w/o emulsifier in the tested creams, was not evaluated [27]. Kovacic A. et al. concluded that trans-

dermal drug delivery using penetration enhancers had not reached its potential yet [28].

Even though most of the studies on the relationship between some physicochemical properties of bases and the *in vitro* release of active substances were performed at a high scientific level, each of them has certain limitations and does not allow evaluating a complex of factors that are important for confirming extended pharmaceutical equivalence.

In emulsion bases, the functional purpose of *CSA* is obvious – it is the w/o emulsifier and a consistency factor [15]. Therefore, it was interesting to study the performance of *CSA* in the bases without an oil phase, as well as the effect of *CSA* on the properties of such bases. In addition, the publications lack the results of the study of the release of the hydrophilic solvent *PG*, which can be a penetration enhancer for active substances.

Considering summarised data of the scientific publications, the purpose of the research was defined.

The aim was to study the effect of cetostearyl alcohol on the rheological properties of bases with different dispersion media, the release of propylene glycol from them, and the ability of these bases to absorb water.

2. Planning (methodology) of the research

Two emulsifiers o/w and w/o: non-ionic surfactant (macrogol 20 cetostearyl ether – *M20CE*) and *CSA*, as well as hydrophilic non-aqueous solvents *PG* and macrogol 400 (*M400*), were planned to be used in the experiments. The first group of research objects consisted of *M20CE* micellar solution and colloidal dispersion systems containing the mixture of *M20CE* and *CSA* in the mixed solvent *PG – M400 – water* (mass ratio 6:4:80) (Table 1). In order to modify the rheological properties of this system the mass ratio *M20CE*: *CSA* was varied [29]. Due to the absence of the oil phase, this dispersed system could be considered as creams [13, 30], where *CSA* was the dispersed phase. *PG* and *M400* with total content of 10 % could be considered moisturisers [15]. The water structure dominated the dispersion medium of these systems [31]. The second group included the mixed hydrophilic non-aqueous solvent *PG – M400* (mass ratio 6:4) [31], the dispersion of *M20CE*, as well as the dispersion of *M20CE* with *CSA* in this solvent (Table 2). The hydrophilic ointment bases were the third group of studied objects. 5 % poloxamer 338, 20 % macrogol 1450 and 5 % macrogol 4000 were the thickening agents in these bases (Table 2) [13, 15]. The fourth group consisted of liquid and cream with a mixed structure of dispersion medium (Table 3) [4].

The rheological properties of these systems should be studied by rotational viscometry at 25 °C. If, due to their properties, these systems could be bases for semi-solid preparations, their flow type and rheological parameters depending on temperature should be determined. It was necessary to determine mass ratios between *M20CE* and *CSA* at which, in their aggregates, structural transitions at the supramolecular level leading to the formation of non-Newtonian systems occurred. Previously, the spin probe method using a lipophilic probe simulating a w/o emulsifier was used for this purpose [29]; it was of interest to study the structural transitions in *M20SE* and *CSA* aggregates using a hydrophilic probe that simulates an o/w emulsifier. After that, it was planned to study this spin probe's electron paramagnetic resonance (EPR) spectra in anhydrous bases. The results of these studies would allow for establishing the difference in the mechanism of interaction *M20CE* with *CSA* in the medium with the dominant structure of water and in the hydrophilic non-aqueous solvent. In turn, this could make it possible to explain the difference in the rheological and osmotic properties of various hydrophilic bases for SSPs.

Non-aqueous solvents (*PG* and *M400*) can perform various functions in different hydrophilic bases. Therefore, it was necessary to find out whether the functions of these excipients remained unchanged or were modified under the influence of *M20CE* and *CSA* in hydrophilic bases with both an anhydrous dispersion medium and a medium with a dominant structure of water. First of all, this could be confirmed by the results of studies on the *in vitro* release of solvents and the water absorption by bases. Furthermore, it was important to determine the difference between the *in vitro* release of *PG* and *M400*.

The results of the planned comprehensive studies could contribute to optimising the pharmaceutical development of semi-solid preparations with hydrophilic bases.

3. Materials and methods

Macrogol cetostearyl ether (Kolliphor® CS 20 – *M20CE*); cetostearyl alcohol (Kolliwax® CSA 50 – *CSA*); poloxamer 338 (Kolliphor® P 338 – *P338*); propylene glycol (Kollisolv® PG – *PG*); macrogol 400 (Kollisolv® PEG 400 – *M400*); purified water (hereinafter referred to as water), macrogol 1450 (Kollisolv® PEG 1450 – *M1450*); macrogol 4000 (Kollisolv® PEG 4000 – M4000) and dexpanthenol were used in the experiments (all substances manufactured by the company «BASF») [15]. They met the pharmacopoeial requirements [13, 30]. The substance benzyldimethyl[3-myristoylamino)propyl] ammonium chloride monohydrate (UA/17990/01/01) (hereinafter referred to as cationic surfactant) was also used.

The formulations of disperse systems are presented in Tables 1–3.

Table 1

Disperse systems (with the predominance of the structure of water in the dispersion medium)

Component	Content (% m/m) in the disperse system:										
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
M20CE	10.0	9.0	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.5
CSA	0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	9.5
PG	6.0										
M400	4.0										
Water	80.0										

Table 2

Anhydrous hydrophilic systems

Component	Content (% m/m) in the disperse system:					
	No. 12	No. 13	No. 14	No. 15	No. 16	No. 17
PG	60.0	54.0	54.0	42.0	36.0	36.0
M400	40.0	36.0	36.0	28.0	24.0	24.0
M20CE	–	10.0	3.0	–	10.0	3.0
CSA	–	–	7.0	–	–	7.0
M1450	–	–	–	20.0	20.0	20.0
M4000	–	–	–	5.0	5.0	5.0
P338	–	–	–	5.0	5.0	5.0

Table 3

Disperse systems (with the mixed structure *water–hydrophilic components*)

Component	Content (% m/m) in:	
	Liquid No. 18	Cream No. 19
Cationic surfactant	0.5	0.5
Dexpanthenol	5.0	5.0
P338	17.0	17.0
CSA	–	6.0
*M4*00	9.0	9.0
PG	10.0	10.0
Water	58.5	52.5

M20CE was dissolved in the mixed solvent *water – PG – M4*00 at 50-60 °C and then cooled to 20–25 °C. To prepare other disperse systems, the mixture of substances was heated to 70–75 °C until the components melted, homogenised, degassed and then cooled to 20–25 °C with stirring. The production method for cream No. 19 is described in the literature [4]. Water, dexpanthenol, surfactant and *CSA* were heated to 70–75 °C and stirred until the surfactant dissolved and *CSA* melted; then, the mixture was emulsified and degassed (phase A). *PG*, *M4*00, and *P338* were heated to 65–70 °C with stirring (phase B). Phases A and B were mixed at ~65–70 °C; the mixture was homogenised and degassed and then cooled to 20–25 °C C with stirring.

Rheograms were obtained at a certain temperature by rotational viscometry [13, 30] using a rotating viscometer «Rheolab QC» with coaxial cylinders CC-27 (in the case of bases for SSPs) and DG42 (for liquids) («Anton Paar GmbH»; software RHEOPLUS, 2.66 version). Rheograms were used to characterise the flow behaviour and determine the yield stress (τ_0) and the apparent viscosity or the dynamic viscosity (η).

Electron paramagnetic resonance (EPR) spectroscopy was used for the research [32]. The spin probe simulating cationic surfactant: 4-(*N,N*-dimethyl-*N*-hexadecyl) ammonio-2,2,6,6tetramethyl-piperidine-1-oxyl iodide (M_r 551.65; CAS [114199-16-5]) (hereinafter referred to as TEMPO-iodide) was used. The probes were added into the disperse systems at the concentration of 10^{-4} mol/l. EPR spectra were obtained using an EPR Spectrometer CMS8400 («Adani»). The type of EPR spectrum (triplet, anisotropic spectrum, singlet, superposition spectrum) was determined. Using the EPR spectra, which were triplets, the height of the low-field, central and high-field peaks (h_{+1}, h_0 та h_{-1}, respectively) as well as the width of the low-field component (ΔH_{+1}) and central component (ΔH_0) were determined. Rotational correlation times of spin probes (τ_{+1}, τ_{-1}, $\tau_{\pm1}$) and anisotropy parameter (ε) were calculated by the following equations [33, 34]:

$$\tau_{+1} = \left(\sqrt{\frac{h_o}{h_{+1}}} - 1\right) \cdot \Delta H_0 / 2 \cdot 10^8, \quad (1)$$

$$\tau_{-1} = \left(\sqrt{\frac{h_o}{h_{-1}}} - 1\right) \cdot \Delta H_0 / 3.6 \cdot 10^9, \quad (2)$$

$$\tau_{\pm1} = \left(\sqrt{\frac{h_{+1}}{h_{-1}}} - 1\right) \cdot \Delta H_{+1} / 6.65 \cdot 10^{-10}, \quad (3)$$

$$\varepsilon = \frac{\sqrt{h_0 / h_{+1}} - 1}{\sqrt{h_0 / h_{-1}} - 1}. \quad (4)$$

The rotational correlation time of the spin probe (τ) is directly proportional to the effective radius of the molecule (*R*) and the microviscosity of its local surrounding (η) and inversely proportional to the absolute temperature (*T*) [32, 33]:

$$\tau = \left(4 \cdot \pi \cdot R^3 \cdot \eta\right) / 3 \cdot k \cdot T. \quad (5)$$

The hyperfine splitting constant (A_N) was determined as the distance (in mT) between the central and high-field components by the EPR spectra, which were triplets; the A_N characterises the micropolarity of the environment in the vicinity of the nitroxyl radical [33].

In the case of superposition spectra, if the signal was split into two lines in the high-field component, the parameter *f* was determined by the formula [32]:

$$f=a:(a+b), \quad (6)$$

where *a* and *b* are the distances from the upper point of the first and second peaks of the high-field component to the central axis of the EPR spectrum, a change in the parameter *f* depending on any variable factor indicates the redistribution of the spin probe from one phase to another [32, 33].

The experiments in regard to the release of *PG* or *M400* from dispersing systems were performed using vertical diffusion cells and semipermeable cellulose membranes (GOST 7730-89); the membranes were pre-soaked in the receptor medium (*water R*) for 24 hours. The tests were performed at 32 °C. The donor chamber contained 3.0 g of the studied system: membrane contact area was 7.065 cm^2; the medium in the receptor chamber was stirred by a magnetic stirrer with mixing rate of 600 rpm. Samples (1.0 ml) were collected from the receptor chamber at 0.5, 1, 2, 3, 4, 5, and 6 h after application of the tested system and the volume withdrawn was replaced with stock receptor medium (*water R*). The concentrations of *PG* or *M400* in receptor medium at different sampling times were measured, and the amount of *PG* or *M400* (mg) released at a given time per unit

area (cm^2) was calculated for each sample. The results were assessed according to the requirements of EMA draft guidelines [9], USP General Chapter <1724> [10] and using accepted approaches [2, 35].

Quantitative determination of *PG* or *M4*00 in the samples was performed by gas chromatography (2.2.28) [13, 30] using Shimadzu GC-2014 gas chromatograph with FID detector and AOC-5000 autosampler («Shimadzu»; software: GC solution version 2.30.00). Quantification of *M4*00 was performed according to the validated analytical procedure [36]. Quantitative determination of *PG* was performed according to the developed analytical procedure as described below.

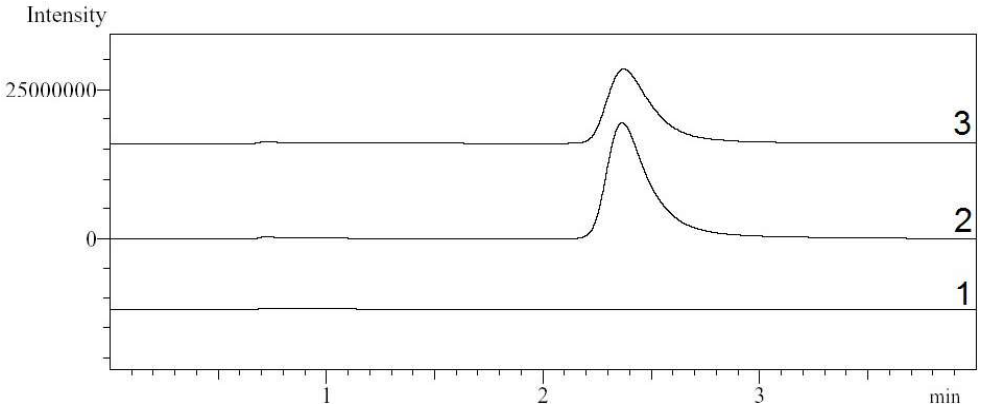


Fig. 1. Chromatograms obtained during the quantitative determination of *PG* (peaks with *Rt*≈2.4 min correspond to *PG*): 1 – solvent; 2 – reference solution; 3 – test solution

Analytical procedure for the quantitative determination of PG.

Test solution. The filtered sample (receptor medium containing released *PG)* is to be tested.

Reference solution. The solution of *PG CRS* (CRS of State Pharmacopoeia of Ukraine, cat. No. P0347) in *water R* 40 mg/ml.

Chromatographic conditions:

– *column:* glass column 110 cm×3.2 mm with stationary phase ethylvinylbenzene-divinylbenzene copolymer *R* (8100 mesh);

– *carrier gas*: nitrogen for chromatography *R*;

– *flow rate:* 25 ml/min;

– *detection:* flame ionisation;

– *injection:* 1 *μl* of the test solution and the reference solution;

– *temperature:* column – 220 °C; injection port – 250 °C; detector – 250 °C;

– *run time:* ~ 4 min; Rt of *PG* peak ~2.4 min.

System suitability (reference solution): column performance calculated by the peak due to *PG* should be at least 300 theoretical plates; the symmetry factor of *PG* peak should be in the range from 0.8 to 2.0, and the relative standard deviation should be≤3.0 %.

Validation of the procedure for the quantitative determination of *PG* was carried out according to the accepted methodology [30, 37]. Acceptance criteria for validation characteristics were calculated following the requirements of general article 5.3.N.2 of State Pharmacopoeia of Ukraine [30].

The specificity of the analytical procedure was confirmed by the fact that on the chromatogram of the solvent (water), there was no peak with a retention time, which would coincide with the retention time of the PG peak (*Rt*≈2.4 min) on the chromatograms of the reference solution and model solution (Fig. 1). In addition, there was no difference in the retention times of the *PG* peaks on the chromatograms of the model solution and reference solution (Fig. 1).

The range of the analytical procedure was chosen, taking into account the results of determining the minimum and maximum concentrations of PG in the samples of receptor medium. As a result, the validation of the procedure for the quantitative determination of *PG* was performed in the range of *PG* concentrations in model solutions from 0.31 mg/ml to 60.82 mg/ml (from 0.8 % to 152.0 % of the nominal concentration of *PG* in the reference solution – 40.0 mg/ml). According to the data presented in Table 4, the procedure for the quantitative determination of *PG* in the studied range met the acceptance criteria for linearity, repeatability and accuracy established by State Pharmacopoeia of Ukraine [30].

The solutions were stable for more than 24 hours because ΔZ for *PG* was less than critical value $\sqrt{2}\times\max\Delta_{As}=4.53$ (Table 5).

Table 4

Validation characteristics of the analytical procedure for the *PG* assay by GC in the receptor medium and their evaluation against the acceptance criteria [30]

Parameter	Value	Criterion (n=10)	Conclusion
Linearity			
b	0.98925	–	–
S_b	0.00505	–	–
α	–0.08167	1) $\leq\lvert S_\alpha\times 1.8595\rvert=\lvert 0.69\rvert$; 2) if it does not meet criterion (1), then $\leq\lvert 1.03\rvert$	Pass with both criteria
S_α	0.36878	–	–
S_0	0.80444	–	–
S_0/b	0.81318	$\leq\lvert 1.72\rvert$	Pass
r	0.9999	$\geq\lvert 0.9998\rvert$	Pass
Repeatability			
standard deviation $SD_{\Delta Zi}$, %	1.68	–	–
confidence interval: $\Delta_{\Delta Zi}=t(95\ \%, 10–1)\times SD_{\Delta Zi}$	3.08	≤3.2 %	Pass
Accuracy			
mean value ΔZ, %	–0.83	–	–
1) statistical insignificance $\lvert\Delta Z\rvert$:	0.83	$\lvert\Delta Z\rvert\leq 3.08:\sqrt{10}=0.97$ %	Pass
2) practical insignificance $\lvert\Delta Z\rvert$:		$\lvert\Delta Z\rvert\leq 0.32\times 3.2$ %=1.02 %	

Table 5

Calculation of stability parameters of model solutions

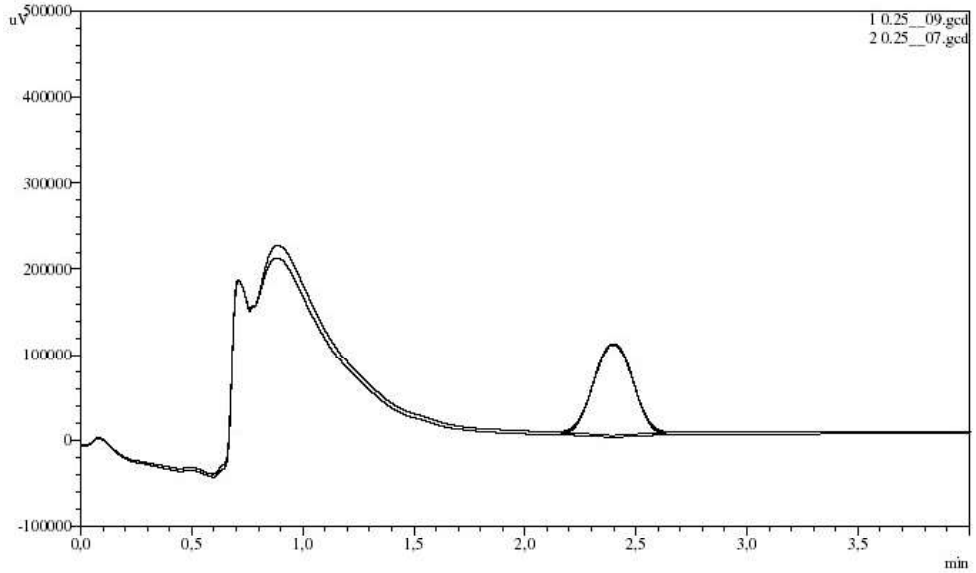
Substance	Z_{first}	Z_{last}	$\lvert\Delta Z_i\rvert$	$\leq\sqrt{2}\times\max\Delta_{As}$	Conclusion
PG	102.02	99.43	2.59	≤4.53	Pass

The chromatograms of *PG* model solution 0.31 mg/ml, where the signal-to-noise ratio for the *PG* peak is 202.51 are shown in Fig. 2. The minimum limit of *PG* quantification (MLQ), calculated from the signal/noise ratio, was 15.31 μg/ml [30].

Water absorption was studied by the dialysis method using cellulose membranes (GOST 7730-89), which were pre-soaked in water for 24 hours. Vertical diffusion chambers were used. The chamber with the test sample (3.0 g) was weighed at regular intervals, and the change in mass of the chamber (Δ*m*) contents was calculated, which approximately corresponded to the mass of absorbed water.

Unless otherwise stated, the experiments were performed at 25 °C. A circulating thermostat Julabo F12-ED («Julabo Labortechnik GmbH», Germany) was used to maintain a necessary temperature.

The microstructure of the systems was studied by optical microscopy [13, 30] using the microscope with a micrometre «Krüss MBL-2100» («A. Krüss Optronic», Germany).



<< SFID1 >>
ID#1 Compound Name: PG

Title	Ret. Time	Area	Resolution(JP)	nber of Theoretical Plate	S/N
0.25__09.gcd	2,399	2059694	--	515	206,50
0.25__07.gcd	2,403	2065069	--	468	198,51
Average	2,401	2062381	--	491	202,51
%RSD	0,136	0,184	0,000	6,652	2,790

Fig. 2. Chromatogram of the *PG* model solution 0.31 mg/ml (peak with *Rt*≈2.4 min corresponds to *PG*)

4. Research results

Studies by spin probe method and rotational viscometry.

The EPR spectra of the probe TEMPO-iodide in the No. 1–11 at 25 °C are shown in Fig. 3, and some parameters of these spectra are presented in Table 6.

With a decrease in the *M20CE* content from 10.0 % to 6.0 % and an increase in the *CSA* content to 4.0 %, the EPR spectra were triplets (Fig. 3). At the same time, there was an increase in the rotational correlation times (τ_{+1}, τ_{-1}, $\tau_{\pm1}$), the width of the low-field component (ΔH_{+1}) and the anisotropy parameter (ε); the A_N constant was about 1.6 mT, but tended to increase (Table 6).

With a further increase in the mass part of *CSA*, the EPR spectra transformed into a superposition of two triplets with the appearance of two signals in the high-field (Fig. 3). The transformation of the EPR spectrum into a superposition evidenced that the probe molecules were distributed in two phases. The nitroxyl radical of the probe TEMPO-iodide in M20CE micelles was localised in their polar part (A_N≈1.6 mT). In the case of the superposition spectrum, the value of A_N for one peak was about 1.6 mT. For the second peak, it was about 1.7 mT (Table 6), which indicated the localisation of the nitroxyl radical in the phase that was in direct contact with water. A more compact packing of molecules characterised this phase at the interface with the polar part. The value of τ_{+1} increased with an increase in the mass part of *CSA* (Table 6); the calculation of τ_{+1} according to equation (1) became incorrect at concentrations of CSA≥3 % m/m. It can be assumed that the lateral diffusion of molecules in this phase was very restricted, and this phase had a solid consistency. The larger the mass part of *CSA*, the smaller the parameter *f* became, which indicated the redistribution of TEMPO-iodide probe molecules to the phase with more compact packing of alkyl chains (Table 6).

The superposition spectra indicate the lateral phase distribution in mixed aggregates of *M20SE* and *CSA*, which are very different in their physicochemical properties. In mixed aggregates of *M20CE* and *CSA*, liquid domains were enriched with non-ionic surfactant *M20CE* and solid domains consisting mainly of *CSA* molecules. A similar effect was found for lipid membranes containing phosphatidylcholine with cholesterol [32].

The change in the EPR spectra correlated with the change in the rheological properties of dispersed systems No. 1–11 (Fig. 4, 5).

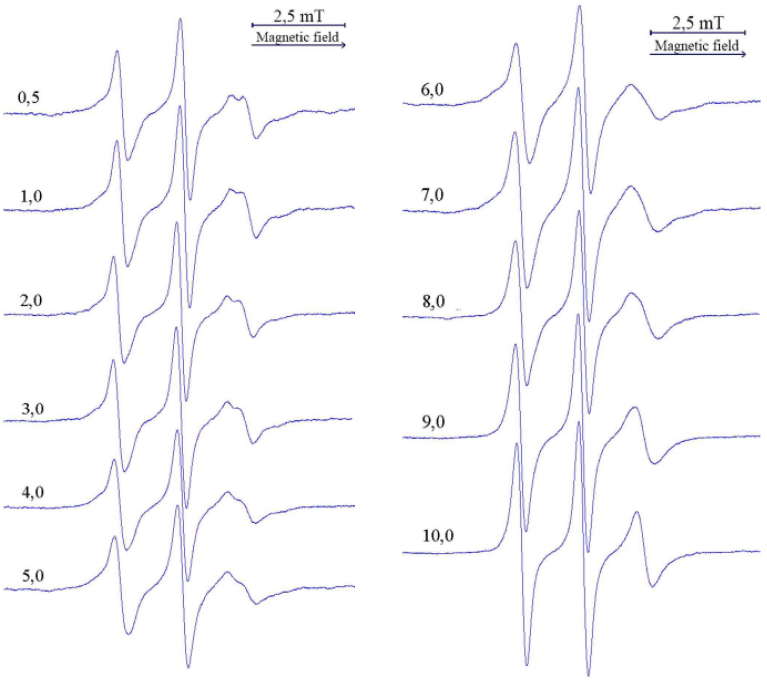


Fig. 3. EPR spectra of the probe TEMPO-iodide in the disperse systems containing mixtures of *M20CE* and *CSA* at their different mass ratios. The number of the spectrum indicates the content of *M20CE* (% m/m). The total content of mixture *M20CE* and *CSA* was 10 %

Table 6

Some parameters of the EPR spectra of the probe TEMPO-iodide in the dispersions containing mixtures of *M20CE* and *CSA* at 25 °C

Content, % m/m		τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	ΔH_{+1}, mT	A_N, mT		*f*
M20CE	*CSA*						phase 1	phase 2	
10.0	0	0.84	0.59	1.28	0.08	0.28	1.59	–	–
9.0	1.0	1.63	0.74	1.54	0.12	0.30	1.61	–	–
8.0	2.0	2.40	0.80	1.68	0.15	0.32	1.62	–	–
7.0	3.0	–	0.98	1.74	0.18	0.33	1.63	–	–
6.0	4.0	–	1.02	1.95	0.19	0.37	1.64	–	–
5.0	5.0	–	–	–	–	0.39	–	1.70	0.74
4.0	6.0	–	–	–	–	0.34	–	1.70	0.69
3.0	7.0	–	–	–	–	0.30	–	1.70	0.59
2.0	8.0	–	–	–	–	0.30	–	1.70	0.57
1.0	9.0	–	–	–	–	0.30	–	1.70	0.55
0.5	9.5	–	–	–	–	0.30	–	1.70	0.52

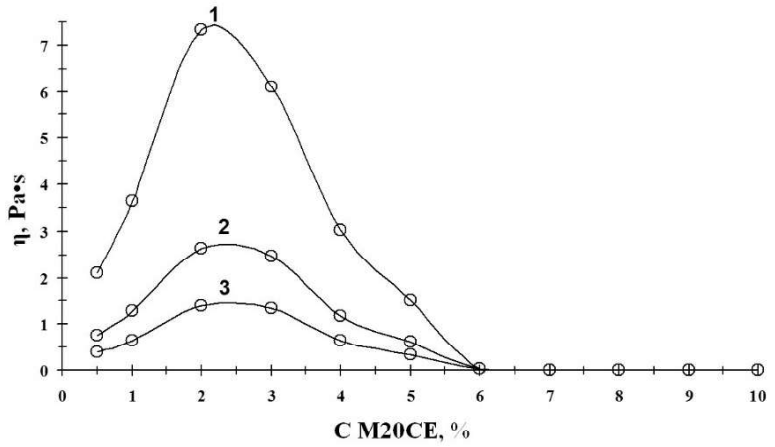


Fig. 4. Apparent viscosity (η) of disperse systems No. 1–11 at 25 °C *vs* *M20CE* content (*C*) at: 1 – D_r 14.6 s^{-1}; 2 – D_r 41.6 s^{-1}; 3 – 82.3 s^{-1}

The change in the structure of the mixed aggregates of *M20SE* and *CSA*, as evidenced by the transformation of the triplet spectra into the superposition spectra, led to a change in the Newtonian flow behaviour of the dispersed system to a plastic flow behaviour with thixotropic properties (Fig. 5) and an increase in the apparent viscosity with the extremum zone (Fig. 4). As a result, M20CE solution was a clear Newtonian liquid, system No. 5 was an opaque white liquid with Newtonian flow behaviour, and systems No. 6–11 were characterised by the plastic flow behaviour (Fig. 5).

The mechanism of change in rheological parameters has been described previously [29]. However, in this study, instead of the lipophilic probe based on palmitic acid, the hydrophilic probe TEMPO-iodide was used. This made it possible to avoid the phenomenon of exchange broadening even when this probe was localised in *CSA* domains. Furthermore, the absence of a singlet in the superposition spectra significantly facilitated their processing, and the obtained data led to novel, unexpected results.

The EPR spectra of the probe TEMPO-iodide in aggregates *M20SE* and *CSA* with their mass ratio of 3.0:7.0 (system No. 8) were obtained at temperatures from 15 °C to 45 °C (Fig. 6).

As the temperature increased from 15 °C to 45 °C, the probe TEMPO-iodide was redistributed from hydrophilic domains formed mainly by *M20CE* to lipophilic domains formed mainly by *CSA*, as evidenced by a decrease in the values of parameter *f* from 0.67 to 0.32 (0.67 at 15 °C, 0.57 at 20 °C, 0.56 at 25 °C, 0.48 at 30 °C, 0.43 at 35 °C, 0.39 at 40 °C, 0.32 at 45 °C). This differentiates hydrophilic probe TEMPO-iodide and lipophilic probe 4-palmitamido-2,2,6,6tetramethylpiperidine1-oxyl (CAS [22977-65-7]), which, in contrast, redistributes to the hydrophilic domains formed by non-ionic surfactants in the case of heating a similar disperse system. This was incorrectly explained by the melting of domains formed by *CSA* [29]. Using the probe TEMPO-iodide, it was found that in the mixed aggregates of *M20CA* and *CSA* (system No. 8), solid areas of *CSA*, necessary for maintaining the cream consistency, persisted even at an elevated temperature of 45 °C.

The data obtained by the spin probe method (Fig. 6) were correlated with the results of the study by rotational viscometry (Fig. 7).

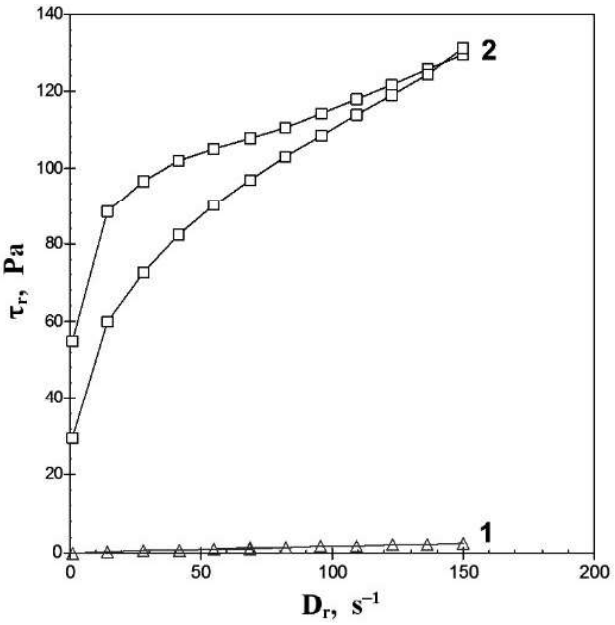


Fig. 5. Rheograms of disperse systems at 25 °C: 1 – No. 5; 2 – No. 8

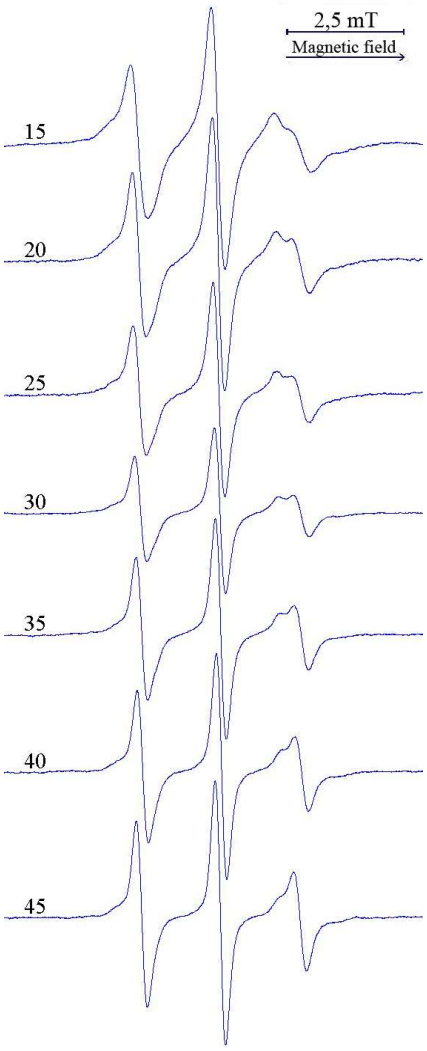


Fig. 6. EPR spectra of the probe TEMPO-iodide in dispersion system No. 8 at different temperatures (ºC), indicated by numbers in the figure

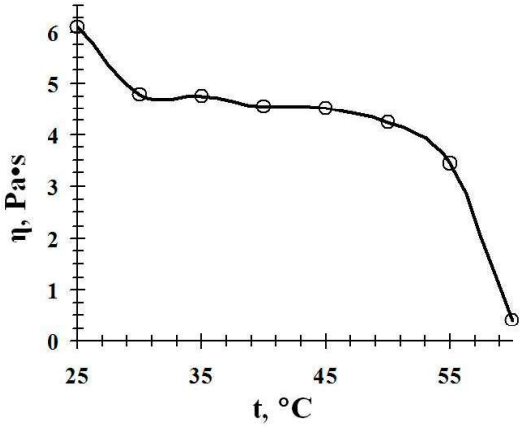


Fig. 7. Apparent viscosity (η) (at D_r=14.6 s^{-1}) of disperse system No. 8 (1) *vs* temperature (*t*)

With an increase in temperature from 25 °C to 40 °C, the apparent viscosity decreased by only 25 %; in the temperature range from 30 °C to 50 °C, the apparent viscosity of dispersion system No. 8 remained at an almost constant level (Fig. 7).

It was interesting to study the associates of *M20CE* and *CSA* in the non-aqueous solvent *PG – M400* (system No. 12) and their influence on the rheological properties of hydrophilic anhydrous systems.

Table 7

Parameters and type of the EPR spectra of probe TEMPO-iodide in water, *M20CE* solution and non-aqueous systems at 25 °C

Disperse system (Table 2)	A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
Water	1.70	0.05	0.03	0.07	0.32	Triplet
10 % *M20CE* solution (Table 1)	1.59	0.84	0.59	1.28	0.08	Triplet
No. 12	1.56	–	0.82	2.08	–0.03	Triplet
No. 13	1.56	–	0.96	2.24	–0.01	Triplet
No. 14	1.56	0.14	0.99	2.31	0.01	Triplet
No. 15	1.57	–	0.94	2.22	–0.01	Triplet
No. 16	1.57	–	0.84	2.12	–0.03	Triplet
No. 17	1.57	–	0.85	2.10	–0.02	Triplet

In *M20CE* micelles formed in a mixed solvent, where the structure of water prevails (dispersed system No. 1, Table 1), the parameters of the EPR spectra of solubilised molecules of probe TEMPO-iodide changed significantly compared to the spectra of the aqueous solution of this probe: τ_{+1} increased in 16.8 times, τ_{-1} – in 19.7 times, $\tau_{\pm1}$ – in 18.3 times, the anisotropy parameter decreased by 4 times, and the A_N value became lower by 0.11 mT (Table 7). This indicates that the microviscosity of the local environment of the spin probe molecules increased significantly in the micelles of the non-ionic surfactant compared to water, and the polarity of the environment of the nitroxyl radical localised among the hydrated polyoxyethyl chains decreased.

When *M20CE* at a concentration of 10 % was added to the non-aqueous solvent *PG – M4*00, the type and

parameters of the EPR spectra of the spin probe TEMPO-iodide almost did not change as well as in the case of adding 3 % *M20CE* and 7 % *CSA* (dispersion system No. 14) (Fig. 8). That is, based on the EPR spectra of the probe TEMPO-iodide, it cannot be concluded that *M20CE* micelles or mixed aggregates of *M20CE* and *CSA* were formed in a non-aqueous hydrophilic medium.

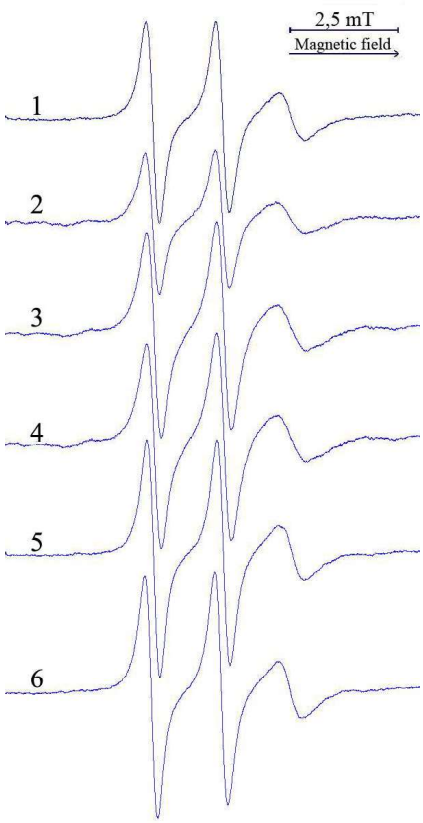


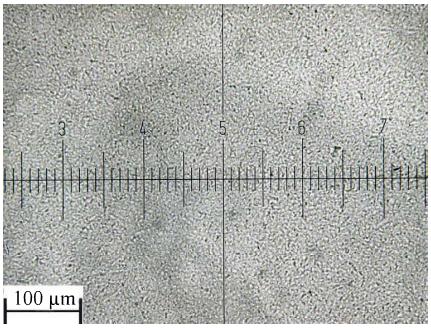
Fig. 8. EPR spectra of probe TEMPO-iodide in mixed non-aqueous solvent *PG – M400* (60: 40 % m/m) (1) and in systems containing this solvent as a dispersive medium: 2 – No. 13; 3 – No. 14; 4 – No. 15; 5 – No. 16; 6 – No. 17

The non-aqueous solvent *PG – M400* is a Newtonian liquid with a dynamic viscosity of ~0.05 Pa·s. *M20CE* was soluble in this solvent, provided heating to ~30.6 °C and then when this solution was cooled to ~29.0 °C *M20CE* separated from the solution as a phase of round particles ≤5 μm in size (Fig. 9, *a*). At 25 °C, an opaque gel was formed. This gel was characterised by a lower yield point (τ_0), pseudoplastic flow behaviour and thixotropic properties (Fig. 10, *a*, Table 8).

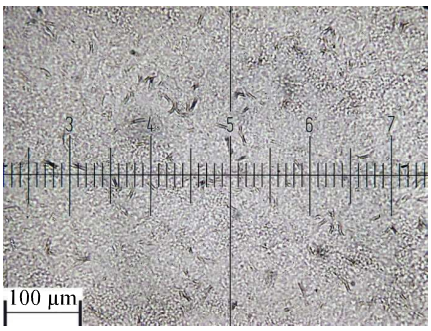
In the dispersion system, No. 14 at 25 °C, an opaque gel with non-Newtonian flow behaviour and thixotropic properties (Fig. 10, *b*) was also formed from *M20CE* particles and larger *CSA* particles, which had a solid state (Fig. 9, *b*).

When heated, the structure of gels on anhydrous bases was destroyed, which was evidenced by a decrease in the area of the hysteresis loop, the lower yield point and apparent viscosity (Fig. 10, 11).

At 35 °C, dispersed system No. 13 became a Newtonian liquid (Fig. 10) with a dynamic viscosity of ~0.03 Pa·s. The gel structure of dispersed system No. 14 was also easily destroyed when heated. The values of the rheological parameters decreased (Fig. 10, *b*). When the temperature increased from 25 °C to 35 °C, the apparent viscosity of dispersed system No. 14 decreased from 8.7 Pa·s to 0.9 Pa·s (Fig. 11), and the area of the hysteresis loop – from 7849 Pa/s to 280 Pa/s. At 40 °C, dispersed system No. 14 became a Newtonian liquid (Fig. 10, *b*). The destruction of gel structures with increasing temperature was caused by the melting and dissolution of *M20CE* and *CSA* particles.



a



b

Fig. 9. Micrographs of disperse systems at 25 °C: *a* – No. 13; *b* – No. 14

Table 8

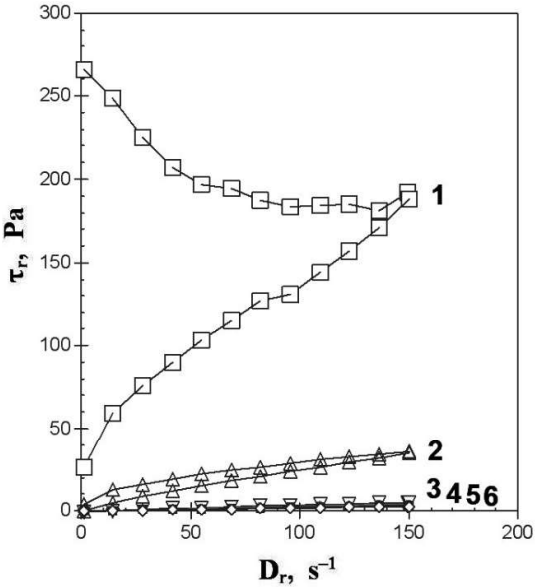
Some rheological parameters of disperse systems with anhydrous hydrophilic medium (Table 2) at 25 °C

Disperse system	τ_0, Pa	A_N, Pa·s^{-1}	η (D_r=14.55 s^{-1}), Pa·s
No. 13	265	12788	17.0
No. 14	96	7849	8.7
No. 15	294	36866	38.5
No. 16	794	62172	79.4
No. 17	508	56605	49.6

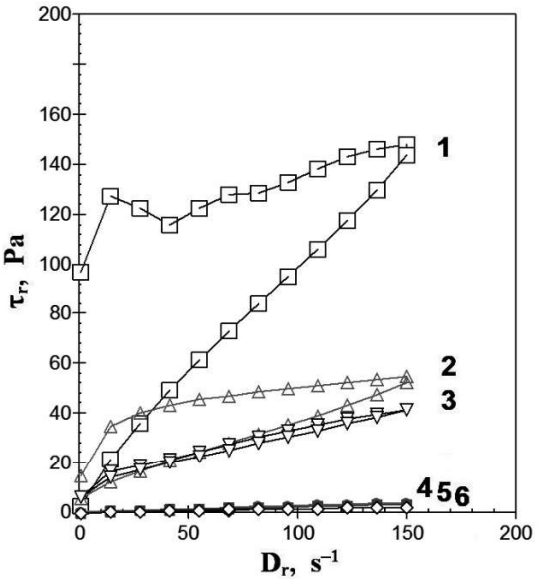
In the presence of a solid phase that contributed to the formation of the gel, the EPR spectra of the probe TEMPO-iodide at 25 °C practically did not change (Fig. 8, Table 7). This may indicate the selective solubility of TEMPO-iodide in the non-aqueous solvent *PG – M400* and the acceptability of this spin probe for studying such mixed solvents.

The mixed non-aqueous solvent *PG – M400* was the dispersion medium of the dispersion system No. 15 (Table 2); the consistency of this ointment base was due to *M1450*, *M4000* and *P338*. 10 % M20CE was added to another ointment base (dispersion system No. 16), and the mixture of 3 % *M20CE* and 7 % *CSA* was added to

dispersion system No. 17 (Table 2). The EPR spectra of the probe TEMPOiodide in all three ointment bases were very similar triplets (Fig. 8), and their parameters were practically identical to those for the EPR spectrum of this probe in a non-aqueous solvent, which is the dispersion medium of these ointment bases (Table 7).



a



b

Fig. 10. Rheograms of disperse systems: *a* – No. 13; *b* – No. 14 at: 1 – 25 °C, 2 – 30 °C; 3 – 35 °C; 4 – 40 °C;5 – 45 °C; 6 – 50 °C

Therefore, the probe TEMPO-iodide in non-aqueous dispersion systems is soluble in a mixed non-aqueous solvent PG – M400. The EPR spectra of this probe do not provide evidence about the formation of any micelles from M20CE molecules or mixed aggregates from M20CE and CSA molecules in anhydrous hydrophilic systems. However, these substances modified the rheological properties of the mixed solvent PG – M400 and hydrophilic ointment base (disperse system No. 15) (Table 8).

The rheograms of the hydrophilic ointment base (disperse system No. 15) at different temperatures are shown in Fig. 12. Ointment bases to which *M20CE* or a mixture of *M20CE* and *CSA* were added were characterised by the similar rheograms.

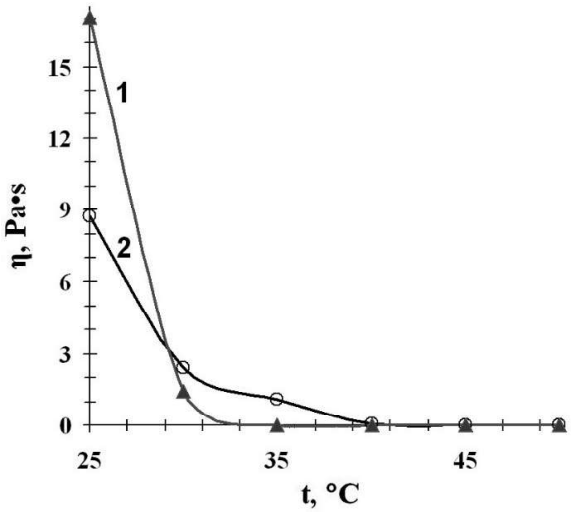


Fig. 11. Apparent viscosity (η) (at D_r=14.55 s^{-1}) of dispersed systems No. 13 (1) and No. 14 (2) *vs* temperature (*t*)

Hydrophilic non-aqueous ointment base No. 15 melted when heated and became a liquid at 35–40 °C (Fig. 12, 13). When *M20CE* or a mixture of 3 % *M20CE* and 7 % *CSA* was added to the formulations, the values of the rheological parameters of these systems at 25–30 °C were greater (Table 8, Fig. 13), but their thermal stability did not increase. At 35 °C, all three ointment bases with the non-aqueous dispersion medium were liquid in consistency.

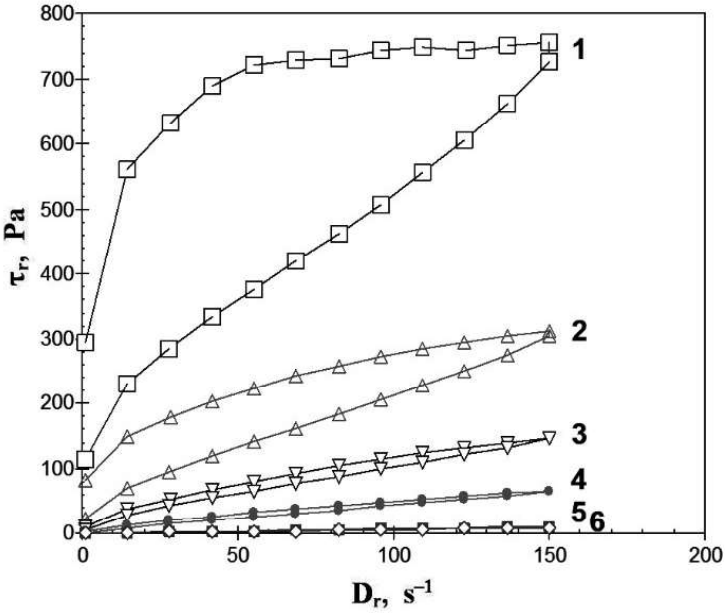


Fig. 12. Rheograms of disperse system No. 15 at: 1 – 25 °C; 2 – 30 °C; 3 – 35 °C; 4 – 40 °C; 5 – 45 °C; 6 – 50 °C

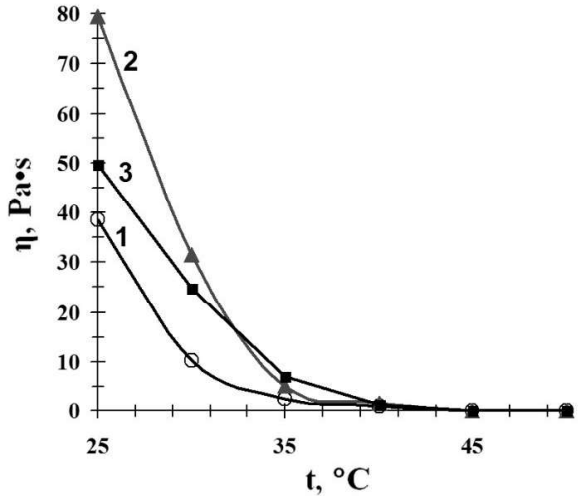


Fig. 13. Apparent viscosity (η) (at D_r=14.55 s^{-1}) of dispersed systems No. 15 (1), No. 16 (2) and No. 17 (3) *vs* temperature (*t*)

Study of PG and M400 release from liquids and bases for semi-solid preparations.

When studying the rheological parameters of disperse systems, it was found that the addition of 3 % *M20CE* and 7 % *CSA* significantly affected the apparent viscosity of dispersed system No. 8, the liquid dispersion medium of which was the mixed solvent *PG – M400 – water*. (Fig. 4, 5). The main functional purpose of *PG* and *M400* on this basis might be related to the moisturising *effect* on the skin [15].

The values of the rheological parameters of the non-aqueous hydrophilic ointment basis were also greater at 25–32 °C if these emulsifiers were included in its composition (disperse systems No. 15 and No. 17) (Fig. 13). *PG* content in such non-aqueous bases is higher, and *PG* can act as a penetration enhancer and promote absorption of exudate during topical treatment of infected wounds. It was interesting to study the effect of the mixture of 3 % *M20CE* and 7 % *CSA* on the *in vitro* release of *PG*. The results of such studies would make it possible to control the release of active substances and their penetration into the affected tissues, as well as to control the dehydration of these tissues under the influence of ointment bases.

Fig. 14 shows the mean release rates of *PG*. The relevant release parameters are given in Table 9.

Plots in Fig. 14 and the values of the correlation coefficients in Table 9 evidence that the dependence of the amount of *PG* released per unit of membrane area on the square root of time was linear in all cycles of the *in vitro* release experiment. This was observed for the release cycles of *PG* from the mixed solvent as well as from the other three dispersed systems No. 8, No. 15, and No. 17. The value of the coefficients of determination was more than 0.99 (acceptance criterion >0.90) [2, 35].

The release rate (*R*) of *PG* from the mixed solvent compared to dispersed system No. 8 was 4.8 times greater. After 6 hours, the cumulative content (*A*), *PG* content in the dialysate (*C*), and *PG* recovery were 4.4 times greater (Table 9). The formation of a spatial network by *M20CE* and *CSA* molecules in a dispersion medium, where water structure was predominant, led to a significant decrease in the parameters of *PG* release from the base in *vitro* experiments.

The release rate (*R*) of *PG* from the mixed solvent compared to the dispersed system No. 15 was 4.7 times less. In the experiment on *PG* release from anhydrous base No. 15, the cumulative content (*A*) and *PG* content (*C*) in the dialysate were 5.4 times greater, and the *PG* recovery was 1.3 times less (Table 9). This was due to the difference in *PG* concentrations in the mixed solvent and hydrophilic ointment base, which were 6 % and 42 % m/m, respectively.

When 3 % M20CE and 7 % CSA were added to the anhydrous ointment base, a significant decrease in *PG* release from dispersion system No. 17 was observed. The average release rate (R) of *PG* decreased by 36.0 %, the cumulative content (*A*) – by 37.9 %, *PG* content in the dialysate (*C*) – by 38.2 %, and *PG* recovery – by 28.0 % (Table 9). That is, the formation of a spatial network by particles or molecules of *M20CE* and *CSA* in a non-aqueous dispersion medium of *PG – M400* led to a certain decrease in the parameters of *PG* release from the hydrophilic water-soluble base.

To continue our research on the development of hydrophilic ointment bases with cationic surfactants [4], a comparative study of the *PG* and *M400* release from solution No. 18 and cream No. 19 was conducted (Table 3). Unlike the systems presented in Tables 1, 2, 17 % P338 was also added to the composition of each of these two disperse systems.

Solution No. 18 was a transparent liquid with Newtonian flow behaviour (Fig. 15) and dynamic viscosity of ~0.29 Ra·s at 25 °C. System No. 19 was a homogeneous white cream with a plastic flow behaviour, thixotropic properties (Fig. 15) and such rheological parameters: τ_0 – 266 Pa, A_N – 15033 Pa·s^{-1}, η (at D_r=14.55 s^{-1}) – 27.86 Pa·s.

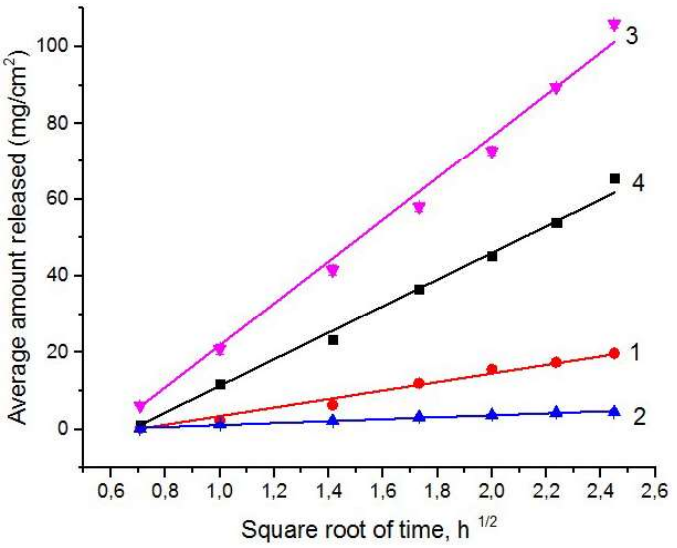


Fig. 14. *PG* release rate plots obtained from the *in vitro* experiments for solvent: 1 – *PG – M400 – water*; 2 – disperse systems (DS) No. 8; 3 – No. 15; 4 – No. 17

Release rate plots for *PG* and *M*400 release from solution No. 18 and cream No. 19 are shown in Fig. 16, and the relevant release parameters are provided in Table 10.

The plots of average release rates (R) in the case of *PG* and *M*400 release from solution No. 18, as well as *PG* release from cream No. 19, were linear since the values of the coefficients of determination were more than 0.90.

The average rate of PG release from solution No. 18 compared to cream No. 19 was 2 times greater. In the case of solution No. 18, the cumulative content, *PG* content in the dialysate, and *PG* recovery were 2.7 times greater compared to the *PG* release from cream No. 19 (Table 10). This can be explained by the different rheological properties of solution No. 18 and cream No. 19 (Fig. 15).

In addition to *PG*, *M*400 was another hydrophilic solvent in solution No. 18 and cream No. 19. Compared to *PG*, the average release rate of *M*400 from solution No. 18 was 2.1 times less. The *M*400 cumulative and *M*400 content in the dialysate were 2.8 times less, and the release rate was 2.5 times less in the case of *M*400 release from solution No. 18 compared to the corresponding parameters of *PG* release from solution No. 18 (Table 10).

First, the difference in the release parameters of PG and M400 from solution No. 18 can be explained by the different molecular weights (M_r) of these solvents; the average M_r *M400* is 383.5 a.m.u. [36]. Furthermore, M_r *PG* is 76.1 a.m.u. [13], i.e., it is 5 times less [13]. Nevertheless, the *M400* release parameters were less, not by 5 times, but by 2.1–2.8 times. One of the factors that slows down the *PG* release is the dipole-dipole interactions between the hydroxyl groups of *PG* and oxygen atoms of P338 [38], the molecules of which were unable to diffuse through the membrane due to high M_r [13].

Table 9

Parameters of *PG* release during *in vitro* experiments (Fig. 14)

Parameter	Results in the case of *PG release from*:			
	Solvent	DS No. 8	DS No. 15	DS No. 17
Release rate (*R*), mg/cm²/h$^{-1/2}$	11.85±0.56 SD: 0.28	2.46±0.03 SD: 0.01	55.97±0.96 SD: 0.48	35.80±0.65 SD: 0.32
Cumulative amount (A) (at the time the point 6 h), mg/cm²	19.78±1.02 SD: 0.50	4.54±0.08 SD: 0.04	105.84±1.94 SD: 0.96	65.39±2.22 SD: 1.10
PG content (C) in the receptor medium (at the time point 6 h), mg/ml	2.329±0.120 SD: 0.06	0.534±0.009 SD: 0.004	12.463±0.229 SD: 0.11	7.699±0.26 SD: 0.13
Correlation coefficient r	0.99344 SD: 0.00188	0.99232 SD: 0.001	0.99640 SD: 0.001	0.99682 SD: 0.00169
Coefficient of determination R^2	0.987	0.985	0.993	0.994
Recovery (at the time point 6 h), %	77.65±3.99 SD: 1.98	17.81±0.30 SD: 0.15	59.35±1.09 SD: 0.54	42.77±1.45 SD: 0.72

Table 10

Parameters of *PG* and *M*400release during *in vitro* experiments (Fig. 16)

Parameter	Value in case of release:			
	PG from liquid No. 18	*PG* from cream No. 19	*M*400 from liquid No. 18	*M*400 from cream No. 19
Release rate (*R*), mg/cm²/h$^{-1/2}$	16.87±1.22 SD: 0.60	8.40±0.31 SD: 0.15	7.92±0.08 SD: 0.04	ND
Cumulative amount (A) (at the time the point 6 h), mg/cm²	39.50±2.49 SD: 1.23	14.93±0.66 SD: 0.33	14.16±0.16 SD: 0.08	ND
Content (C) in the receptor medium (at the time point 6 h), mg/ml	4.651±0.293 SD: 0.15	1.758±0.078 SD: 0.04	1.668±0.02 SD: 0.01	ND
Correlation coefficient *r*	0.99522 SD: 0.00305	0.99661 SD: 0.00121	0.99377 SD: 0.00027	ND
Coefficient of determination R^2	0.990	0.993	0.988	ND
Recovery (at the time point 6 h), %	93.02±5.86 SD: 2.91	35.17±1.56 SD: 0.77	37.06±0.42 SD: 0.21	ND

Note: ND – M400 was not detected in the samples of receptor medium

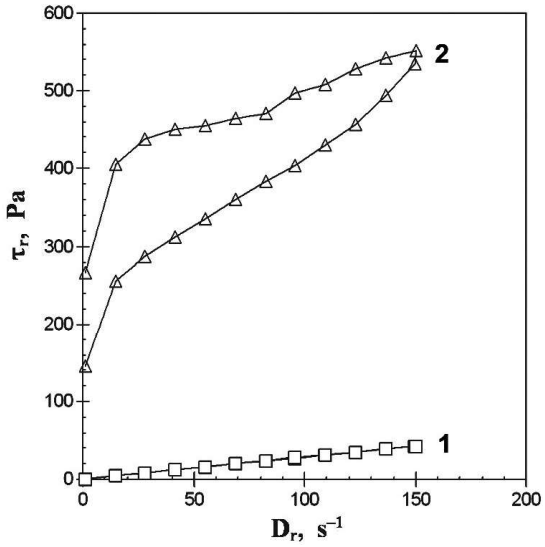


Fig. 15. Rheograms of: 1 – liquid No. 18; 2 – cream No. 19

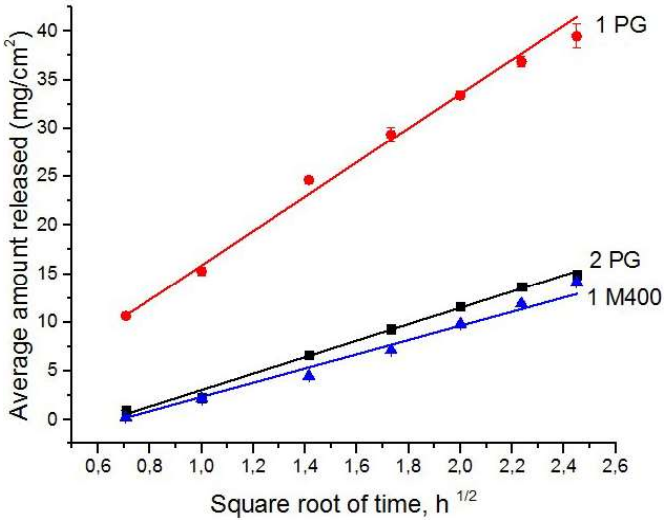


Fig. 16. *PG* and *M*400 release rate plots obtained from the *in vitro* experiments for liquid No. 18 (1) and cream No. 19 (2)

The parameters of *PG* release significantly decreased in the case of the cream base No. 19. In addition, during *in vitro* experiments, neither M400 fraction was released from cream No. 19 (Table 10). Such an effect regarding the release of solvents cannot be explained only by the high rheological parameters of the base No. 19. This was probably due to a change in the functional properties of the cationic surfactant [4]. This phenomenon needs to be further studied using different methods.

Study of water diffusion into the chamber with disperse system.

If a permeable membrane separates water and the hydrophilic system, there are two oppositely directed diffusion processes:

1) *PG* and *M400* penetrate a chamber with water;

2) water diffuses into a chamber with a disperse system [14].

The quantitative determination of water in a chamber with a hydrophilic system can be performed by the K. Fisher method [13, 14], but, generally, this study can be performed by the evaluation of change in mass (Δm) of the contents in the chamber with a hydrophilic system.

If the diffusion chamber contains solutions of hydrophilic substances with a high *Mr* that do not diffuse through the membrane, this approach is quite correct. However, this approach does not take into account the diffusion of hydrophilic substances with low *Mr* into the chamber with water. In this case, the researcher does not receive exact quantitative results but can assess the kinetics of the process and the conditional mass of absorbed water per 1 cm^2 of the membrane area.

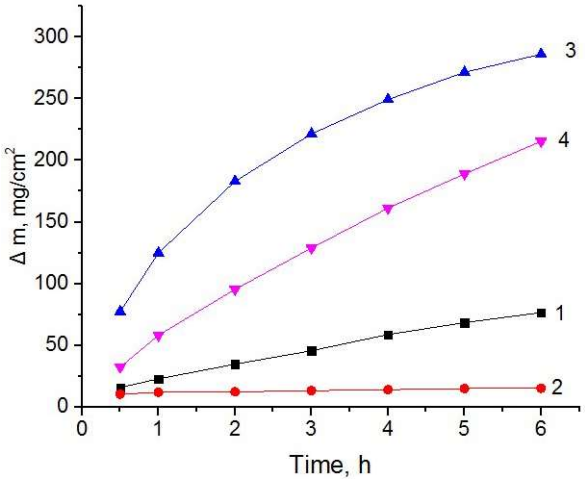


Fig. 17. Kinetics of changes in the conditional mass of absorbed water (Δm) in the case of solvent: 1 – *PG – M400 – water*; 2 – disperse system No. 8; 3 – disperse system No. 15; 4 – disperse system No. 17

As shown in Fig. 17, all 4 studied systems absorbed water for 6 hours. The conditional amount of water absorbed by the solvent *PG – M400 – water* was 78.2 mg/cm^2, and by the dispersed system No. 8 – 15.5 mg/cm^2, which was 5.0 times less. The conditional amount of water absorbed by dispersion system No. 15 was 282 mg/cm^2, and by dispersion system No. 8 – 219 mg/cm^2, which was 1.3 times less.

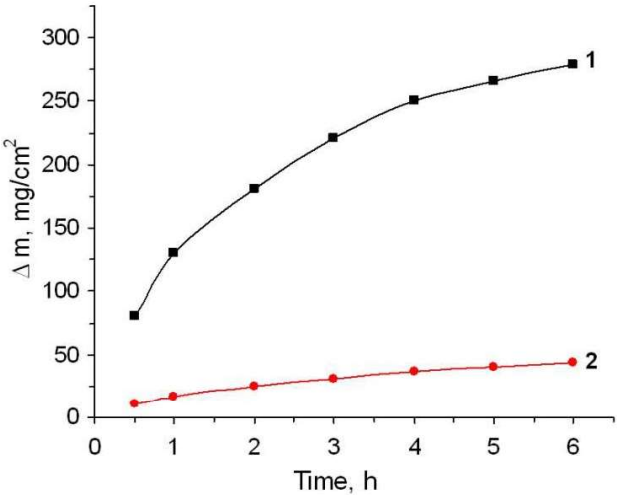


Fig. 18. Kinetics of changes in the conditional mass of absorbed water (Δm) in the case of: 1 – solution No. 18; 2 – cream No. 19

The cumulative amount (A) of *PG* after its release from the mixed solvent *PG – M400 – water* was 19.78 mg/cm^2, and from dispersed system No. 8 – 4.54 mg/cm^2, which was 4.4 times less. The cumulative amount of *PG* after its release from dispersed system No. 15 was 105.84 mg/cm^2, and from dispersed system No. 17 – 65.39 mg/cm^2, which was 1.6 times less (Table 9).

According to the results of the research, the addition of 3 % *M20CE* and 7 % *CSA* into dispersed systems was a significant factor in reducing *PG* release and the water absorption by systems with the prevalence of water structure and hydrophilic systems with an anhydrous medium.

Solution No. 18 and cream No. 19 contained about 50 % m/m of water. During 6 hours, the conventional amount of water absorbed by solution No. 18 was 279.25 mg/cm^2 (Fig. 18). That is, the amount of water absorbed by this solution exceeded the total cumulative amount of *PG* and *M400* 53.66 mg/cm^2 (Table 10), which diffused into the chamber with water, by approximately 5.2 times.

If 6.0% CSA was added to cream No. 19 (Table 3), the conditional amount of absorbed water was 44.0 mg/cm^2 (Fig. 18), i.e., it was 6.3 times less than in the case of solution No. 18. The cumulative amount of *PG* released into the chamber with water from cream No. 19 within 6 hours was about 15.0 mg/cm^2 (Table 10). That is, the conventional amount of water absorbed by cream No. 19 exceeded the cumulative amount of *PG* diffused into the chamber with water by only about 2.9 times. Thus, the introduction of CSA to cream No. 19 changed the ratio between solvent release and water absorption.

5. Discussion of research results

Studies were conducted with three types of bases-vehicles, which differed in the structure of the dispersion medium. A significant factor that increased the

value of their rheological parameters for the studied bases, reduced the release rate and amount of *PG* during *in vitro* experiments. Reduced water absorption by the bases was *CSA*, which was included in their composition in certain mass ratios with surfactants. However, the mechanisms of this influence were different depending on the structure of the dispersion medium of the base vehicle.

In the bases, where the structure of water prevailed, in the supramolecular structures formed by surfactants and CSA at certain mass ratios, lateral phase separation occurred with the formation of liquid domains formed mainly by *M20CE* and solid domains formed mainly by *CSA* [29]. The solid state of those domains is one of the prerequisites for the formation of coagulation structures in the system (Table 6, Fig. 5). This phenomenon was previously studied using the lipophilic spin probe 4-palmitamido-2,2,6,6-tetramethylpiperidine-1-oxyl (CAS [22977-65-7]) [29]. Upon heating, the lipophilic probe was redistributed into domains formed mainly by hydrophilic surfactant. According to the changes in the shape of the EPR spectra, the solubilisation of the lipophilic probe was wrongly associated with the melting of solid *CSA* domains [29]. In this work, a probe simulating a cationic surfactant was used. In the case of the hydrophilic probe, with increasing temperature, on the contrary, there was a redistribution into the phase formed mainly by *CSA* (Fig. 6), which was evidenced by a decrease in the *f* parameter. That is, the solid-state of *CSA* domains remained at fairly high temperatures, up to 45 °C (Fig. 6). This was the reason that the apparent viscosity of the bases up to the *CSA* melting temperature remained at an almost constant level (Fig. 7).

The formation of the base with a plastic flow behaviour and thixotropic properties with the addition of 3 % *M20CE* and 7 % *CSA* (Fig. 5) reduced the release rate of PG by 4.8 times, the recovery – by 4.4 times (Table 9) and the mass of water absorbed by the base – by 5.0 times (Fig. 17) compared to the mixed solvent *PG – M400 – water*.

The inclusion of *M20CE* and *CSA* into the mixed non-aqueous solvent *PG – M400* led to the formation of coagulation structures at 25 °C (Fig. 10). But in anhydrous medium micelles did not form from *M20CE* molecules as well as mixed aggregates did not form from *M20CE* and *CSA* molecules (Table 7, Fig. 8). *M20CE* and *CSA* at 25 °C became the dispersed phase of suspensions (Fig. 9), which contributes to the formation of gels. Nevertheless, when the temperature rose, the structures of these gels on anhydrous bases were destroyed due to the dissolution and/or melting of *M20CE* and *CSA* (Fig. 10).

The addition of *M20CE* and *CSA* to the ointment base with a hydrophilic anhydrous medium also led to an increase in its apparent viscosity (Fig. 13) but did not enhance the thermal stability; at 35 °C, ointment bases with a hydrophilic non-aqueous dispersion medium became a liquid for the above-mentioned reasons (Fig. 12, 13).

The inclusion of 3 % *M20CE* and 7% *CSA* into hydrophilic anhydrous bases reduced the release rate of *PG* by 1.6 times, the *PG* recovery – by 1.4 times (Table 9), and the conditional amount of water absorbed by the base – by 1.3 times (Fig. 17). Thus, the influence of *M20CE* and *CSA* on *PG* release and water absorption was significantly less compared to bases with the medium where the structure of water dominated.

The addition of 6.0 % CSA into the solution containing 41 % hydrophilic non-aqueous components and 0.5 % cationic surfactant led to significant changes in the rheological properties: Newtonian flow behaviour changed to plastic flow, the thixotropic properties appeared, and apparent viscosity became high (Fig. 15). As a result, the release rate of PG decreased by 2 times, and the release amount (recovery) – by 2.7 times (Table 10). It should be noted that the absorption of water by the base compared to the solution decreased by 6.3 times. Thus, this cream base is characterised by the low capacity to absorb water (Fig. 18) with fairly high parameters of *PG* release (Table 10). The apparent viscosity of this bases type depended little on temperature [4].

M400 was released from solution No. 18 during *in vitro* experiments (Table 10). The release rate and released amount of *M400* from solution No. 18 compared to *PG* were 2.1 and 2.5 times less, respectively, with a 5-fold difference in their molecular weights. This difference can be explained by the interaction between *PG* and *P338* [38], which reduced the PG release. From cream No. 19, to which 6 % *CSA* was added, *M400* was not released during *in vitro* experiments (Table 10).

Study limitations. *M400* was not released from cream No. 19, probably due to complex interactions between the components of the base, which should be further investigated.

CSA is a mixture of solid aliphatic alcohols, mainly cetyl and stearyl alcohols and may contain up to 10 % of other alcohols [13]. A limitation of this work is the study using only one brand of *CSA*, i.e. the lack of data on the risks of changes in apparent viscosity, other rheological parameters, and *in vitro* release of PG when using *CSA* with a different ratio between cetyl and stearyl alcohols.

Prospects for further research. This work should get the attention of researchers to the fact that the composition of emulsifiers (in particular, *CSA* content) in creams and ointments on hydrophilic bases might be a critical factor in the study to confirm an extended pharmaceutical equivalence. In addition, the results of this work could be used for the choice of base vehicles for the development of semi-solid preparations according to the specific requirements imposed on them, for example, the rheological properties of topical semi-solid preparations during their administration, that is, when the temperature changes from 25 °C to 32–36 °C. *CSA* should not be considered an inert excipient that affects only rheological parameters [23] since the *PG* release, and the water absorption by hydrophilic bases significantly depend on *CSA*. It is also not correct to assess the effect of surfactants or *PG* on the *in vitro* release of active substances [23, 27] separately because, in the presence of *CSA*, their release can change significantly.

6. Conclusions

The addition of *CSA* in combination with surfactants into the bases for semi-solid preparations is a significant factor for modifying their rheological parameters, the kinetics of *PG* release from them as well as water absorption in experiments *in vitro*. The mechanisms of such an effect are different and depend on the composition and structure of the dispersion medium of the base.

Conflict of interests

The authors declare that they have no conflict of interest in relation 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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References

1. Buckingham, R. (Ed.) (2020). Martindale: The Complete Drug Reference. London: Pharmaceutical Press, 4912.

2. Ilić, T., Pantelić, I., Savić, S. (2021). The Implications of Regulatory Framework for Topical Semisolid Drug Products: From Critical Quality and Performance Attributes towards Establishing Bioequivalence. Pharmaceutics, 13 (5), 710. doi: https://doi.org/10.3390/pharmaceutics13050710

3. Shanley, A. (2016). Topical Formulation: Moving from Art to Science. APIs, Excipients, and Manufacturing 2016 Supplement to Pharmaceutical Technology, 40 (9).

4. Bezuglaya, E., Lyapunov, N., Lysokobylka, O., Liapunov, O., Klochkov, V., Grygorova, H., Liapunova, A. (2021). Interaction of surfactants with poloxamers 338 and its effect on some properties of cream base. ScienceRise: Pharmaceutical Science, 6 (34), 4–19. doi: https://doi.org/10.15587/2519-4852.2021.249312

5. Wu, K., Yeoh, T., Hsieh, Y. L., Osborne, D. W.; Langley, N., Michniak-Kohn, B., Osborne, D. W. (Eds.) (2019). Quality Assesment of API in Topical Drug Products. The Role of Microstructure in Topical Drug Product Development. Cham: Springer, 36, 109–154. doi: http://doi.org/10.1007/978-3-030-17355-5_4

6. Guideline on the Investigation of Bioequivalence (2010). CPMP/EWP/QWP/1401/98 Rev. 1 / Corr **. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf

7. Raghavan, L., Brown, M., Michniak-Kohn, B., Sammeta, S.; Langley, N., Michniak-Kohn, B., Osborne, D. W. (Eds.) (2019). Quality Assesment of API in Topical Drug Products. The Role of Microstructure in Topical Drug Product Development. Springer: Cham, 36, 47–87. doi: http://doi.org/10.1007/978-3-030-17355-5_2

8. SUPAC-SS. Guidance for Industry Nonsterile Semisolid Dosage Forms. Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation (1997). Rockville: Center for Drug Evaluation and Research (CDER). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/supac-ss-nonsterile-semisolid-dosage-forms-scale-and-post-approval-changes-chemistry-manufacturing

9. Draft guideline on quality and equivalence of topical products (2018). CHMP/QWP/708282/2018. Available at: www.ema.europa.eu/en/quality-equivalence-topical-products

10. The United States Pharmacopoeia, 41 – NF 36 (2018). The United States Pharmacopoeial Convention. Rockville. Available at: https://www.worldcat.org/title/united-states-pharmacopeia-2018-usp-41-the-national-formulary-nf-36/oclc/1013752699

11. Miranda, M., Veloso, C., Brown, M., C. Pais, A. A. C., Cardoso, C., Vitorino, C. (2022). Topical bioequivalence: Experimental and regulatory considerations following formulation complexity. International Journal of Pharmaceutics, 620, 121705. doi: https://doi.org/10.1016/j.ijpharm.2022.121705

12. Pleguezuelos-Villa, M., Merino-Sanjuán, M., Hernández, M. J., Nácher, A., Peris, D., Hidalgo, I. et. al. (2019). Relationship between rheological properties, in vitro release and in vivo equivalency of topical formulations of diclofenac. International Journal of Pharmaceutics, 572, 118755. doi: https://doi.org/10.1016/j.ijpharm.2019.118755

13. The European Pharmacopoeia (2019). European Directorate for the Quality of Medicines & HealthCare of the Council of Europe. Strasbourg, 5224.

14. Bezuglaya, E., Ivashchenko, H., Lyapunov, N., Zinchenko, I., Liapunova, A., Stolper, Y. et. al. (2021). Study of factors affecting the in vitro release of diclofenac sodium from hypromelose-based gels. ScienceRise: Pharmaceutical Science, 5 (33), 12–31. doi: https://doi.org/10.15587/2519-4852.2021.243040

15. Sheskey, P. J., Hancock, B. C., Moss, G. P., Goldfarb, D. J. (Ed.) (2020). Handbook of Pharmaceutical Excipients. London: Pharm. Press, 1296.

16. D'souza, A. A., Shegokar, R. (2016). Polyethylene glycol (PEG): a versatile polymer for pharmaceutical applications. Expert Opinion on Drug Delivery, 13 (9), 1257–1275. doi: https://doi.org/10.1080/17425247.2016.1182485

17. Bodratti, A., Alexandridis, P. (2018). Formulation of poloxamers for drug delivery. Journal of Functional Biomaterials, 9 (1). doi: https://doi.org/10.3390/jfb9010011

18. Mangas-Sanjuán, V., Pleguezuelos-Villa, M., Merino-Sanjuán, M., Hernández, M. J., Nácher, A., García-Arieta, A. et. al. (2019). Assessment of the Inter-Batch Variability of Microstructure Parameters in Topical Semisolids and Impact on the Demonstration of Equivalence. Pharmaceutics, 11 (10), 503. doi: https://doi.org/10.3390/pharmaceutics11100503

19. Miranda, M., Pais, A. A. C. C., Cardoso, C., Vitorino, C. (2019). aQbD as a platform for IVRT method development – A regulatory oriented approach. International Journal of Pharmaceutics, 572, 118695. doi: https://doi.org/10.1016/j.ijpharm.2019.118695

20. Miranda, M., Cova, T., Augusto, C., Pais, A. A. C. C., Cardoso, C., Vitorino, C. (2020). Diving into Batch-to-Batch Variability of Topical Products-a Regulatory Bottleneck. Pharmaceutical Research, 37 (11). doi: https://doi.org/10.1007/s11095-020-02911-y

21. Xu, Z., Mangas-Sanjuán, V., Merino-Sanjuán, M., Merino, V., García-Arieta, A. (2020). Influence of Inter- and Intra-Batch Variability on the Sample Size Required for Demonstration of Equivalent Microstructure of Semisolid Dosage Forms. Pharmaceutics, 12 (12), 1159. doi: https://doi.org/10.3390/pharmaceutics12121159

22. Benaouda, F., Brown, M. B., Ganguly, S., Jones, S. A., Martin, G. P. (2012). Discriminating the Molecular Identity and Function of Discrete Supramolecular Structures in Topical Pharmaceutical Formulations. Molecular Pharmaceutics, 9 (9), 2505–2512. doi: http://doi.org/10.1021/mp300127f

23. Miron, D. S., Rădulescu, F. Ștefan, Voicu, V. A., Mînea, A., Cardot, J.-M., Shah, V. P. (2021). Rheological and in vitro release measurements of manufactured acyclovir 5% creams: confirming sensitivity of the in vitro release. Pharmaceutical Development and Technology, 26 (7), 779–787. doi: https://doi.org/10.1080/10837450.2021.1945625

24. Benaouda, F., Jones, S. A., Martin, G. P., Brown, M. B. (2015). Localized Epidermal Drug Delivery Induced by Supramolecular Solvent Structuring. Molecular Pharmaceutics, 13 (1), 65–72. doi: https://doi.org/10.1021/acs.molpharmaceut.5b00499

25. Carrer, V., Alonso, C., Pont, M., Zanuy, M., Córdoba, M., Espinosa, S. et. al. (2019). Effect of propylene glycol on the skin penetration of drugs. Archives of Dermatological Research, 312 (5), 337–352. doi: https://doi.org/10.1007/s00403-019-02017-5

26. Zhang, W., Harty, B., Zheng, Y., Zhang, Z., Li, X., Wang, D., Kohane, D. S. (2021). Permeation of polyethylene glycols across the tympanic membrane. Giant, 6, 100057. doi: https://doi.org/10.1016/j.giant.2021.100057

27. Laffleur, F., Pschick, S., Barthelmes, J., Hauptstein, S., Bernkop-Schnurch, A. (2018). Impact of Surfactants on Skin Penetration of Dexpanthenol. Current Drug Delivery, 15 (3), 351–356. doi: https://doi.org/10.2174/1567201814666170503142707

28. Kováčik, A., Kopečná, M., Vávrová, K. (2020). Permeation enhancers in transdermal drug delivery: benefits and limitations. Expert Opinion on Drug Delivery, 17 (2), 145–155. doi: https://doi.org/10.1080/17425247.2020.1713087

29. Liapunov, M. O., Ivanov, L. V., Bezuhla, O. P., Zhdanov, R. I., Tsymbal, L. V. (1992). Doslidzhennia ahrehativ poverkhnevo-aktyvnykh rechovyn (PAR) metodom spinovykh zondiv. Farmatsevtychnyi zhurnal, 5-6, 40–45.

30. Derzhavna Farmakopeia Ukrainy. Tom 1 (2015). Kharkiv: Derzhavne pidpryiemstvo «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv», 1128.

31. Krasnoperova, A. P., Sytnik, O. Iu., Iukhno, G. D., Bezuglaia E. P., Liapunov, N. A., Krichevskii, A. O. (2002). Nekotorye zakonomernosti rastvoreniia i solvatatcii mikonazola v dvoinykh i troinykh smeshannykh rastvoriteliakh na osnove polietilenglikolia-400. Zhurnal prikladnoi khimii, 75 (12), 1972–1975.

32. Berliner, L. (Ed.) (1979). Metod spinovykh metok. Teoriia i primenenie. Moscow: Mir, 635.

33. Likhtenshtein, G. I. (1974). Metod spinovykh zondov v molekuliarnoi biologii, Moscow: Nauka, 256.

34. Kuznetcov, A. N. (1976). Metod spinovogo zonda (Osnovy i primenenie). Moscow: Nauka, 210.

35. Tiffner, K. I., Kanfer, I., Augustin, T., Raml, R., Raney, S. G., Sinner, F. (2018). A comprehensive approach to qualify and validate the essential parameters of an in vitro release test (IVRT) method for acyclovir cream, 5 %. International Journal of Pharmaceutics, 535 (1-2), 217–227. doi: https://doi.org/10.1016/j.ijpharm.2017.09.049

36. Bezuglaya, E., Lyapunov, N., Bovtenko, V., Zinchenko, I., Stolper, Y. (2021). Study of pressurised metered dose inhalers for the purpose of standardization of quality attributes characterizing uniformity of dosing. ScienceRise: Pharmaceutical Science, 4 (32), 11–23. doi: https://doi.org/10.15587/2519-4852.2021.238294

37. Note for Guidance on Validation of Analytical Procedures: Text and Methodology, Step 5 (1995). CPMP/ICH/381/95 (ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology).

38. Ivanova, R., Alexandridis, P., & Lindman, B. (2001). Interaction of poloxamer block copolymers with cosolvents and surfactants. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 183-185, 41–53. doi: https://doi.org/10.1016/s0927-7757(01)00538-6

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