STUDY OF THE FORMATION OF MICELLES AND THEIR STRUCTURE BY THE SPIN PROBE METHOD

Elena Bezuglaya, Nikolay Lyapunov, Valentyn Chebanov, Oleksii Liapunov

The aim. *To study the surfactant solutions depending on the type and concentration of surfactants as well as their interaction with some excipients by spin probe method.*

Materials and methods. *Solutions of ionic and nonionic surfactants containing 4 spin probes differing in molecular structure and solubility were studied. Electronic paramagnetic resonance (EPR) spectra were obtained and their type and parameters were determined. The critical micelle concentration (CMC) was determined from the surface tension isotherm, and the rheological parameters were studied by rotational viscometry.*

Results. *The shape of the EPR spectra and the spectral parameters of the spin probes depended on both the surfactant concentration and the molecular structure and solubility of these spin probes. There was a concentration range in which associations with surfactants formed at surfactant concentrations below the CMC. At surfactant concentrations above the CMC and up to 1 %, the structure of the surfactant micelles did not change. In the micelles, the surfactant modelling probes rotated rapidly about the long axis of the molecule and perpendicular to it, while they were fixed in the radial direction. The rotational diffusion of probes dissolved in water was much faster. The micelle cores formed by nonionic surfactant and P338 were more viscous compared to ionic surfactants. Surfactant micelles were anisotropic in viscosity, and different segments of the alkyl chains of surfactant modelling probes had different dynamic properties. The packing of molecules in the micelles was more ordered and compacted at the level of the fifth carbon atom. The interactions between surfactant and probe and between cationic surfactant and disodium edetate were determined from the parameters of the EPR spectra. The relationship between the changes in the parameters of the EPR spectra with increasing temperature, the P338 content in the solutions, and the sol-gel transition was revealed. Solubilization of lipophilic substances by P338 solutions increased due to the interaction of propylene glycol and P338.*

Conclusions. *The shape and parameters of the EPR spectra in real solutions and micellar solutions of surfactants were different and also depended on the structure and solubility of spin probes. Surfactant micelles were anisotropic in viscosity, and different segments of the alkyl chains of surfactant modelling probes had different dynamic properties. The packing of molecules in the micelles was more ordered and compacted at the level of the fifth carbon atom. The EPR spectra and/or their parameters changed due to the interaction between surfactant and probe, surfactant and other substances, or sol-gel transitions in P338 solutions*

Keywords: *surfactant, poloxamer P338 (P338), solution, micelles, spin probe, EPR spectrum, spectrum parameters, viscosity*

1. Introduction

Surfactants are widely used in pharmacy due to their physicochemical, biological and functional properties, mainly as excipients [1]. Among them, hydrophilic surfactants with low molecular mass and a diphilic structure of molecules classified as non-ionic and ionic (anionic, cationic, and ampholytic) are of greatest importance for practical application. Surfactants are used as emulsifiers, solubilizers, foaming agents, wetting agents, release agents, etc. Cationic surfactants are mainly used as antimicrobial preservatives and antiseptics.

Poloxamers, copolymers consisting of a hydrophobic polyoxypropylene segment and two hydrophilic polyoxyethylene chains, also possess surface-active properties and are non-ionic surfactants [1, 2]. Poloxamers are used as emulsifying, solubilizing, stabilizing, and dispersing agents, as binders for tablets and coatings, and as bioadhesive materials or wetting agents in topical preparations, etc. [1, 2]. Solutions of some poloxamers are thermoreversible systems, i.e., Newtonian fluids could be converted into gels by heating and became fluids again after cooling [3, 4]. This phenomenon is exploited for drug development [5].

The ability to reduce the surface tension (σ) of aqueous solutions and to form micelles and lyotropic liquid crystals are the key properties of hydrophilic surfactants. Poloxamers in aqueous solutions also form micelles; lyotropic liquid crystals with different structures can be formed provided high content of poloxamers [6]. Non-ionic surfactant can influence the surface-active properties of poloxamers [7]. The physicochemical properties of surfactants and poloxamers are very important

for pharmaceutical development because the performance characteristics of medicinal products depend on them [8, 9]. Micelles could solubilize lipophilic substances. Thermally induced sol-gel transition in solutions of poloxamers and solubilization of hydrophobic substances by their micelles have been studied to develop extended-release subcutaneous preparations and drug delivery systems with controlled release of active substances [9, 10]. Solubilization depends on the structure of the poloxamer micelles, which can be influenced by components of the dispersion medium, e.g., glycols [11, 12].

The formation of spherical micelles occurs when the surfactant content reaches the critical micelle concentration (CMC), which can be measured by various techniques. The most commonly used method is the direct determination of CMC by the electrical conductivity method or the use of the isotherm of surface tension, osmotic pressure, turbidity, etc. [13]. An abrupt change in the property as a function of surfactant concentration marks CMC [13]. In addition, indirect methods associated with the observation of materials other than the micelle-forming surfactant (e.g., fluorescent probes) are used to study CMC and micelles [14]. Spectrometric methods are the most widely used in this type of CMC measurement [13].

It is worth mentioning the topicality of studies on the formation and structure of micelles and on the dynamic properties of surfactant molecules. There are numerous practical studies and theoretical papers dealing with this problem. For example, the low-angle X-ray scattering method with the construction of triangular phase diagrams has been used to determine different forms of lyotropic liquid crystals formed by poloxamer 407 [11]. The concept of micelles as two-dimensional liquid and one-dimensional solid objects is developed, the thermodynamics of micelles as phase particles, the electrostatics of ionic micelles, the theory of polymorphism and polydispersity of micelles are substantiated [15].

In recent decades, electronic paramagnetic resonance spectroscopy (EPR) has been most widely used to study model and biological membranes [16]. Two approaches should be mentioned here: first, obtaining information through the EPR spectra of spin labels covalently bonded to proteins [17, 18], and second, using spin probes (e.g., based on phospholipids or fatty acids) containing nitroxyl radicals in molecules to study lipid layers [19, 20]. The spin probe technique was used for the first time to obtain information on the mobility and spatial arrangement of molecules in lipid membranes [16].

The spin probe method is also very promising for the study of surfactant associations. The various spin probes simulating surfactants containing nitroxyl radicals in different segments of the alkyl chain, as well as hydrophilic probes that could be localized in the polar part of the micelles, can be used to obtain comprehensive information about the structure of surfactant micelles and the hydration of radicals [16, 21]. Using the EPR spectra of the probe 4 palmitamido-2,2,6,6-tetramethylpiperidine-1-oxyl, it was shown that the micelle nuclei are two-dimensionally liquid in terms of consistency and

anisotropic in terms of viscosity at 25 °C, but the solid-like state in the radial direction remained the subject of discussion [22]. In another work, the results of studies on the microviscosity of micelles for three ionic surfactants are presented using the EPR spectra of 5-doxylstearic acid (5-DSC) and 16-DSC [23]. It was found that the microviscosity increased abruptly at higher surfactant concentration due to the solubilization of the probes dissolved in water by micelles, which allowed the determination of CMC. However, these lipophilic probes are insoluble in water and their EPR spectra in water could not be triplets with narrow lines. Such spectra are typical for water-soluble probes [24], so the results of [23] should be critically evaluated.

The aim was the study of surfactant solutions depending on the type and concentration of surfactants and their interaction with some excipients using the spin probe method.

2. Planning (methodology) of the research

It was planned to use cationic surfactant (benzalkonium chloride), anionic surfactant (sodium lauryl sulphate), non-ionic surfactants (macrogol 20 cetostearyl ether, macrogol 40 stearate) and poloxamer 338 (P338). The research objects should be:

1) aqueous solutions of surfactants and P338 at different concentrations;

2) solutions of P338 and gels formed from P338 solutions with increasing temperature;

3) solutions of benzalkonium chloride and disodium edetate used as preservative systems [2];

4) solutions of P338 in mixed solvents water-propylene glycol (PG), since PG interacts with the oxygen atoms of both the oxyethyl and oxypropyl groups of P338 by forming hydrogen bonds [11].

A series of spin probes were selected for the study, one simulating water-soluble ionic surfactants and three probes simulating lipophilic surfactants based on fatty acids with different positions of the nitroxyl radicals, allowing an evaluation of the dynamic properties of the different segments of the alkyl chain in the micelle nuclei [16]. The spin probe TEMPO was also used, which is partially soluble in water and partially dissolvable by micelles [16].

Where appropriate, experiments using the spin probe method should be supplemented by the determination of CMC of surfactants and rheological studies of liquids and gels. The results of complex studies with different methods could provide an opportunity to discuss changes in the parameters of EPR spectra of different spin probes depending on changes in the physico-chemical properties of the studied objects and to explain the mechanisms of these changes. On the other hand, the results of such research could provide a rationale for the choice of surfactants and P338 at the stage of development of drugs with certain performance characteristics.

3. Materials and methods

Benzalkonium chloride (BC) («Fef Chemicals A/S»); sodium laurilsulfate (Kolliphor® SLS) – (SLS)

(«BASF»); macrogol cetostearyl ether (Kolliphor® CS 20) – (M20CE) («BASF»); macrogol 40 stearate (Crodet S40) – (**M40S**) («Croda»); poloxamer 338 (Kolliphor® P 338) – (P338) («BASF»); propylene glycol (Kollisolv® PG) – (PG) («BASF»); disodium edetate – (DSE) («Sigma-Aldrich»); water purified (water) were used for the experiments. These substances met the requirements of the monographs of the European Pharmacopoeia [1].

Solutions of surfactants at concentrations up to 1 % were prepared by mass/volume and at higher concentrations – by mass. Surfactants were dissolved in water at 50–60 °C and then solutions were cooled up to 25 °C. P338 was dissolved in water or in mixed solvent *water – PG* at 6–8 °C followed by degassing of the solutions and heating them to 25 °C.

Surface tension (σ) was measured by maximum bubble pressure method at (25,0±0,1) °C; CMC values was determined using surface tension isotherms [13].

Rheograms (plots of the shear stress (τ_r) vs the shear rate (D_r)) were obtained at certain temperature by rotational viscometry [1] using a rotating viscometer «Rheolab QC» with coaxial cylinders CC-27 (for creams and gels) and DG-42 (for liquids) («Anton Paar GmbH»; software RHEOPLUS, 2.66 version). Rheograms were used to characterize the flow behavior as well as to determine the yield stress (τ_0) and the apparent viscosity (η) of non-Newtonian fluids or the dynamic viscosity (η) of Newtonian liquids.

Electron paramagnetic resonance (EPR) spectroscopy was used for the research [16, 25]. The following spin probes were used:

– **probe 1**: 4-[(N,N-dimethyl-N-hexadecyl)ammonio]-2,2,6,6-tetramethyl-piperidine-1-oxyl iodide (M_r 551,65; CAS [114199-16-5]) (TEMPO-iodide);

– **probe 2**: 4-Palmitamido-2,2,6,6-tetramethylpiperidine-1-oxyl (M_r 409,67; CAS 22977-65-7]) (4-palmitamido-TEMPO);

– **probe 3**: 5-DOXYL Stearic acid (M_r 384.57; CAS [29545-48-0]) (5-DSA);

– **probe 4**: 16-DOXYL Stearic acid (M_r 384,57; CAS [53034-38-1]) (16-DSA);

– **probe 5**: 2,2,6,6-Tetramethylpiperidine 1-oxyl (M_r 156,25; CAS: [2564-83-2]) (TEMPO).

Probe 1 simulates cationic surfactants that can be dissolved in water as well as localized in surfactant micelles. Probes 2, 3, and 4 are practically insoluble in water and can be dissolved by surfactant micelles. The free radical in probes 2, 3 and 4 is located at the level of the different carbon atoms of the alkyl chains. The probe TEMPO could be simultaneously soluble in water and solubilized by micelles or lipophilic phases. When the phases of the probe localization differ significantly in the polarity of the radical environment, the EPR spectrum of the TEMPO probe was superimposed and the signal was split into two lines in the high field [16, 26].

The spin probes were added to the studied systems at a concentration of 10^{-4} mol/l. The EPR spectra were recorded with the "ESR Spectrometer CMS8400" ("Adani"). The type of EPR spectra (triplet, anisotropic spectrum, singlet, superposition spectrum) was determined. The hyperfine splitting constant (A_N) characteriz-

ing the micropolarity of the environment near the vicinity of radical, the peak heights, and the linewidth at the low field (ΔH_{+1}) and central (ΔH_0) components were determined. The rotational correlation times of the spin probes (τ_{+1}, τ_{-1}, $\tau_{\pm1}$) and the anisotropy parameter (ε) were calculated using the following equations [16, 25]:

$$\tau_{+1} = \left(\sqrt{\frac{h_0}{h_{+1}}} - 1 \right) \cdot \Delta H_0 / 2 \cdot 10^8, \qquad (1)$$

$$\tau_{-1} = \left(\sqrt{\frac{h_0}{h_{-1}}} - 1 \right) \cdot \Delta H_0 / 3.6 \cdot 10^9, \qquad (2)$$

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

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

where h_{+1}, h_0 and h_{-1} are the peak-to-peak heights at the low-field, central and high-field components of EPR spectrum; ΔH_{+1} and ΔH_0 are the linewidth at low-field and central components, respectively.

The reorientation of nonspherical molecules dissolved in a liquid is characterized by two different correlation times: τ_{-1}, which is associated with fluctuations in directions perpendicular to the long axis of the probe, and τ_{+1}, which is associated with its rotation around the long axis [25]; in addition, the values of $\tau_{\pm1}$ were calculated [26]. The rotational correlation time of the spin probe (τ) is directly proportional to the effective radius of the molecule (R) and to the microviscosity of its local surrounding (η) and inversely proportional to the absolute temperature (T) [16, 25]:

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

In the case of triplets, the A_N constant was determined as the distance (mT) between the central and high-field components [25]. In the case of anisotropic spectra, the A_N constant and the order parameter (S) were calculated by the equations after determining the hyperfine splitting constants $A_\parallel$ and $A_\perp$ [16]:

$$A_N = \left(A_\parallel + 2A_\perp \right) / 3, \qquad (6)$$

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

The parameter (γ), which characterizes the half-amplitude of molecular motion, was determined by the order parameter (S) using the calibration graph [16].

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.

4. Research results

BC reduces the surface tension (σ) of water (Fig. 1). By the isotherm of the surface tension of aqueous solutions the CMC of BC is about 0.15 % m/v at 25 °C [27].
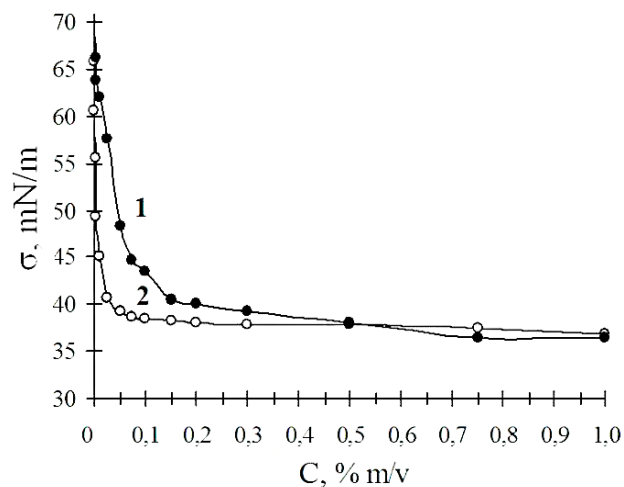

Fig. 1. Surface tension (σ) isotherms for BC aqueous solutions (1) and BC aqueous solutions containing 0.5 % DSE (2) at 25 °C

Lipophilic probe 2 is insoluble in water, so the EPR spectra of probe 2 in water and ionic solutions of BC were singlets due to the interaction between their molecules (Fig. 2, *a*). At higher BC concentrations of 0.05 % to 0.15 %, the EPR spectra were superpositions of singlet and triplet, with the proportion of triplet increasing with increasing BC content (Fig. 2, *a*). In this concentration range, probe 2 was partially included in the BC associates. At the CMC, the EPR spectrum still showed the phenomenon of exchange broadening. At BC concentrations above the CMC, the EPR spectra transformed into characteristic triplets, indicating complete solubilization of the probe 2 molecules by the micelles and their uniform distribution in the micelles. The nitroxyl radical of probe 2 was localized in the polar part of the micelles, as evidenced by the constant $A_N \approx 1.63$ mT.

With increasing BC content from 0.2 % to 1.0 %, the rotational correlation times of probe 2 almost did not change ($\tau_{-1} \approx 0.13$ ns; $\tau_{\pm 1} \approx 0.30$ ns). Probe 2 in the micelles was in state of rapid rotation. The viscosity of its environment was about 2 times less than the viscosity of liquid paraffin ($\tau_{-1} \approx 0.28$ ns; $\tau_{\pm 1} \approx 0.70$ ns) [28]. Using of lipophilic probe 2 it was shown that there was BC concentration range, in which BC associates were formed before the CMC. At BC concentrations exceeding the CMC, probe 2 was completely solubilized by the BC micelles, and the micelle structure at the BC content from 0.2 % to 1.0 % did not change significantly.

When DSE was added to BC solutions, the CMC of BC decreased to about 0.05-0.10 % due to the interaction between BC cations and DSE anions (Fig. 1). Accordingly, the transformation of the EPR spectra of probe 2 into triplets was observed at lower concentrations of BC (Fig. 2). Furthermore, due to this interaction, the packing density of ions in the surfactant micelles increased. This was evidenced by the increase in the value of $\tau_{\pm 1}$ of probe 2, localized in the micelles. Thus, at 25 °C $\tau_{\pm 1}$ was equal to 0.29 ns in the case of 0.3 % aqueous solution of BC and $\tau_{\pm 1}$ was equal to 0.39 ns in the case of 0.15 % aqueous solution of BC containing 0.5 % DSE, which was 34.5 % more.

A cationic surfactant was simulated by hydrophilic probe 1, which is soluble in water and its EPR spectra in water, ionic, and micellar solutions of BC were triplets (Fig. 3). Upon formation of a micellar solution, a linewidth broadening at high field was observed in the EPR spectra of probe 1 (see spectrum 2 and spectrum 5 in Fig. 3); at the same time, the anisotropy parameter ε decreased approximately tenfold from 0.30 to 0.03, indicating a change in the localization environment, which is now more ordered.
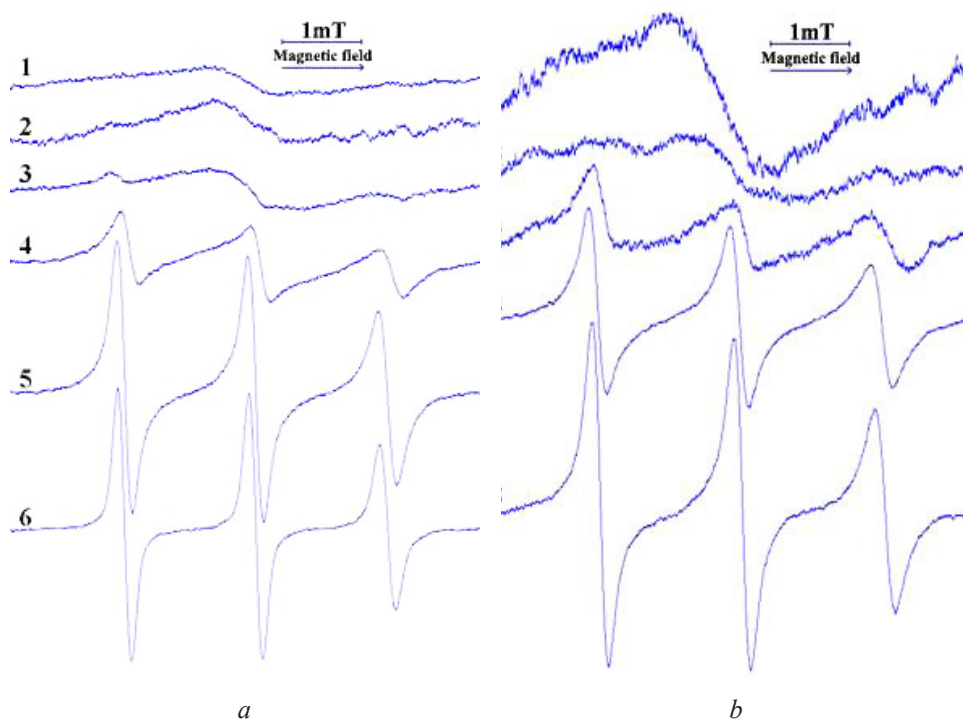

a   *b*

Fig. 2. EPR spectra of lipophilic probe 2 in: *a* – water (1) and aqueous solutions of BC at concentration: 2 – 0.05 %, 3 – 0.1 %, **4 – 0.15 % (CMC)**, 5 – 0.3 %, 6 – 1.0 %; *b* – aqueous solutions containing: 1 – 0.5 % DSE, 2 – 0.5 % DSE+0.025 % BC, **3 – 0.5 % DSE+0.05 % BC (CMC)**, 4 – 0.5 % DSE+0.10 % BC, 5 – 0.5 % DSE+0.15 % BC
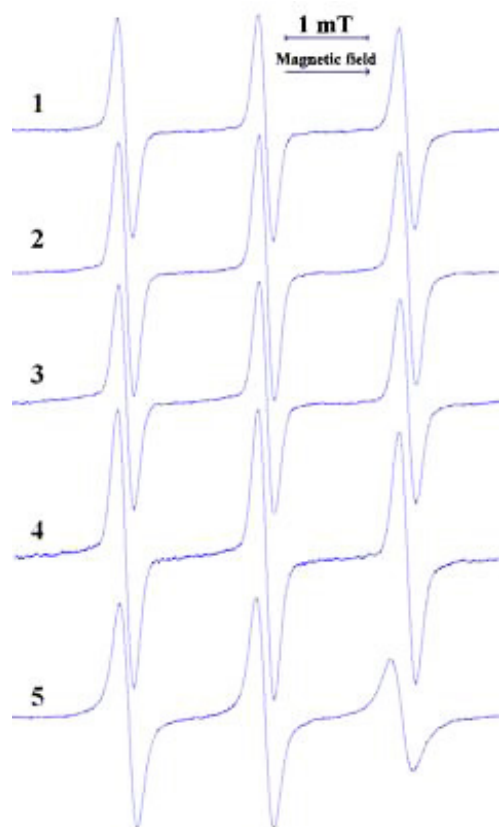
Fig. 3. EPR spectra of probe 1 in water (1) and aqueous solutions of BC at concentrations: 2 – 0.005 %, 3 – 0.075 %, 4 – 0.10 %, 5 – 1.0 %

At BC concentration of up to 0.1 %, the rotational correlation times of probe 1 remained at a constant level ($\tau_{-1} \approx 0.037$ ns; $\tau_{\pm1} \approx 0.078$ ns) (Fig. 4). At BC critical micelle concentration (0.15 %), the rotational correlation times of probe 1 increased sharply and continued to increase up to BC concentration of 0.3 %, and then were almost the same with the increase in BC content ($\tau_{-1} \approx 0.25$ ns; $\tau_{\pm1} \approx 0.60$ ns) (Fig. 4). Compared to the ionic solution, the values of τ_{-1} and $\tau_{\pm1}$ in micelles increased by approximately 6.8 and 7.7 times respectively.

Probes 1 and 2 were incorporated into the BC associates, which were forming below CMC starting at BC concentration of 0.1 %. But the τ values of probe 1 increased with increasing BC content from 0.1 % to 0.3 %, while in the case of probe 2 the τ values were the same at BC concentrations above 0.2 %.

The study was also performed with the probe TEMPO, which has a significantly different molecular structure and a much smaller hydrophobic fragment:
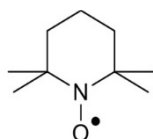
The probe TEMPO is soluble in water. Its EPR spectra in water, ionic and micellar solutions of BC were triplets (Fig. 5). In the case of all these objects, the nitroxyl radical of the probe TEMPO localized in water ($A_N \approx 1.73$ mT).
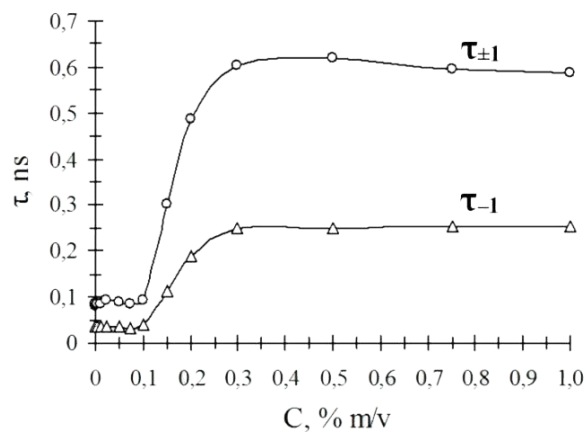

Fig. 4. Rotational correlation time (τ) of probe 1 vs concentration (C) of BC solutions

In contrast to the EPR spectra of probe 1, the shape of the EPR spectra of the probe TEMPO did not change significantly with increase of BC concentration, and there was not signal splitting into two lines at high field, since the polarity of the environment of nitroxyl radical was the same in water, ionic solution, and micelles (Fig. 5).
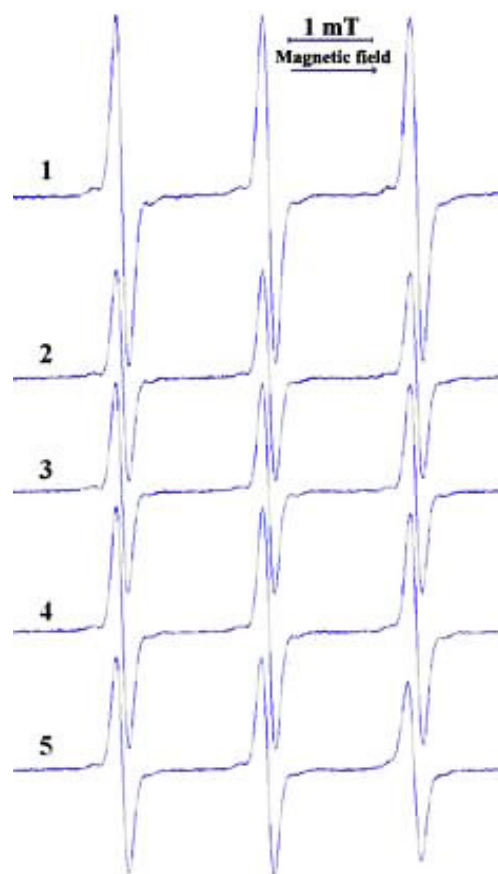

Fig. 5. EPR spectra of probe TEMPO in water (1) and aqueous solutions of BC at concentrations: 2 – 0.01 %, 3 – 0.1 %, 4 – 0.3 %, 5 – 1.0 %

The rotational correlation time of probe TEMPO (probe 5) depended significantly on the concentration of BC solutions (Fig. 6).

In the range of BC concentrations from 0.001 % to 0.15 %, the values of τ for the probe TEMPO re-

mained at a constant level ($\tau_{-1} \approx 43.3$ ps; $\tau_{\pm 1} \approx 96.7$ ps). Starting from BC concentration of 0.2 %, which exceeded the CMC, the values of τ_{-1} and $\tau_{\pm 1}$ were growing almost linearly and increased by nearly an order of magnitude as the BC concentration had increased to 1.0 %.
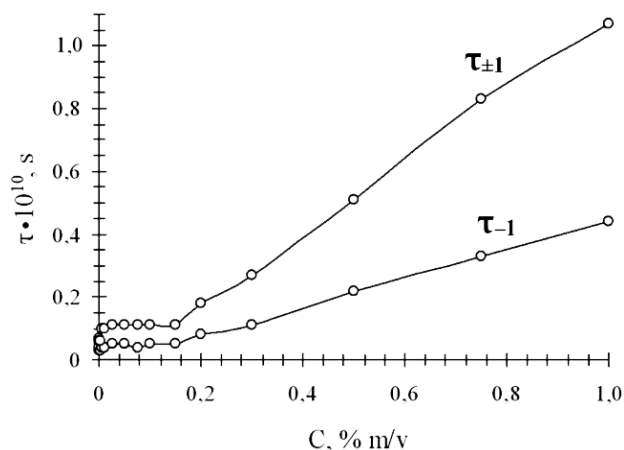

Fig. 6. Rotational correlation time (τ) of probe 5 *vs* concentration (C) of BC solutions

Thus, studies of cationic surfactant solutions with three different spin probes determined the different dependencies of their rotational correlation times on surfactant concentration. In this context, the exact determination of the critical BC micelle concentration by the spin probe method is questionable.

The same experiments were carried out with anionic surfactant SLS and non-ionic surfactant M20CE. According to the surface tension isotherms (at 25 °C), CMC of SLS was 0.15 % m/v and CMC of M20SE was 0.10 % m/v.

The EPR spectra of lipophilic probe 2 in solutions of anionic and non-ionic surfactants with increasing their concentration (Fig. 7, 8) changed like the EPR spectra in the case of solutions of cationic surfactant BC (Fig. 2). For ionic or molecular solutions, these EPR spectra initially were singlets; with an increase in the surfactant concentration and the appearance of low number of surfactant associates, the EPR spectra transformed into a superposition of singlet and triplet; the part of triplet increased with rising of surfactant content. It should be noted, that at CMC of three surfactants under study the EPR spectra of probe 2 still illustrated the exchange broadening. The shape of EPR spectra in the case of solutions at CMC differed compared to micellar solutions containing enough micelles to solubilize probe 2. At CMC, the nitroxyl radical of probe 2 is localized in the polar part of both SLS micelles ($A_N \approx 1.65$ mT) and M20CE micelles ($A_N \approx 1.61$ mT). At concentrations above CMC with an increase in surfactant content up to 1.0 %, the rotational correlation time of probe 2 in SLS solutions tended to decrease, and in M20CE solutions – to increase (Table 1). Probe 2 in surfactant micelles was in a state of rapid rotation, as evidenced by the shape of the spectra and rotational correlation times (Table 1). It could be concluded that the packing of molecules in the cores of micelles in the case of non-ionic surfactants was more compacted compared to ionic surfactants, since the values of τ_{-1} and $\tau_{\pm 1}$ for M20CE solutions were greater. Surfaceactive anions in SLS micelles could be considered the least ordered, as evidenced by the largest values of the anisotropy parameter ε.

Probe 1 simulated a cationic surfactant, and EPR spectra of this probe in the case of aqueous solutions of anionic and non-ionic surfactants with increasing their concentration differed significantly from the EPR spectra for BC solutions (Fig. 2). In a certain concentration range of non-ionic surfactant solutions, the EPR spectra of hydrophilic probe 1 were superpositions of two triplets (Fig. 9); at the high-field of the EPR spectrum, a narrow line (A_N=1.71 mT) corresponded to the localization of probe 1 in water, and a wider one (A_N=1.60 mT) – in surfactant associates. As the M20CE content increased, the part of the narrow line decreased, and the part of the broad line increased. But at CMC, the EPR spectrum still illustrated the phenomenon of exchange broadening (Fig. 9).

Table 1

Some parameters of rotational diffusion of probe 2 in surfactant micelles

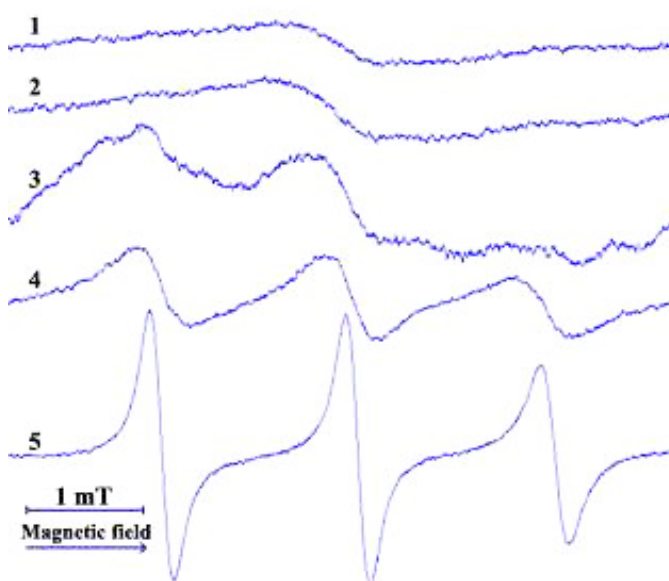| $C_{surfactant}$, %	BC			SLS			M20CE		
	τ_{-1}, ns	$\tau_{\pm 1}$, ns	ε	τ_{-1}, ns	$\tau_{\pm 1}$, ns	ε	τ_{-1}, ns	$\tau_{\pm 1}$, ns	ε
0.2	0.13	0.31	0	0.15	0.34	0.03	0.24	0.49	–0.02
0.3	0.13	0.29	–0.06	0.13	0.30	0	0.25	0.55	–0.03
0.5	0.13	0.31	–0.03	0.12	0.30	0.02	0.27	0.62	–0.02
0.75	0.13	0.29	–0.05	0.12	0.28	0	0.29	0.66	–0.07
1.0	0.13	0.30	–0.05	0.12	0.27	–0.1	0.29	0.67	–0.07

Fig. 7. EPR spectra of probe 2 in water (1) and aqueous solutions of SLS at concentrations: 2 – 0.075 %; 3 – 0.10 %; **4 – 0.15 % (CMC);** 5 – 30 %

Thus, in the case of M20CE solutions, there was a surfactant concentration range where the probe 1 cations were in equilibrium between the ionic solution and surfactant associates, which were forming below CMC. At concentrations above CMC, on the EPR spectra there were no lines indicating that cations of probe 1 were dissolved in water (Fig. 9). That is, the flip-flop motion of cations of probe 1 were not detected by the EPR method, and it can be assumed that their state in the micelles in the radial direction is fixed. This also may arise from the ion-dipole interaction between the cations of probe 1 and the oxygen atoms of the polar part of the non-ionic surfactant. In the case of lipophilic probe 2, fixation of its molecules in micelles in the radial direction is due to insolubility of the probe in water.

There was an ion-ion interaction between the probe 1 cations and the SLS anions in the aqueous solution, which is why the EPR spectra were singlets at low concentrations of the anionic surfactant (Fig. 10). With increasing SLS content, the EPR spectra were transformed to singlet-triplet superpositions; the proportion of triplets increased with increasing SLS content; at CMC, the EPR spectrum still showed exchange broadening and its configuration was different compared to the characteristic EPR spectrum of probe 1 in micelles (Fig. 10).

At concentrations above CMC with an increase in surfactant content up to 1.0 %, the rotational correlation time of probe 1 in SLS solutions did not change significantly as well as in M20CE solutions (Table 2). Cations of probe 1 in BC micelles were in a state of rapid rotation, as evidenced by the rotational correlation times (Table 2).

In micelles of anionic surfactant, the rotational diffusion of probe 1 cations slowed down both around the long axis and in directions perpendicular to it. The rotational correlation times of probe 1 cations in micelles of non-ionic surfactant increased even more. Thus, $\tau_{\pm1}$ was equal to 0.59 ns in a 1.0 % BC solution, 1.04 ns – in 1.0 % SLS solution, 1.55 ns – in 1.0 % M20CE solution, which was greater by 1,8 and 2.6 times, respectively. Greater values of τ in the case of probe 1 cations in micelles of non-ionic surfactants could be due to both more compacted packing of M20CE molecules compared to ionic surfactants, and to ion-dipole interactions between probe 1 cations and the polar part of surfactants.

According to the surface tension isotherm at 25 °C the CMC of P338 was approximately 1 % (Fig. 12). But the EPR spectra of lipophilic probe 2 in P338 aqueous solutions at the concentrations up to 5 % were singlets; at P338 concentration of 6 % the EPR spectrum transformed into singlet-triplet superposition, and only at P338 concentration of 10 % the EPR spectrum transformed into triplet (Fig. 11). That is, the solubilization of molecules of probe 2 occurred at concentrations of P338 that significantly exceeded its CMC. It makes P338 different from low molecular weight surfactants (BC, SLS, and M20CE), which completely solubilized probe 2 at concentrations only slightly above their CMC.
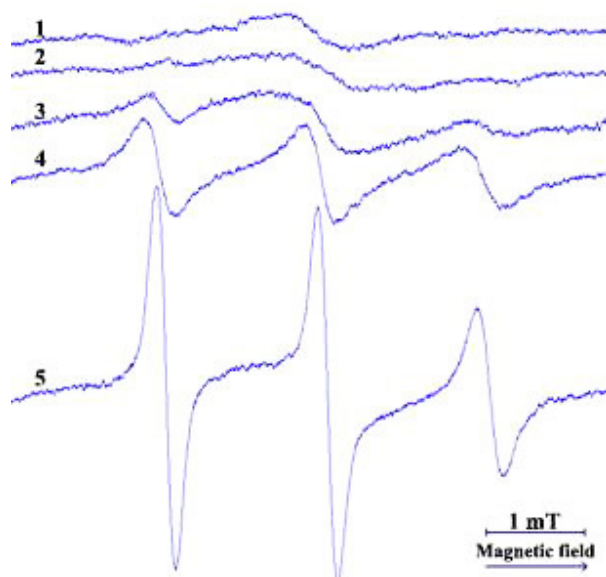

Fig. 8. EPR spectra of probe 2 in water (1) and aqueous solutions of M20CE at concentrations: 2 – 0.005 %; 3 – 0.025 %; **4 – 0.10 % (CMC);** 5 – 0.50 %
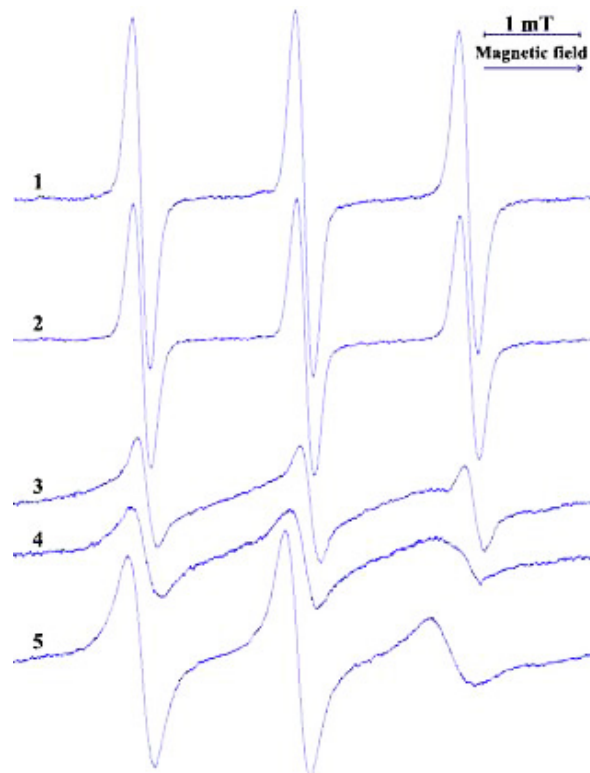

Fig. 9. EPR spectra of probe 1 in water (1) and aqueous solutions of M20CE at concentrations: 2 – 0.005 %; 3 – 0.025 %; **4 – 0.10 % (CMC);** 5 – 0.50 %

Table 2

Some parameters of rotational diffusion of probe 1 in surfactant micelles

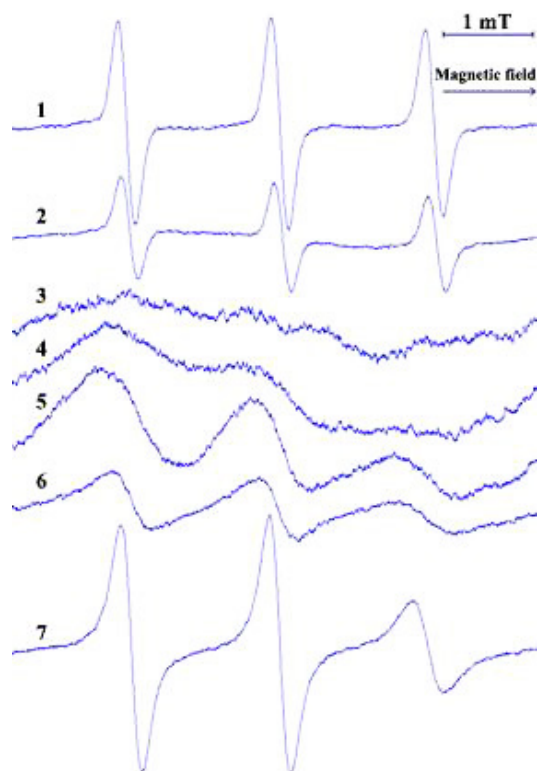| $C_{surfactant}$, %	BC				SLS				M20CE			
	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε
0.2	0.46	0.19	0.49	0.04	1.02	0.44	1.12	0.06	1.20	0.57	1.42	0.10
0.3	0.58	0.25	0.60	0.03	1.00	0.45	1.08	0.04	1.26	0.57	1.38	0.10
0.5	0.60	0.25	0.62	0.02	0.96	0.43	1.02	0.04	1.33	0.64	1.70	0.09
0.75	0.55	0.25	0.57	0.03	0.93	0.45	1.00	0.05	1.33	0.57	1.54	0.10
1.0	0.57	0.26	0.59	0.03	0.97	0.42	1.04	0.04	1.34	0.61	1.55	0.09

Fig. 10. EPR spectra of probe 1 in water (1) and aqueous solutions of SLS at concentrations: 2 – 0.0025 %; 3 – 0.005 %; 4– 0.10 %; 5 – 0.125 %; **6 – 0.15 % (CMC);** 7 – 0.5 %
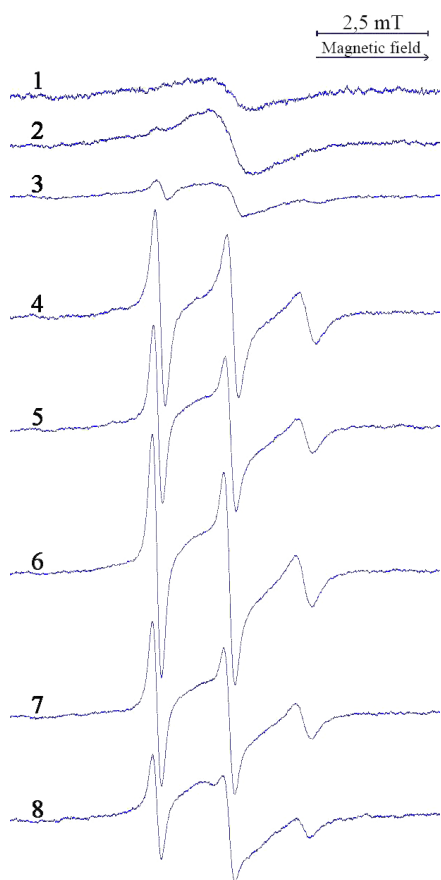

Fig. 11. EPR spectra of spin probe 2 in aqueous solutions of P338 at concentrations: 1 – 1 %; 2 – 5 %; 3 – 6 %; 4 – 10 %; 5 – 15 %; 6 – 17 %; 7 – 20 %; 8 – 25 %
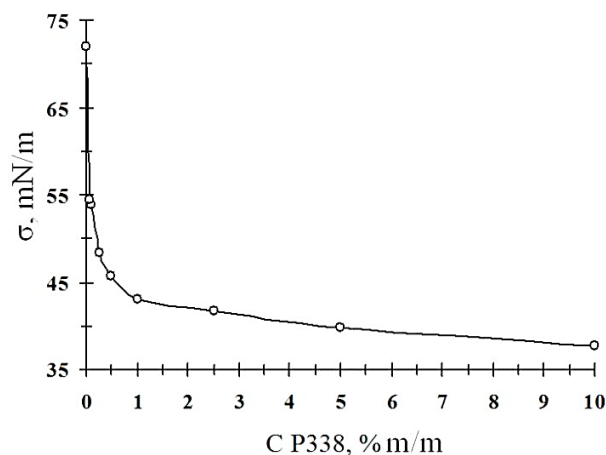

Fig. 12. Surface tension (σ) isotherms for P338 aqueous solutions at 25 °C

With an increase in the P338 content from 10 % to 20 %, the rotational correlation times of probe 2 in P338 solutions did not change significantly, and the nitroxyl radical was localized in the polar part of the P338 associates, as evidenced by A_N the values (Table 3). The rotational correlation times of probe 2 in P338 associates were several times greater than in the micelles of the non-ionic surfactant M20SE but values of the anisotropy parameters ε of probe 2 in these objects were close (Tables 1, 3).

At P338 content of 25 % the EPR spectrum of probe 2 transformed into triplet-singlet superposition (Fig. 12). This indicated the structural changes in P338 micelles, because of which the ability to solubilize the probe decreased and a *sol→gel* transition occurred in the solution (Fig. 13).

Table 3

Some parameters of the EPR spectra of spin probe 2 in P338 aqueous solutions and the dynamic viscosity (η) of these solutions at 25 °C

C_{P338}, %	Parameters of the EPR spectra				η, mPa·s
	A_N, mT	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	
10	1.65	0.52	1.41	–0.12	7.2
15	1.65	0.74	1.71	–0.06	26.5
17	1.64	0.71	1.54	–0.06	60.4
20	1.64	0.70	1.60	–0.05	283.7

Aqueous solutions with P338 content up to 20 % at 25 °C were transparent Newtonian fluids; their dynamic viscosity increased with increasing P338 content (Table 3). At P338 concentration of 25 %, the transparent gel with a plastic flow behaviour and thixotropic properties was formed. The yield stress (τ_0) was equal to 351 Pa, and the apparent viscosity (η) at Dr=14.59 s^{-1} was 26.5 Pa·s and was 93.5 times greater than the dynamic viscosity of 20 % P338 solution. This is probably due to change in the shape of P338 associates which also led to deterioration in the solubilization of molecules of lipophilic probe 2.

The dynamic viscosity of liquid paraffin (comparator liquid) was equal to 128 mPa·s at 25 °C, and $\tau_{\pm1}$

of probe 2 was≈0.7 ns. By the calculations, the viscosity of the microenvironment of probe 2 in P338 associates was on average 2.2 times greater than the dynamic viscosity of liquid paraffin, which approximately corresponds to the viscosity of soft white paraffin [28]. In micelles of M20CE, the viscosity of the microenvironment of probe 2 was on average about 85 % of the dynamic viscosity of liquid paraffin. These imputations are approximate, since liquid paraffin is an isotropic liquid, and the cores of micelles are anisotropic in viscosity [15, 28]. Moreover, the dynamic properties of different segments of alkyl chains of spin probe 2 are different (Fig. 14, 15) [16].
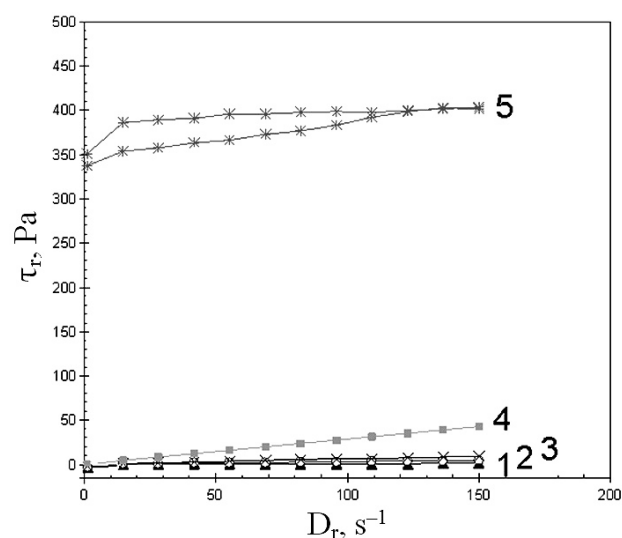

Fig. 13. Rheograms of the aqueous solutions of P338 at 25 °C at concentrations: 1 – 10 %; 2 – 15 %; 3 – 17 %; 4 – 20 %; 5 – 25 %

Representative EPR spectra of spin probes 1–4 in 1 % solution of BC and in 1 % solution of M20CE are shown in Fig. 14. The nitroxyl radicals of these probes are located in different segments of the alkyl chains, which makes it possible to detect certain areas of surfactant micelles. In contrast to BC micelles, there was an ion-dipole interaction between the cations of probe 1 and the polar part of M20CE micelles, which affected the parameters of the EPR spectra of probe 1. The parameters of the EPR spectra of the above-mentioned probes in 1 % solutions of BC, SLS and M20CE are presented in Table 4.

The nitroxyl radicals of probes 1 and 2 are localized in the polar part of the micelles, and their alkyl chains are located in the nonpolar cores, which makes them promising for studying the interface of polar and nonpolar parts of surfactant associates.

Probe 2 rapidly rotated around the long axis of the molecule, which is

why its EPR spectra were characterized by a higher intensity of the low-field line compared to the central line; accordingly, the anisotropy parameter ε in the micelles of the studied surfactants had a negative value (Fig. 14, Table 4). The values of τ_{-1} also indicated the rapid rotation of probe 2 [25]. As it was shown above, according to the rotational diffusion parameters of probe 2, the cores of BC, SLS and M20CE micelles at the interface with the polar part are liquid in consistency.

Table 4
Parameters of the EPR spectra of spin probes in 1 % aqueous solutions of surfactants

Surfactant	Probe	A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	S	γ
BC	1	1.63	0.57	0.26	0.59	0.03	–	–
	2	1.63	–	0.13	0.30	–0.05	–	–
	3	1.49	1.44	0.71	1.76	0.09	0.13	77
	4	1.50	0.41	0.18	0.44	0.05	0.07	83
SLS	1	1.61	0.97	0.42	1.04	0.04	–	–
	2	1.64	–	0.12	0.27	–0.10	–	–
	3	1.53	0.87	0.42	1.07	0.11	0.13	77
	4	1.55	0.26	0.13	0.30	0.11	0.07	83
M20CE	1	1.60	1.34	0.61	1.55	0.09	–	–
	2	1.62	–	0.29	0.67	–0.07	–	–
	3	1.38	2.12	0.99	4.48	0.32	0.25	68
	4	1.41	0.65	0.30	0.76	0.09	0.08	82

The difference in the rotational diffusion parameters of probe 2 and probe 1, whose nitroxyl radical is also localized in the polar part of the micelles, was due to their different molecular structures as well as the interaction of probe 1 cations with SLS anions or oxygen atoms of the polar part of M20CE micelles.
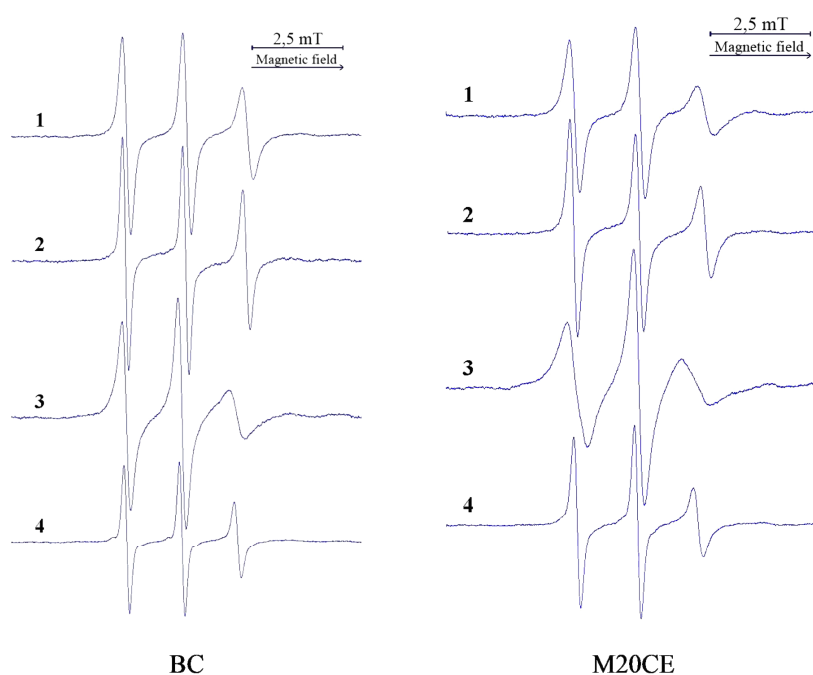

Fig. 14. EPR spectra of the spin probes in 1 % aqueous solutions of surfactants at 25 °C: 1 – TEMPO-iodide; 2 – 4-palmitamido-TEMPO; 3 – 5-DSA; 4 – 16-DSA

Based on the EPR spectra of 5-DSA and the parameters of these spectra, it can be concluded that the packing density of alkyl chains and their orderliness at the level of the 5th carbon atom was significantly higher than at the interface with the polar part of the micelles (Fig. 14, Table 4). The A_N value of the EPR spectra of 5-DSA were less than the A_N value of the EPR spectra of probes 1 and 2 indicating the nonpolar environment of the doxyl radical of probe 3. According to the 5-DSA EPR spectra, the A_N value was the lowest in micelles of non-ionic surfactants compared to micelles of SLS and BC (Table 4), which is probably due to the higher content of water molecules in the cores of micelles of ionic surfactants.

Compared to the 5-DSA probe, the rotational correlation times of the 16-DSA probe decreased, the order parameter S was lower, and the γ parameter increased accordingly (Table 4). According to research results, wedge-shaped packing of alkyl chains in the cores of micelles took place up to approximately the 5th carbon atom. In terms of dynamic properties, segments of alkyl chains at the level of the 16th carbon atom were more mobile, and as regards their structure, they were less ordered. It can be assumed that the microviscosity of the micelle cores at the level of 16th carbon atom was lower than at the level of 5th carbon atom of alkyl chains.

The EPR spectra of probes 1–4 in micelles of non-ionic surfactant M40S with a high degree of oxyethylation and in micelles of P338, which is a block copolymer with a high molecular weight, are presented in Fig. 15.

The EPR spectra of the spin probe 5-DSA in aqueous solutions of M40S or P338 were anisotropic (Fig. 15). Compared with the micelles of ionic surfactants and non-ionic surfactant M20CE, the values of S parameter at the level of the 5th carbon atom of the probe 5-DSA in the M40S and P338 micelles were significantly greater, and the values of γ parameter were lower, which indicated that packing of molecules were more ordered and their rotation angle was smaller (Tables 4, 5).

Changes in the parameters of the EPR spectra of probes 2–4 in micelles of M40S or P338 were like those observed in the case of micelles of three other surfactants: rapid rotation of probe 2, the largest value of the order parameter for the EPR spectra of probe 5-DSA as well as rapid rotation and a significantly lower value of the order parameter for the EPR spectra of the probe 16-DSA. However, the rotational correlation times of probes 1–4 in solutions M40S or P338 were several times greater than in solutions of ionic surfactants (Table 4, 5).
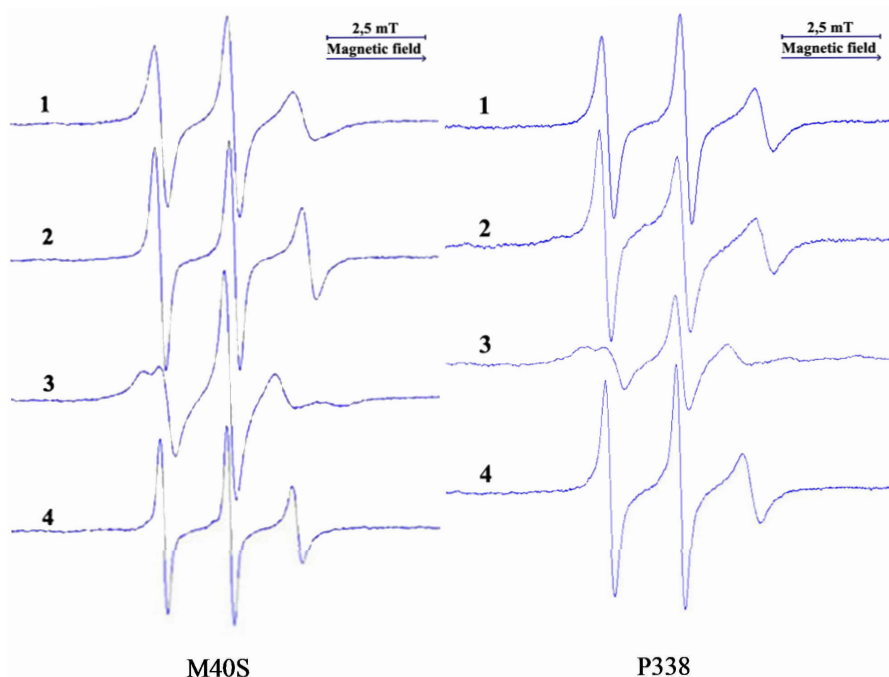

Fig. 15. EPR spectra of the spin probes in 7 % aqueous solutions of M40S and in 10 % aqueous solution of P338 at 25 °C: 1 – TEMPO-iodide; 2 – 4-palmitamido-TEMPO; 3 – 5-DSA; 4 – 16-DSA

The spin probe method is promising for researching the structural and dynamic properties of surfactant associates and elucidating the mechanisms of changes in some physicochemical properties of surfactants that could affect the functional properties of medicinal products.

Table 5

Parameters of EPR spectra of spin probes in solutions of M40S and P338

Surfactant	Probe	A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	S	γ
M40S	1	1.60	1.57	0.75	1.97	0.11	–	–
	2	1.65	0.88	0.35	0.91	0.02	–	–
	3	1.46	–	–	–	–	0.42	56
	4	1.49	0.60	0.30	0.72	0.11	0.11	80
P338	1	1.62	0.83	0.51	1.14	0.09	–	–
	2	1.65	–	0.52	1.41	–0.12	–	–
	3	1.46	–	–	–	–	0.43	55.5
	4	1.45	0.62	0.47	1.01	0.07	0.11	80

Glycerol, PG and ethanol interact with poloxamer 407 [11]. The formation of P338 micelles, which can solubilize lipophilic probe 2, occurred at high content of P338 – 10 % or more (Fig. 12, Table 3). It was of interest to find out whether the interaction between glycol and poloxamer can affect the formation of P338 micelles, their ability to solubilize lipophilic probe 2, and its spectral parameters.

The EPR spectra of probe 2 in solutions containing 5.0 % P338 and PG in various concentrations are illustrated in Fig. 16 and the parameters of these spectra are given in Table 6.

In aqueous solutions, molecules of probe 2 are solubilized at P338 content of 10 % (Fig. 12). When 5 %

of P338 was dissolved in mixed solvents PG–water containing PG up to 10 % m/m, the EPR spectra of probe 4-palmitamido-TEMPO were singlets, which indicated that its molecules were not solubilized (Fig. 16). At PG content from 15 % to 50 %, the EPR spectra were triplets. That is, because of the interaction between poloxamer and PG [11], the structure of P338 micelles changed, which contributed to the solubilization of molecules of a lipophilic substance at P338 concentration that was 2 times lower. It should be noted that at PG content of 50 %, molecules of probe 2 were localized precisely in P338 micelles, since 4-palmitamido-TEMPO dissolves in a mixed solvent PG–water at concentrations of PG above 65-70 % m/m (at PG content of 55 % m/m the EPR spectrum of probe 2 was still a singlet).

Table 6
Parameters of EPR spectra of spin probe 2 in solutions of P338 containing PG at various concentrations at 25 °C

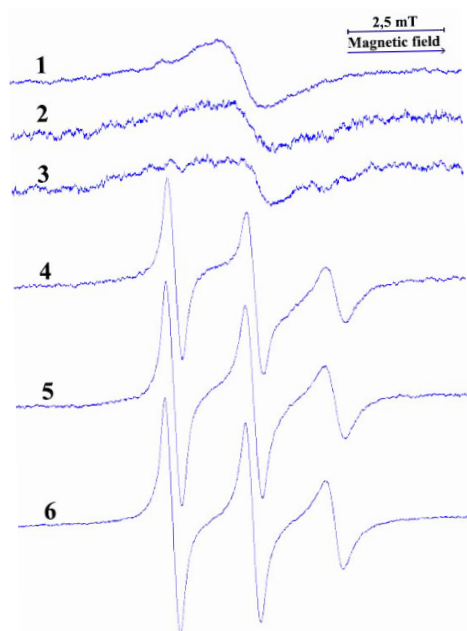| C_{P338}, %	C_{PG}, %	A_N, mT	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε
5	15	1.65	0.49	1.26	–0.08
5	20	1.64	0.49	1.16	–0.12
5	50	1.63	0.33	0.92	–0.16
10	0	1.65	0.52	1.42	–0.12
10	20	1.65	0.50	1.40	–0.14
10	40	1.63	0.44	1.30	–0.18
10	50	1.63	0.42	1.20	–0.17

Fig. 16. EPR spectra of spine probe 2 in 5 % solutions of P338 containing PG at concentrations: 1 – 0 %; 2 – 5 %; 3 – 10 %; 4 – 15 %; 5 – 20 %; 6 – 50 %

With an increase in concentration of PG, there was a tendency to decrease the rotational correlation times (τ_{-1}, $\tau_{\pm1}$) of probe 2 and the anisotropy parameter (ε). At a constant content of PG, it was possible to note a tendency for an increase of the τ_{-1} and $\tau_{\pm1}$ values with the raising concentration of P338 (Table 6).

When aqueous solutions of poloxamers are heated, gels could form, which upon cooling transform into liquids again [8, 10] (Fig. 13, 17).

When the temperature increased from 25 °C to 40 °C, 10 % aqueous solution of P338 remained a Newtonian liquid; the flow behaviour of solutions containing 15 %, 17 % and 20 % of P338 changed from Newtonian to plastic, and these solutions turned into transparent gels (Fig. 13, 17). As the temperature increased to 40 °C, the values of the rheological parameters of all these objects increased albeit to varying degrees (Table 7).

The EPR spectra of probes 1–4 in 17 % aqueous solution of P338 at 25 °C and 40 °C are shown in Fig. 18, and the parameters of EPR spectra of these probes in 10 % and 17 % aqueous solutions of P338 are provided in Table 8.
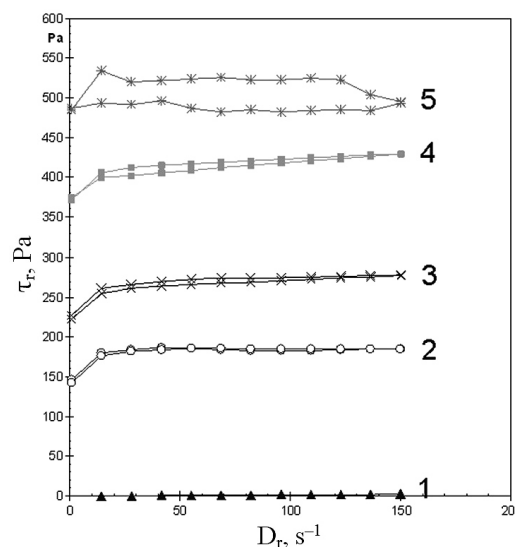

Fig. 17. Rheograms (at 40 °C) of aqueous solutions of P338 at concentration: 1 – 10 %; 2 – 15 %; 3 – 17 %; 4 – 20 %; 5 – 25 %

Table 7
Rheological parameters of P338 aqueous solution at 25 °C and 40 °C

C_{P338}, % m/m	Temperature	τ_0, Pa	η, mPa·s, at D_r:		
			14.59 s^{-1}	41.66 s^{-1}	82.31 s^{-1}
10 %	25 °C	0	7.2	7.2	7.2
	40 °C	0	16.3	16.3	16.3
15 %	25 °C	0	26.5	26.5	26.5
	40 °C	146	12340	4486	2221
17 %	25 °C	0	60.4	60.4	60.4
	40 °C	227	17950	6461	3330
20 %	25 °C	0	283.7	283.7	283.7
	40 °C	372	27920	9975	5113
25 %	25 °C	351	26520	9394	4838
	40 °C	485	36700	12540	6353

The nature of the change in the parameters of the EPR spectra of spin probes 2–4 in P338 micelles with an increase in its concentration from 10 % to 17 % was as described above in the case of surfactant micelles. P338 micelles were characterized by rapid rotation of probe 2, anisotropic EPR spectra of probe 5-DSA with a high

enough order parameter, and then was a transition to rapid rotation of probe 16-DSA with a significantly lower order parameter (Fig. 18, Table 8). As the temperature increased from 25 °C to 40 °C, the rotational correlation times of probes 1, 2 and 4 decreased, which characterized lower packing density of molecules in micelles, however, an increase in temperature by 15 °C had little effect on the order parameter of probe 3 (Table 8).

It can be assumed, that at 40 °C the volume of P338 micelles increased with keeping of structural factors necessary for the gel formation. At P338 content of 10 %, the same changes occurred in the EPR spectra parameters of spin probes with increasing temperature (Table 7, Fig. 15, 18). So, it can be concluded that the gel can be formed only provided enough micelles in certain volume; at P338 content of 10 % number of micelles is not enough to create a spatial network and form a gel.
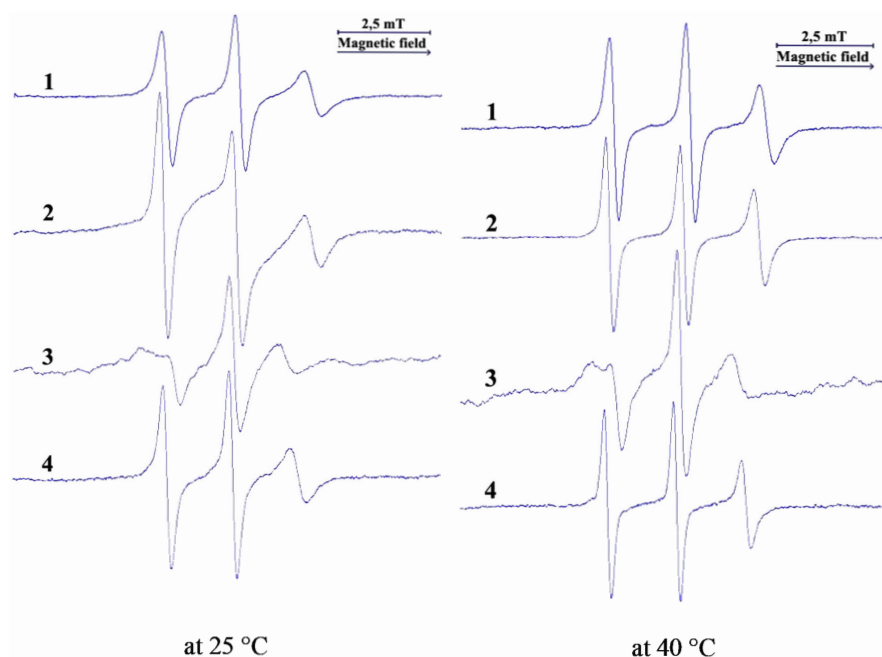

Fig. 18. EPR spectra of the spin probes 1–4 in 17 % aqueous solution of P338

Table 8

Parameters of EPR spectra of spin probes 1, 2, 3 and 4 in P338 aqueous solutions at 25 °C and 40 °C

Probe	C_{P338}	t, °C	A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm 1}$, ns	ε	S	γ
1	10 %	25	1.62	0.83	0.51	1.14	0.09	–	–
		40	1.62	0.49	0.35	0.75	0.08	–	–
	17 %	25	1.60	0.92	0.57	1.22	0.09	–	–
		40	1.61	0.48	0.34	0.73	0.08	–	–
2	10 %	25	1.65	–	0.52	1.41	–0.12	–	–
		40	1.61	–	0.22	0.60	–0.10	–	–
	17 %	25	1.64	–	0.71	1.54	–0.06	–	–
		40	1.59	–	0.19	0.52	–0.11	–	–
3	10 %	25	1.46	–	–	–	–	0.43	55.5
		40	1.51	–	–	–	–	0.38	60
	17 %	25	1.52	–	–	–	–	0.46	55
		40	1.49	–	–	–	–	0.43	55.5
4	10 %	25	1.45	0.60	0.47	1.01	0.07	0.11	80
		40	1.47	0.25	0.23	0.49	0.06	0.07	83
	17 %	25	1.43	0.62	0.50	1.10	0.07	0.12	79
		40	1.47	0.26	0.23	0.52	0.06	0.07	83

5. Discussion of research results

The spin probe method is an indirect technique for studying solutions of surfactants and poloxamers. By the EPR spectra of spin probes and their parameters, information about the studied object can be obtained depending on certain variable factors [16]. The behaviour of the probe in the phase in which it is localized can be used to assess the state of this phase. The estimation of EPR spectra is necessary to determine the localization and distribution of the probe molecules in the surfactant solutions to identify the object of study. In addition, the structure of the surfactant micelles and the dynamic properties of the probes in the different segments of the alkyl chains should be determined; for this research, the probe molecules should be solubilized by the micelles. It is rational to correlate changes in the shape of EPR spectra and their parameters with changes in the physical and chemical properties of the research object, e.g., formation of micelles in surfactant solutions and their structure, solubilization of substances, changes in rheological properties of solutions, etc. The changes in these properties can contribute to the performance characteristics of the medicinal product, and the results of the studies using the spin probe method make it possible to determine the mechanisms of the phenomena taking place.

It was shown that the shape of the EPR spectra and the spectral parameters of the spin probes depended on the surfactant concentration and micelle formation in surfactant solutions, as well as on the molecular structure and hydrophilic-lipophilic properties of the probes themselves. It was found that there was a concentration range in which surfactant associates were forming at concentrations below the CMC (Fig. 2, 3, 7, 8). At concentrations above the CMC and up to 1.0 % m/v, the micelle structures of cationic, anionic, and non-ionic surfactants did not change significantly (Table 1). In the micelles, the molecules of the probes simulating the surfactants were fixed in the radial direction but rotated rapidly around the long axis of the molecule and perpendicular to it. The rotational correlation times (τ) of the probes dissolved in water were much lower than in micelles.

For hydrophilic probes in aqueous surfactant solutions, τ is an additive value composed of τ of the probe molecules dissolved in water and τ of the probe molecules localized in micelles.

The different dependencies of the total value τ of the probes TEMPO-iodide and TEMPO on the surfactant concentration are due to the different molecular structure of these probes and their different ability to be solubilized by surfactant micelles (Fig. 4, 6).

At a certain surfactant concentration, the probe TEMPO-iodide was completely solubilized by the micelles.

In this case, the τ-values did not change significantly with a subsequent increase in surfactant content (Fig. 4) and characterized the structure of the micelles. In the case of probe TEMPO, the dynamic equilibrium between the molecular and micellar solutions is probably most strongly shifted in favour of the molecular solution. Therefore, in the CMC, the contribution of the τ-values of the probe TEMPO molecules localized in the micelles was negligible. With an increase in surfactant concentration and number of micelles, there was an almost linear increase in τ-values (Fig. 6), which was related to the solubilization of the probe TEMPO, but not to the change in micelle structure.

The assessment of EPR spectra makes it possible to reveal the interaction between the components of system. Thus, because of the interaction of the probe TEMPO-iodide with an anionic surfactant, its EPR spectra, depending on the surfactant content, became similar in shape to the EPR spectra of the lipophilic probe 4-palmitamido-TEMPO (Fig. 1, 10). The transformation of the EPR spectra of the probe 4-palmitamido-TEMPO into triplets at low BC concentrations due to the interaction between BC cations and DSE anions could serve as another example (Fig. 2). At the same time, the packing density of ions in surfactant micelles increased by more than 30 %, and the critical micelle concentration of BC decreased (Fig. 1). This indicates a decrease in the hydrophilicity of surfactants, which can increase the antibacterial activity of cationic surfactants and affect the physical stability of dispersed systems, if cationic surfactants are used for their physical stabilization. This indicates a decrease in the hydrophilicity of surfactants, which might reinforce the antibacterial activity of cationic surfactants and affect the physical stability of dispersed systems, if cationic surfactants were used for their physical stabilization.

The use of the spin probe method for accurate determination of CMC is possible only in the case of development and validation of analytical procedures for specific types of surfactants, provided that the spin probe with a specific molecular structure and its optimal concentration is properly selected. For example, in the study of P338 solutions, it was impossible to determine the critical micelle concentration of P338 using a lipophilic probe because the CMC was about 1 % according to the surface tension isotherm (Fig. 11) and the solubilization of the probe 4-palmitamido-TEMPO occurred at a P338 content of 10 % and more (Fig. 12).

It was found that with an increase in the content of P338, the structure of its associates changed and the ability to solubilize the probe 4-palmitamido-TEMPO decreased. However, due to the interaction of P338 with PG, solubilization of the lipophilic probe 4-palmitamido-TEMPO occurred at a lower concentration of P338 (Fig. 16). That is, PG can be regarded as a modulator of the solubilization of lipophilic substances in poloxamer solutions.

In studying the structure of micelles and the dynamic properties of spin probes, the viscosity of micelles was compared with the viscosity of liquid paraffin by calculating the τ values of the probe 4-palmitamido-TEMPO in micelles and liquid paraffin. This approach should be considered as conditional, since liquid paraffin is an isotropic substance, while micelles have an anisotropic viscosity according to the results of the studies using the spin probe method. However, it can be noted that compared to micelles of ionic surfactants, the cores of micelles of non-ionic surfactants and P338 were more viscous (Tables 1, 2, 3, 5). Here, the viscosity of the microenvironment of the probe 4-palmitamido-TEMPO in P338 associates approximately the same as the viscosity of soft white paraffin.

The dynamic properties of various segments of spin probes in surfactant micelles were studied. The nitroxyl radicals of the probes TEMPO-iodide and 4-palmitamido-TEMPO were localized in the polar part of the micelles, and their alkyl chains were in the nonpolar cores. These probes were intended to study the interface of polar and nonpolar parts of surfactant associates. The probe 4-palmitamido-TEMPO rapidly rotated around the long axis of the molecule; it was why the anisotropy parameter ε in the micelles of the studied surfactants had a negative value (Fig. 14, Table 4). The values of τ_{-1} characterizing the diffusion in the directions perpendicular to the long axis of the probe 2 molecules were greater, but also were typical for rapid rotations. The different rotational diffusion parameters of probes 4-palmitamido-TEMPO and TEMPO-iodide is due to the difference in their molecular structures and the interaction of TEMPO-iodide cations with SLS anions or the polar part of non-ionic surfactant micelles.

For all surfactants studied, the nature of the change in the rotational diffusion parameters of the EPR spectra of probes 4-palmitamido-TEMPO, 5-DSA and 16-DSA in the micelles was the same: fast rotation of probe 4-palmitamido-TEMPO, the largest value of the order parameter for probe 5-DSA and a significantly lower value of the order parameter for probe 16-DSA as well as its fast rotation (Table 4, 5, 8). According to the research results, a wedge-shaped packing of alkyl chains took place in the cores of the micelles up to about the 5th carbon atom.

The A_N value of the EPR spectra of probe 5-DSA were lower compared to the A_N values of the EPR spectra of the probe 4-palmitamido-TEMPO, which indicated the nonpolar environment of the doxyl radical of the probe 5-DSA in surfactant micelles. The lowest A_N value was in the case of localization of probe 5-DSA in micelles of the non-ionic surfactant (Table 4). Larger values of the A_N probe 5-DSA in micelles of ionic surfactants are probably due to higher content of water molecules in the cores of their micelles. At the same time, the rotational correlation times of these four probes in solutions of ionic surfactants were several times lower than in solutions of non-ionic surfactants and P338 (Tables 4, 5).

Aqueous solutions of P338 up to a concentration of 20 % inclusive at 25 °C were transparent Newtonian liquids; gel formation occurred at P338 content of 25 % (Fig. 13). As the temperature increased to 40 °C, the flow behaviour of solutions with P338 content from 15 % to 20 % changed to plastic flow and these liquids transformed into transparent gels (Fig. 13, 17). With an increase in temperature from 25 °C to 40 °C, the rotational correlation times of the probes 4-palmitamido-TEMPO and 16-DSA decreased, which characterized a decrease in the

packing density of molecules in the micelles, but an increase in temperature by 15 °C had little effect on the order parameter of the probe 5-DSA (Table 8). It can be assumed that upon heating the volume of P338 micelles increased with the keeping of their structural factors necessary for gel formation. At P338 content of 10 % with increasing temperature the same changes occurred in the rotational diffusion parameters of the probes (Table 7), but the concentration of 10 % was insufficient to create a spatial network from micelles and to form a gel (Fig. 17).

Study limitations. Complementary comparative studies regarding determination of the CMC of surfactants and the structure of their micelles using the hydrophilic probe 5-DOXYL Stearic acid, ammonium salt (CAS [2315262-05-4]) and the lipophilic probe 5-DSA could provide more comprehensive data to discuss the micelle structure and packing density of alkyl chains.

Prospects for further research. The results of studies concerning the formation of micelles of surfactant or P338, the structure of these micelles, as well as the interaction of surfactant and P338 with some excipients using the EPR spectra and rotational diffusion parameters of spin probes with different molecular structures are provided in this article. Considering the obtained results, similar studies with other poloxamers 188, 237, 407 could be considered reasonable [1, 2].

6. Conclusions

The shape of EPR spectra and spectral parameters of spin probes depended on the surfactant concentration and the micelle formation in surfactant solutions as well as on the molecular structure and hydrophilic-lipophilic properties of the probes themselves. There was a concentration range where surfactant associates were forming at concentrations below CMC. At surfactant concentration above CMC and up to 1 % m/v the structure of surfactant micelles did not change significantly. In micelles, surfactant-modelling probes were in state of rapid rotation around the long axis of the molecule and perpendicular to it and they were fixed in the radial direction. Rotational diffusion of probes dissolved in water was much faster. The micelle cores formed by non-ionic surfactant and P338 were more viscous compared to ionic surfactants. Surfactant micelles were anisotropic in viscosity, and different segments of the alkyl chains of surfactant-modelling probes had different dynamic properties. The packing of surfactant molecules in micelles was more ordered and compacted at the level of the 5th carbon atom of alkyl chain, however the value of hyperfine splitting constant was the lowest. The interaction between the surfactant and the probe, as well as cationic surfactant and DSE, which affected the parameters of the EPR spectra, were revealed. The shape and parameters of the EPR spectra of spin probes changed with increasing temperature and concentration of P338 solutions, which characterized structural transformations in P338 associates that cause sol-gel transitions. Due to the interaction of P338 with PG, the ability of P338 solutions to solubilize lipophilic substances increased, so, PG can be considered as solubilization modulator.

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 paper.

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Elena Bezuglaya*, PhD, Senior Researcher, Head of Laboratory, Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution «Institute for Single Crystals» of National Academy of Sciences of Ukraine, Nauky ave. 60, Kharkiv, Ukraine, 61072

Nikolay Lyapunov, Doctor of Pharmaceutical Sciences, Professor, Leading Researcher, Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution «Institute for Single Crystals» of National Academy of Sciences of Ukraine, Nauky ave. 60, Kharkiv, Ukraine, 61072

Valentyn Chebanov, Doctor of Chemical Sciences, Professor, Corresponding Member of NAS of Ukraine, First Deputy General Director, Director of Department, Department of Chemistry of Functional Materials, State Scientific Institution «Institute for Single Crystals» of National Academy of Sciences of Ukraine, Nauky ave. 60, Kharkiv, Ukraine, 61072

Oleksii Liapunov, PhD, Researcher, Laboratory of Technology and Analysis of Medicinal Products, State Scientific Institution «Institute for Single Crystals» of National Academy of Sciences of Ukraine, Nauky ave. 60, Kharkiv, Ukraine, 61072

*Corresponding author: Elena Bezuglaya, e-mail: bezugla.op@gmail.com