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# STUDY OF AQUEOUS SOLUTIONS OF POLOXAMERS BY ROTATIONAL VISCOMETRY AND SPIN PROBE METHOD

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***The aim.*** *To study aqueous solutions of different poloxamers by spin probe method and rotational viscometry depending on the temperature and poloxamer content.*

***Materials and methods.*** *The aqueous solutions of poloxamers 188, 237, 338 and 407 were studied. The solutions were studied by rotational viscometry at different temperatures; the flow behaviour, yield stress ($\tau_0$), and dynamic or apparent viscosity ($\eta$) were determined. Five spin probes differing in molecular structure, solubility, and radical localisation were introduced into the solutions. Electron paramagnetic resonance (EPR) spectra were obtained. The EPR spectra were used to determine their type and to calculate parameters.*

***Results.*** *Three factors are important for gel formation: the poloxamer type, its concentration in aqueous solution, and temperature. As the temperature of aqueous solutions of poloxamers 237, 338, and 407 increases, the rotational correlation times of fatty acid-based spin probes and the order parameters of their EPR spectra decrease. This indicates a decrease in the packing density and orderliness of the polypropylene oxide (PPO) chains in the non-polar part of the poloxamer associates, leading to an increase in the volume fraction of micelles/mesophases and promoting the formation of gels. As the temperature decreases, the opposite processes occur, leading to a gel→sol transition. At 37 °C, non-polar micelle cores could be characterised as two-dimensionally liquid and one-dimensionally solid. The rotational correlation times of the hydrophilic spin probe 4-OXO-TEMPO in 25 % aqueous solutions of poloxamers 338 and 407 are approximately constant or increasing despite an increase in the temperature. This indicates that in the polar part of the poloxamer associates, where this probe is partially localised, structural rearrangements occur with increasing temperature, which probably prevents hydrophobic hydration of the PPO chains.*

***Conclusions.*** *The rheological properties of aqueous solutions of poloxamers depend on their type, concentration, and temperature. According to the parameters of the EPR spectra of fatty acid-based spin probes, it was found that with increasing temperature, the packing density and the orderliness of the PPO chains in the non-polar part of the poloxamer associates decrease, probably leading to an increase in the volume of the micelles and causing a sol→gel transition*

***Keywords:*** *poloxamer, solution, gel, viscosity, micelle, spin probe, EPR spectrum, spectrum parameters*

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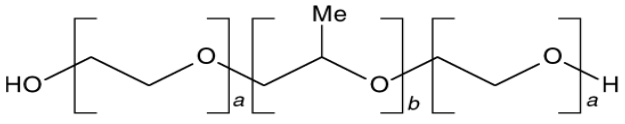
## 1. Introduction

Poloxamers are block copolymers of ethylene oxide and propylene oxide represented by the following general formula [1, 2]:




Five types (grades) of poloxamers are described in the monograph «Poloxamers» of the European Pharmacopoeia (Table 1) [1].

The nonproprietary name «poloxamer» is followed by a number, the first two digits of which, when multiplied by 100, correspond to the approximate average molecular weight ($M_r$) of the polyoxypropylene (PPO) portion of the copolymer and the third digit, when multiplied by 10, corresponds to the percentage by weight of the polyoxyethylene (PEO) portion (% *m/m*) [2]. Poloxamer 124 is a liquid, and poloxamers 188, 237, 338 and 407 are white or off-white waxy powders, granules or flakes with a melting point of above 50 °C. Poloxamer 188 is soluble in water, and poloxamers 124, 237, 338 and 407 are very soluble in water [1, 2].

Poloxamers are non-ionic surfactants; their PPO segment is hydrophobic, while the PEO chains are hydrophilic [3]. Accordingly, poloxamers from solutions are adsorbed on interfacial surfaces [4]. At low concentrations and temperatures, poloxamer molecules exist in solution as monomers (unimers) in which both the PPO block and the PEO chains are hydrated [5]. As the copolymer concentration and/or solution temperature increases, dehydration of the PPO chains occurs and, as a result of hydrophobic interactions, spherical micelles are formed in solution; the PPO chains are their cores, and the outer shells consist of hydrated PEO chains. The critical micelle concentration (CMC) and critical micelle temperature (CMT) decrease with increasing PPO block

content and molecular weight of the block copolymer [6]. The results of the CCM and CTM studies indicate that the formation of associates in aqueous solutions and the surface activity of poloxamers depend on their molecular characteristics [7, 8]. The thermodynamics of micelle formation in poloxamer solutions has been described by R. Alexandridis and co-workers [8]; this process is highly endothermic; during the micellisation, the entropy of the system increases due to the elimination of the hydrophobic hydration of the PPO chains. Poloxamer micelles have the ability to solubilise hydrophobic compounds [3], in particular, hydrophobic active substances used in pharmaceuticals, for example, in the development of anti-cancer drugs [9].

Table 1

Pharmacopoeial types of poloxamers

| Poloxamer type | Ethylene oxide units (*a*) | Propylene oxide units (*b*) | Content of oxyethylene (%) | Average relative molecular mass |
|---|---|---|---|---|
| 124 (P124) | 10–15 | 18–23 | 44.8–48.6 | 2090–2360 |
| 188 (P188) | 75–85 | 25–30 | 79.9–83.7 | 7680–9510 |
| 237 (P237) | 60–68 | 35–40 | 70.5–74.3 | 6840–8830 |
| 338 (P338) | 137–146 | 42–47 | 81.4–84.9 | 12700–17400 |
| 407 (P407) | 95–105 | 54–60 | 71.5–74.9 | 9840–14600 |

As the poloxamer content increases, the volume fraction of micelles increases. When the critical volume fraction of micelles is reached ($c \geq 0.53$), interactions between micelles occur. In the aqueous solution, lyotropic liquid crystals of cubic structure are formed from spherical micelles, causing the sol→gel transition [10, 11]. Lyotropic liquid crystals can also be hexagonal or lamellar [3, 10]. The temperature at which gel formation occurs increases as the length of the PEO chains increases. At concentrations of about 20 % *m/m* in aqueous solutions of certain poloxamers at temperatures close to body temperature, a thermoreversible sol→gel transition occurs. In other words, when their solutions are heated, poloxamers contribute to the formation of gels (thermoreversible systems) [3, 10]. This peculiarity of poloxamers is widely used in the development of prolonged medicinal products intended for use in various fields of medicine (ophthalmology [12], gynaecology [13], surgery [14], oncology [9], etc.) and proposed for different routes of administration: ophthalmic, injection, vaginal, transdermal, intra-articular [15–17]. At body temperature, transparent and stiff hydrogels are formed with an ordered structure formed by cubic lyotropic liquid crystals consisting of closely-packed spherical micelles [11]. These hydrogels are thought to result from a phase transition from a micellar solution state to a solid micellar cubic state [3]. Based on small-angle neutron scattering (SANS) and rheology results, the proposed mechanism of gelation is that it involves repulsive interactions among close-packed spherical micelles rather than aggregation or transitions in micelle morphology to rods or lamellae [11].

Various methods have been used to study poloxamer solutions, such as methods for determining surface and interfacial tension [18], cryogenic temperature transmission electron microscopy (cryo-TEM), small-angle neutron scattering (SANS) and rotational viscometry [5, 11], $^{1}$H NMR spectroscopy [19], small-angle X-ray scattering (SAXS) [19, 20], capillary viscometry [21], dynamic and static light scattering, dye solubilisation technique, differential scanning calorimetry [6, 22], $^{2}$D NMR, IR-, UV- and fluorescence spectroscopy, dynamic and static light scattering [21, 22], electric birefringence [18], etc.

Raman spectroscopy coupled to computational approaches has been exploited by M. T. Cook and co-authors in their work [23] to study thermally-induced processes in 20 % aqueous solutions of P407. The authors of this work emphasise that due to the importance of poloxamer-based thermoresponsive materials, there remains a highly warranted need to acquire a deeper understanding of the thermally-induced gelation mechanism in these materials. The authors conclude that the results of their research on thermally induced sol→gel transitions can be explained by the following processes:

1) the structure of the hydrophobic PPO segments in the cores of the micelles becomes more ordered and, as a result, their intermolecular interactions with water molecules are weakened,

2) PEO chains occupying the outer region of these micelles undergo a transition to a more disordered state with increased interactions with surrounding water molecules.

In our opinion, a more specific and promising method for studying surfactant associates, and poloxamer associates in particular, is the spin probe method. Electron paramagnetic resonance (EPR) spectroscopy has been most widely used to study models and biological membranes [24]. Two approaches can be distinguished: firstly, obtaining information from the EPR spectra of spin labels covalently bound to proteins [25, 26], and secondly, the use of spin probes (e.g., based on phospholipids or fatty acids) containing nitroxyl radicals in their molecules [27, 28]. The spin probe method was the first to provide information on the mobility and order of molecules in lipid membranes [24].

The spin probe method has also been used to study surfactant associates [29–31]. By using different spin probes, it is possible to study different areas of the micelle cores, the interface between the hydrophobic core and the hydrophilic shell, as well as the polar part of the micelles. This makes it possible to obtain comprehensive information about the structure of surfactant micelles, the consistency and viscosity of their non-polar core, the orderliness of the microenvironment surrounding the spin probe, as well as the hydration of radicals localised in different areas of the micelles [24]. In particular, using the spin probe method, it was shown that the packing density of hydrophobic PPO chains in P338 micelles is significantly higher than the packing density of alkyl chains in micelles of non-ionic, cationic and anionic surfactants [30]. In the study of P338 micelles, it was found that with an increase in temperature from 25 °C to 40 °C, the packing density and orderliness of the hydrophobic PPO chains in the micelle cores decreased. This leads to

an increase in the volume of the P338 micelles, which could probably cause a thermally induced sol→gel transition [31]. The consistency of P338 micelle cores at 40 °C in the case of gel formation can be characterised as two-dimensional liquid and one-dimensional solid (in the radial direction) [31, 32]. Such conclusions diverge from the assumptions of the authors based on the results of research using Raman spectroscopy and quantum mechanical calculations [23], as well as from ideas about the solid state of micellar cubic structure [3].

It is reasonable to use the spin probe method to study the structure of associates of different poloxamers, particularly at sol→gel transitions induced by increasing the temperature and concentration of block copolymers. The results of the studies by spin probe method should be compared with the rheological properties of poloxamer solutions. Rheological studies of solutions for those poloxamers described in the European Pharmacopoeia are also rational, as the comparative studies by these two methods are not described in the scientific literature. Most of the articles are devoted to studies of poloxamer 407 [9, 10, 23] or its derivatives [33]. Furthermore, due to the acceptable variability in the molecular structure of poloxamers in different batches [1], studies by both methods should be carried out using the same samples of aqueous solutions of poloxamers.

**The aim** was the study of aqueous solutions of different poloxamers by spin probe method and rotational viscometry depending on the temperature and poloxamer content.

**2. Planning (methodology) of the research**

The experiment was designed to use aqueous solutions of poloxamers 188, 237, 338 and 407 with different concentrations at which thermally induced sol→gel transitions occurred or did not occur. The study was to be conducted at temperatures of 25 °C (storage temperature), 32 °C (skin temperature) and 37 °C (rectal and vaginal temperature), and in some cases at temperatures below 25 °C.

It was planned to study the rheological properties of aqueous solutions of poloxamers depending on the type of poloxamers, their content, and temperature. It was necessary to determine the rheological parameters of poloxamer solutions depending on the variables and to distinguish between the conditions under which aqueous solutions of poloxamers are Newtonian liquids and those under which they are gels.

It was planned to use the spin probe method to detect changes in supramolecular structures formed by poloxamers in aqueous solutions, accompanied by changes in the rheological properties of these solutions. The spin probe method is indirect; it provides information about the object of study based on the EPR spectra of spin probes and the parameters of the obtained spectra depending on certain variables [24]. That is, information can be obtained about the behaviour of the probe in the phase of its localisation, which can be used to assess the state of this phase. It was of interest to establish the relationship between the change in the parameters of the EPR spectra and the sol→gel transitions in aqueous solutions of poloxamers. For the study, 5 spin probes were used to detect associates of poloxamers at the interface of polar and non-polar parts, at different levels of the hydrophobic core, and in the hydrophilic shell of micelles.

These extensive studies were designed to determine how and why poloxamer-based gels form at elevated temperatures.

**3. Materials and methods**

The following substances were used in the experiments: poloxamer 188 (Kolliphor® P 188 – P188), poloxamer 237 (Kolliphor® P 237 – P237), poloxamer 338 (Kolliphor® P 338 – P338), poloxamer 407 (Kolliphor® P 407 – P407) (BASF) and purified water (hereinafter – water) [1].

The aqueous solutions with poloxamer contents of 10 % m/m, 15 % m/m, 20 % m/m and 25 % m/m were the subjects of the study. The poloxamers were dissolved in water at about 12 °C, and the solutions were then degassed. These solutions were tested at 25 °C, 32 °C, and 37 °C as well as below 25 °C.

Rheological properties were studied by rotational viscometry [1]. Rheograms (plots of the shear stress ($\tau_r$) *vs* the shear rate ($D_r$)) were obtained using a rotating viscometer «Rheolab QC» with coaxial cylinders CC-27 (for gels) and DG-42 (for liquids) («Anton Paar GmbH»; software RHEOPLUS, 2.66 version). Rheograms were used to characterise the flow behaviour and to determine the dynamic viscosity (η) of Newtonian liquids or the apparent viscosity (η) of gels as well as the yield stress ($\tau_0$) of gels [1, 2].

The viscosity (η) was calculated using the following equation:

$$\eta=\tau_r/D_r. \quad (1)$$

Electron paramagnetic resonance (EPR) spectroscopy was used for the research [24, 34, 35]. The following spin probes were used:

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

– probe 2: 4-[(N,N-dimethyl-N-hexadecyl)ammonio]-2,2,6,6tetramethyl-piperidine-1-oxyl iodide ($M_r$ 551.65; CAS [114199-16-5]);

– probe 3: 5-DOXYL Stearic acid, ammonium salt ($M_r$ 401.61) (5-DSA, $NH_4$ salt);

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

– probe 5: 4-Oxo-2,2,6,6-tetramethyl-1-piperidinyloxy ($M_r$ 170.23; CAS: [2896-70-0]) (4-OXO-TEMPO).

Probe 1 and probe 4 simulated lipophilic surfactants. Probe 2 and probe 3 simulated cationic and anionic surfactants, respectively. During the solubilisation of probe molecules by micelles, the free radicals of probe 1 and probe 2 are localised in the hydrophilic part, and their alkyl chains are localised in the hydrophobic core. The doxyl radicals of probes 3 and 4 are located, near the $5^{th}$ and $16^{th}$ carbon atoms, respectively, of the alkyl chains localised in the hydrophobic core of the micelles.

The probe 4-OXO-TEMPO could be simultaneously soluble in water and localised in the hydrophilic part of micelles/mesophases formed by non-ionic surfactants. When the phases of the probe localisation differed significantly in the polarity of the radical environment, the EPR spectrum of the probe 4-OXO-TEMPO was superimposed, and the signal was split into two lines in the high field [23, 34, 35].

The spin probes were added to the studied solutions at a concentration of $10^{-4}$ mol/l. The EPR spectra were recorded using the «ESR Spectrometer CMS8400» («Adani»). The type of EPR spectra (triplet, anisotropic spectrum, singlet, superposition spectrum), the peak heights, and the linewidth at the low-field ($\Delta H_{+1}$), central ($\Delta H_0$) and high-field ($\Delta H_{-1}$) components were determined. The rotational correlation times of the spin probes ($\tau_{+1}$, $\tau_{-1}$, $\tau_{\pm1}$) and the anisotropy parameter (ε) were calculated using the following equations [24, 34, 35]:

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

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

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

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

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; $\Delta H_{+1}$ and $\Delta H_0$ are the linewidth at low-field and central components, respectively.

The reorientation of nonspherical molecules dissolved in a liquid is characterised by two different correlation times: $\tau_{-1}$, which is associated with fluctuations in directions perpendicular to the long axis of the probe, and $\tau_{+1}$, which is associated with its rotation around the long axis [34]; in addition, the values of $\tau_{\pm1}$ were calculated [35]. 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 ($T$) [24, 34]:

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

In the case of the triplet spectra for probes 1, 2 and 5, the $A_N$ constant was determined as the distance (mT) between the central and high-field components [34]. In the case of the EPR spectra for probe 3 and probe 4, the $A_N$ constant and the order parameter ($S$) were calculated after the determination of the hyperfine splitting constants $A_{\parallel}$ and $A_{\perp}$ according to the equations [24]:

$$A_N = \left(A_{\parallel} + 2A_{\perp}\right) / 3; \quad (7)$$

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}} \cdot 1.66. \quad (8)$$

The parameter γ characterising the half-amplitude of molecular motion was determined by the order parameter ($S$) using the calibration graph [24].

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

**4. Research results**

**4. 1. Study of aqueous solutions of poloxamers by rotational viscometry**

At 25 °C, 15 % aqueous solutions of poloxamers are Newtonian liquids (Fig. 1) whose dynamic viscosity depends on the type of poloxamer: it was 6.7 mPa·s in the case of P237, 7.4 mPa·s – P188, 23.7 mPa·s – P338 and 45.2 mPa·s – P407.

At temperatures between 25 °C and 37 °C, P188 solutions are Newtonian liquids in the concentration range of 10 % to 25 %. The dynamic viscosity of the 25 % solution of P188 is 22 mPa·s at 25 °C, 19 mPa·s at 32 °C, and 17 mPa·s at 37 °C. That is, the dynamic viscosity of the 25 % P188 solution decreases with increasing temperature.

On the contrary, as the temperature is increased from 25°C to 32°C, the dynamic viscosity of a 25 % P237 solution increases from 31 mPa·s to 110 mPa·s. With a further increase in temperature to 37 °C, a sol→gel transition occurs (Fig. 2). The gel is characterised by plastic flow behaviour with a lower yield stress of 612 Pa and apparent viscosity of 66870 mPa·s (at $D_r$=14.6 $s^{-1}$), 25210 mPa·s (at $D_r$=41.6 $s^{-1}$), 13250 mPa·s (at $D_r$=82.3 $s^{-1}$).

In the case of P338, the sol→gel transition occurs for 20 % aqueous solution when the temperature is raised to 32 °C (Fig. 3, *a*), and 25 % P338 solution is a gel with a plastic flow behaviour at 25 °C, 32 °C and 37 °C (Fig. 3, *b*). When cooled to 20 °C and 15 °C respectively, this gel becomes a Newtonian liquid with a dynamic viscosity of 290 mPa·s and 80 mPa·s (Table 2).

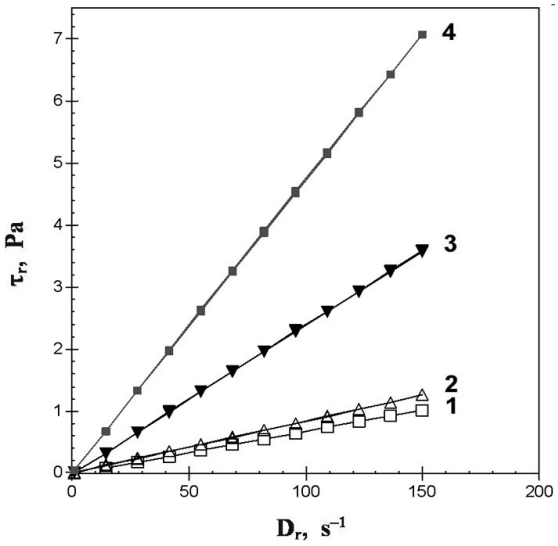



Fig. 1. Rheograms of 15 % aqueous solutions of poloxamers: 1 – P237; 2 – P188; 3 – P338; 4 – P407 (at 25 °C)

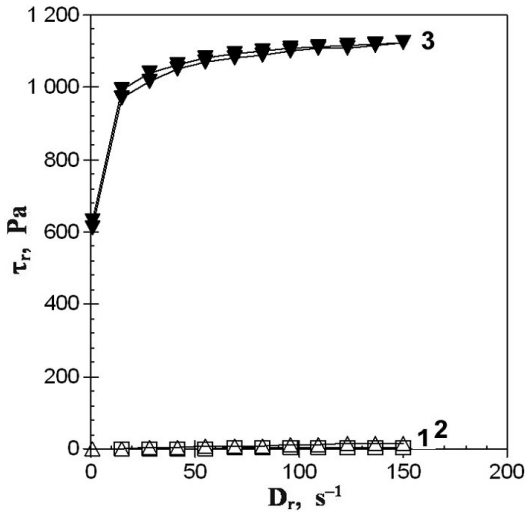
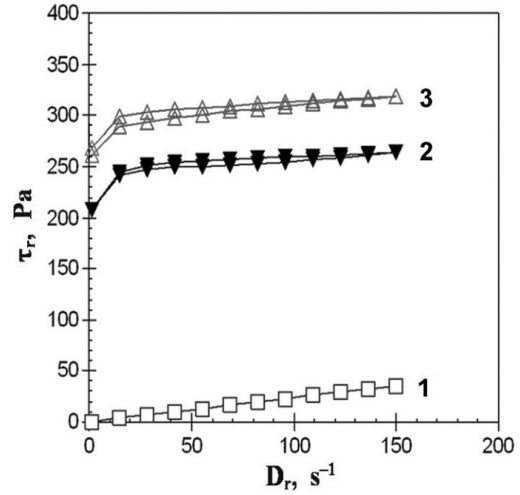
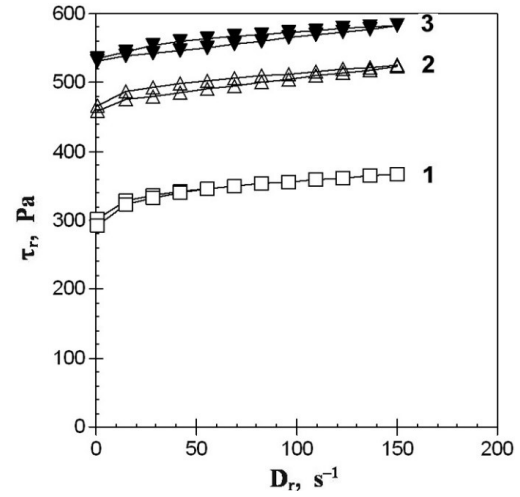



Fig. 2. Rheograms of 25 % P237 solution at: 1 – 25 °C; 2 – 32 °C; 3 – 37 °C




*a*




*b*

Fig. 3. Rheograms of P338 solutions: *a* – 20 %; *b* – 25 %; at: 1 – 25 °C; 2 – 32 °C; 3 – 37 °C

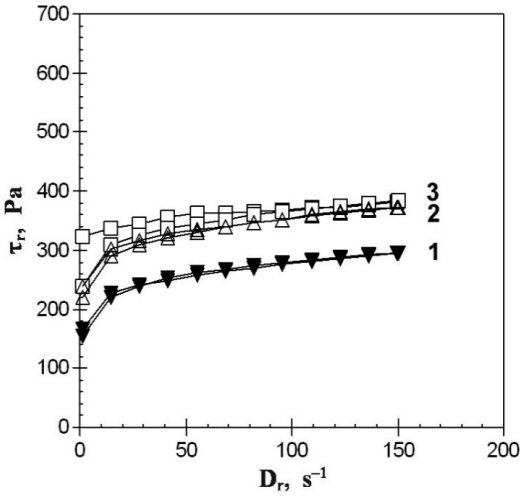
The dynamic viscosity (η) of P338 solutions, which are Newtonian liquids, as well as the rheological parameters ($\tau_0$, η at different $D_r$) of the gels, increases with increasing P338 concentration and temperature (Table 2).

Table 2

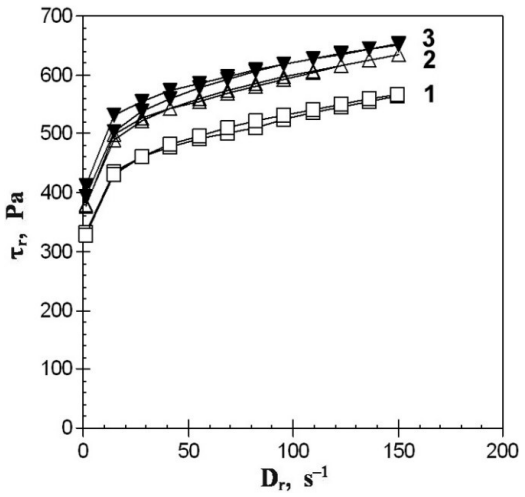
Rheological parameters of P338 solutions at various poloxamer concentrations (*C*) and different temperatures (*t*)

| *t* | *C*, % *m/m* | $\tau_0$, Pa | η (mPa·s) at $D_r$ | | |
|---|---|---|---|---|---|
| | | | 14.6 $s^{-1}$ | 41.6 $s^{-1}$ | 82.3 $s^{-1}$ |
| 15 °C | 25 % | 0 | 80.0* | | |
| 20 °C | | 0 | 290* | | |
| 25 °C | 10 % | 0 | 7.0* | | |
| | 15 % | 0 | 23.7* | | |
| | 20 % | 0 | 110.0* | | |
| | 25 % | 301.2 | 22600** | 8220** | 4290** |
| 32 °C | 10 % | 0 | 12.4* | | |
| | 15 % | 0 | 62.0* | | |
| | 20 % | 207.1 | 16600** | 6000** | 3090** |
| | 25 % | 466.4 | 33500** | 12000** | 6190** |
| 37 °C | 10 % | 0 | 16.4* | | |
| | 15 % | 0 | 94.0* | | |
| | 20 % | 267.3 | 20600** | 7350** | 3780** |
| | 25 % | 534.8 | 37470** | 13420** | 6930** |

*Note: * – dynamic viscosity; ** – apparent viscosity*




*a*




*b*

Fig. 4. Rheograms of P407 solutions: *a* – 20 %; *b* – 25 %; at: 1 – 25 °C; 2 – 32 °C; 3 – 37 °C

In the case of P407, the change from Newtonian to non-Newtonian flow behaviour occurs even for 15 % aqueous solution when the temperature is increased to

37 °C (Table 3). At 25 °C, 32 °C, and 37 °C, 20 % and 25 % P407 solutions are gels with plastic flow behaviour (Fig. 5). At 20 °C, the 25 % P407 solution is still a gel, and at 15 °C it is a Newtonian liquid (Table 3).

Table 3

Rheological parameters of P407 solutions at various poloxamer concentrations (*C*) and different temperatures (*t*)

| *t* | C, % *m/m* | $\tau_0$, Pa | η (mPa·s) at $D_r$ | | |
|---|---|---|---|---|---|
| | | | 14.6 $s^{-1}$ | 41.6 $s^{-1}$ | 82.3 $s^{-1}$ |
| 10 °C | 25 % | 0 | 60.0* | | |
| 15 °C | | 0 | 190.0* | | |
| 20 °C | | 233.2 | 23660** | 9310** | 5120** |
| 25 °C | 10 % | 0 | 7.7* | | |
| | 15 % | 0 | 45.2* | | |
| | 20 % | 166.4 | 15600** | 5970** | 3280** |
| | 25 % | 331.7 | 29880** | 11500** | 6200** |
| 32 °C | 10 % | 0 | 9.2* | | |
| | 15 % | 0 | 80.0* | | |
| | 20 % | 240.1 | 20780** | 7850** | 4200** |
| | 25 % | 378.1 | 34230** | 13020** | 7060** |
| 37 °C | 10 % | 0 | 10.5* | | |
| | 15 % | 25.1 | 2158** | 892** | 530** |
| | 20 % | 323.6 | 23100** | 8530** | 4440** |
| | 25 % | 410.9 | 36590** | 13760** | 7390** |

As in the case of aqueous solutions of P338, the dynamic viscosity (η) of aqueous solutions of P338, which are Newtonian fluids, as well as the values of the rheological parameters ($\tau_0$, η at different $D_r$) of the gels increase with increasing concentration of P407 and temperature (Table 2).

When comparing the rheological parameters of gels based on 25 % aqueous solutions of different poloxamers at 37 °C, the highest values of apparent viscosity and lower yield stress are characteristic of the gel with P237. The rheological parameters of gels with P407 are generally slightly higher than those with P338, but they are comparable.

**4. 2. Study of aqueous solutions of poloxamers by the spin probe method**

The EPR spectra of fatty acid-based spin probes solubilised in poloxamer associates have a characteristic shape; they are triplets or anisotropic spectra (in the case of the probe 3 – 5-DSA NH4 salt) from which certain spectral parameters can be calculated. EPR spectra are singlet if the lipophilic probe is not solubilised and its molecules form associates in an aqueous medium. EPR spectra are also singlet when domains from probe molecules are formed in poloxamer associates. Representative EPR spectra of lipophilic probe 1 in solutions of poloxamers are shown in Fig. 5, and the parameters of these EPR spectra are given in Table 4.

In a 25 % P188 solution at 25 °C, the lipophilic probe 1 is almost not solubilised; its EPR spectrum is predominantly a singlet (Fig. 5, *a*). However, with increasing temperature, the ability of P188 associates to solubilise the probe increases and at 32 °C the EPR spectrum transforms into a triplet (Fig. 5, *a*). The EPR spectra of probe 1 in 25 % P237solution, 20 % P338 solution and 15 % P407solution are triplet (Fig. 5, *b*, Table 4), indicating that molecules of probe 1 are localised in the poloxamer associates, are uniformly distributed there and undergo rapid lateral diffusion. The nitroxyl radical of probe 1 is localised in the hydrophilic part of the poloxamer associates formed by hydrated PEO chains, as evidenced by the values of the $A_N$ constant (Table 4). The alkyl chain of probe 1 is localised in the hydrophobic part of the poloxamer associates formed by PPO chains.

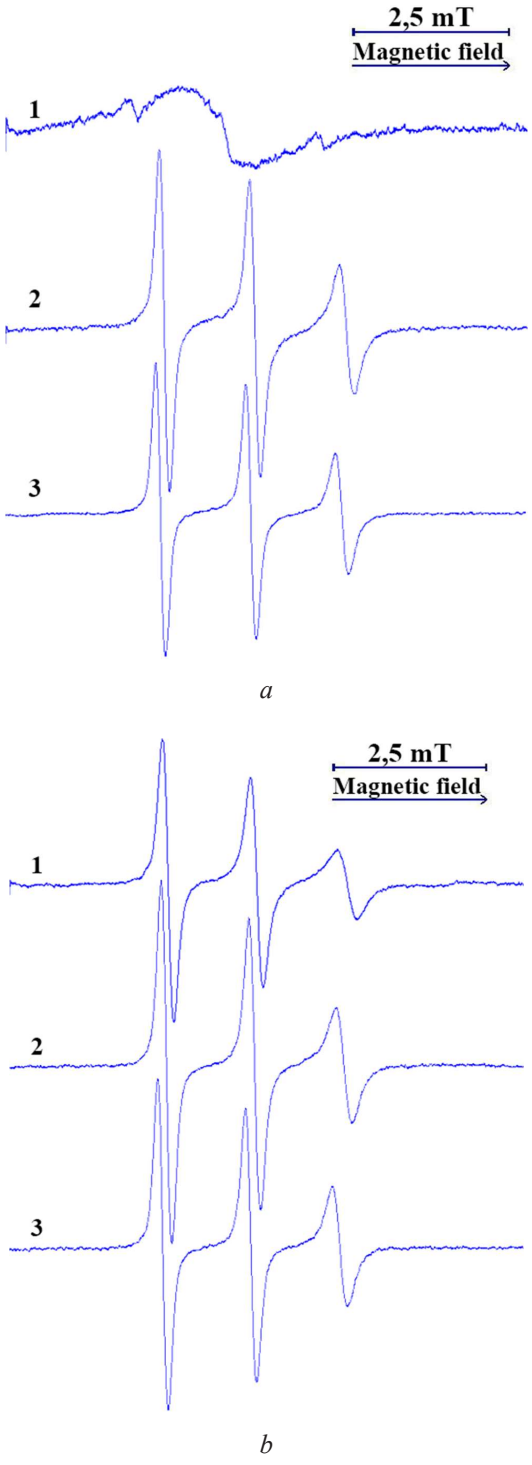



Fig. 5. Representative EPR spectra of lipophilic probe 1 in: *a* – 25 % P188 solution; *b* – 20 % P338 solution; at: 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

Table 4

Parameters of the EPR spectra of spin probe 1 in poloxamer solutions at different temperatures (*t*)

| Object | *t*, °C | $A_N$, mT | $\tau_{-1}$, ns | $\tau_{\pm1}$, ns | ε | Spectrum type |
|---|---|---|---|---|---|---|
| 25 % P188 solution | 25 | – | – | – | – | singlet |
| | 32 | 1.62 | 0.27 | 0.75 | –0.13 | triplet |
| | 37 | 1.61 | 0.23 | 0.62 | –0.15 | triplet |
| 25 % P237 solution | 25 | 1.63 | 0.47 | 1.39 | –0.18 | triplet |
| | 32 | 1.61 | 0.32 | 0.97 | –0.18 | triplet |
| | 37 | 1.60 | 0.28 | 0.81 | –0.19 | triplet |
| 20 % P338 solution | 20 | 1.66 | 0.75 | 2.37 | –0.14 | triplet |
| | 25 | 1.64 | 0.48 | 1.42 | –0.19 | triplet |
| | 32 | 1.62 | 0.34 | 0.97 | –0.18 | triplet |
| | 37 | 1.60 | 0.27 | 0.84 | –0.18 | triplet |
| 15 % P407 solution | 25 | 1.61 | 0.59 | 1.47 | –0.12 | triplet |
| | 32 | 1.60 | 0.33 | 0.94 | –0.18 | triplet |
| | 37 | 1.59 | 0.28 | 0.80 | –0.20 | triplet |

25 % P237 solution is the gel at 37 °C (Fig. 2), 20 % P338 solution is the gel at 32 °C and 15 % P407solution is the gel at 37 °C (Table 3). The parameters of the EPR spectra of probe 1 in the poloxamer associates change with increasing temperature (Table 4). First, the rotational correlation times ($\tau_{-1}$, $\tau_{\pm1}$) decrease by a factor of 1.4 to 1.8 at 32 °C and by a factor of 1.7 to 2.1 at 37 °C. The linewidth at the high-field component of the spectra ($\Delta H_{-1}$) shown in Fig. 5, *b*, decreases with increasing temperature, and it is 0.35 mT at 25 °C, 0.29 mT at 32 °C, and 0.26 mT at 37 °C. Secondly, there is a tendency for the values of the $A_N$ constant to decrease, indicating a decrease in the polarity of the nitroxyl radical environment. In addition, in the case of 15 % P407 solution, there is a decrease in the anisotropy parameter (ε) with increasing temperature from 25 °C to 37 °C. In the case of 25 % P237 solution and 20 % P338 solution, the values of the parameter ε are almost the same with increasing temperature.

It is reasonable to compare the values of $\tau_{-1}$ and $\tau_{\pm1}$ of spin probe 1 in solutions of poloxamers (Table 4), where their molecules are solubilised by micelles, with the values of $\tau_{-1}$ and $\tau_{\pm1}$ of spin probe 1 in liquid paraffin and white soft paraffin (Table 5). This comparison is somewhat tentative as liquid paraffin and white soft paraffin are isotropic substances, whereas micelle cores are anisotropic in viscosity. However, this comparison can be used to assess the consistency of the micelle cores.

Table 5

Parameters of the EPR spectra of the spin probe 1 in liquid paraffin and white soft paraffin at 25 °C

| Object | $\tau_{-1}$, ns | $\tau_{\pm1}$, ns | ε | $A_N$, mT |
|---|---|---|---|---|
| Liquid paraffin | 0.28 | 0.70 | –0.21 | 1.56 |
| White soft paraffin | 0.47 | 1.34 | –0.18 | 1.56 |

At 25 °C the values of $\tau_{-1}$ and $\tau_{\pm1}$ of probe 1 in solutions of poloxamers P237, P338, and P407 are close to the values of $\tau_{-1}$ and $\tau_{\pm1}$ of probe 1 in the case of white soft paraffin (Tables 4, 5). At 37 °C the values of $\tau_{-1}$ and $\tau_{\pm1}$ of probe 1 in solutions of poloxamers P188, P237, P338 and P407 are similar to the values of $\tau_{-1}$ and $\tau_{\pm1}$ of probe 1 in the case of liquid paraffin at 25 °C (Tables 4, 5). This means that at the temperature increase of 12 °C, the micelle cores formed by the poloxamers melt and their consistency changes from semi-solid to liquid. At 37 °C (the temperature at which solutions of poloxamers P237, P338 and P407 are gels), the values of $\tau_{-1}$ and $\tau_{\pm1}$ of probe 1 are in the rapid rotation range. Since the position of the PPO chains in the micelle cores is fixed in the radial direction, their consistency can be characterised as two-dimensionally liquid and one-dimensionally solid [32]. With decreasing temperature, the viscosity of micelle cores increases; for example, at 20 °C, the $\tau_{\pm1}$ value of spin probe 1 in P338 micelles is 2.37 ns (Table 4), which corresponds to the slow rotation range [24, 34, 35].

The nitroxyl radical of the hydrophilic spin probe 2 is also localised in the hydrophilic part of the poloxamer associates and the alkyl chain – in their hydrophobic part. Representative EPR spectra of hydrophilic probe 2 in a 20 % P338 solution are shown in Fig. 6, and the parameters of these spectra are given in Table 6.

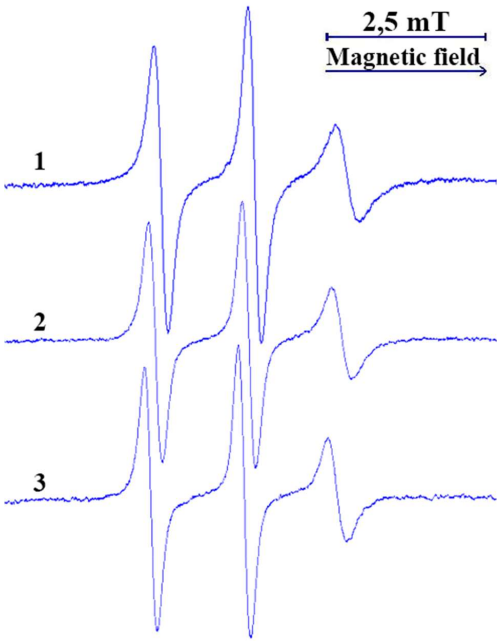



Fig. 6. Representative EPR spectra of hydrophilic probe 2 in 20 % P338 solution at 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

In the case of the EPR spectra of the hydrophilic probe 2, a decrease in the values of $\tau_{+1}$, $\tau_{-1}$, $\tau_{\pm1}$ was also observed with increasing temperature, and there were tendencies to decrease in the values of the $A_N$ constant and the anisotropy parameter (ε) (Table 6).

Table 6

Parameters of the EPR spectra of spin probe 2 in 20 % P338 solution at different temperatures (*t*)

| *t*, °C | $A_N$, mT | $\tau_{+1}$, ns | $\tau_{-1}$, ns | $\tau_{\pm1}$, ns | ε | Spectrum type |
|---|---|---|---|---|---|---|
| 20 | 1.64 | 1.58 | 0.76 | 1.55 | 0.12 | triplet |
| 25 | 1.62 | 0.94 | 0.55 | 1.15 | 0.10 | triplet |
| 32 | 1.61 | 0.59 | 0.44 | 0.98 | 0.08 | triplet |
| 37 | 1.60 | 0.57 | 0.41 | 0.85 | 0.07 | triplet |

The EPR spectra of probe 3 (5-DSA NH4 salt) in solutions of poloxamers P237, P338 and P407 were anisotropic spectra (Fig. 7, *b*, 8) characterised by the order parameter (*S*) and the parameter γ of probe 3 (Table 7).

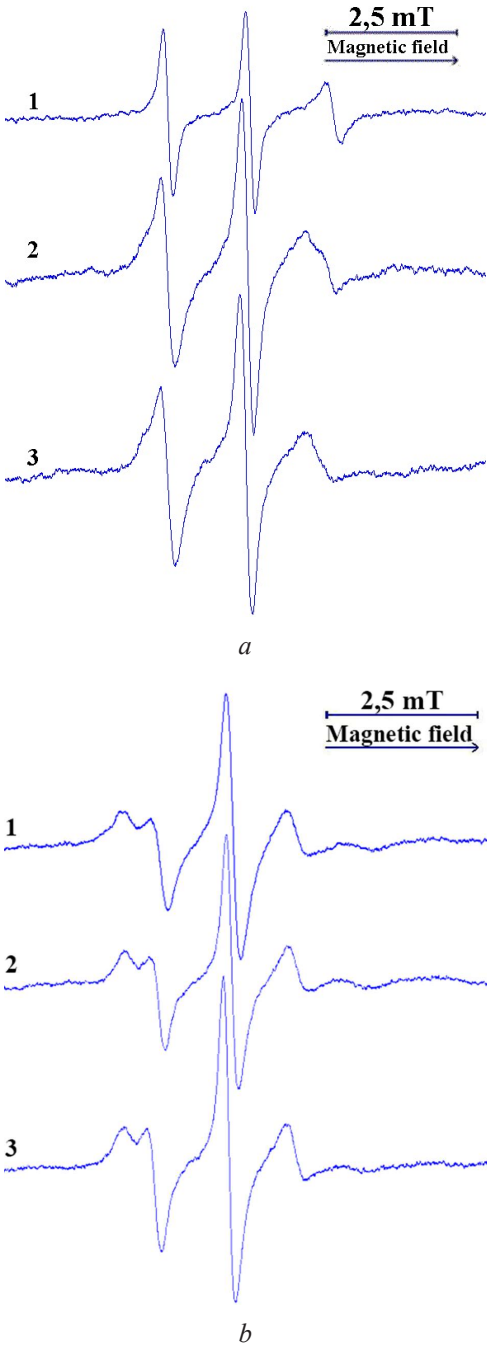



Fig. 7. EPR spectra of probe 3 in: *a* – 20 % P188 solution; *b* – 20 % P338 solution; at 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

The doxyl radical of probe 3 is localised in the hydrophobic part of poloxamer associated at the level of the fifth carbon atom in the alkyl chain of this probe. In the case of spin probe 3 introduced into solutions of poloxamers P237, P338 and P407, the order parameter (S) of the EPR spectra decreases with increasing temperature from 25 °C to 37 °C. Thereby, the parameter γ increases (Table 7). This indicates that the microenvironment of probe 3 becomes less ordered with increasing temperature. In contrast, at 25 °C the EPR spectrum of probe 3 in a 20 % P188 solution is a triplet (*S*=0.09); at 32 °C the EPR spectrum is a superposition of two triplet spectra and at 37 °C it becomes an anisotropic spectrum (*S*=0.35) (Table 7, Fig. 7, *a*). The EPR spectra of probe 3 in P188 solution (Fig. 7, *a*), which does not form a gel, are very different from the EPR spectra of probe 3 in solutions of poloxamers P237, P338 and P407, which form gels (Fig. 7).

The values of the $A_N$ constant indicate that the environment of the doxyl radical of probe 3 is non-polar. With increasing temperature, in the case of micelles of poloxamers P188 and P237, a slight tendency to increase in the values of the $A_N$ constant was observed, which could be due to the penetration of water molecules into the non-polar core. In the case of micelles of poloxamers P338 and P407, there was only a slight increase in the value of the $A_N$ constant at 37 °C. This means that, against the background of a significant decrease in the packing density of the PPO chains in the micelle cores, there is almost no penetration of water into the non-polar core at the level of the 5$^{th}$ carbon atom of the alkyl chain of spin probe 3.

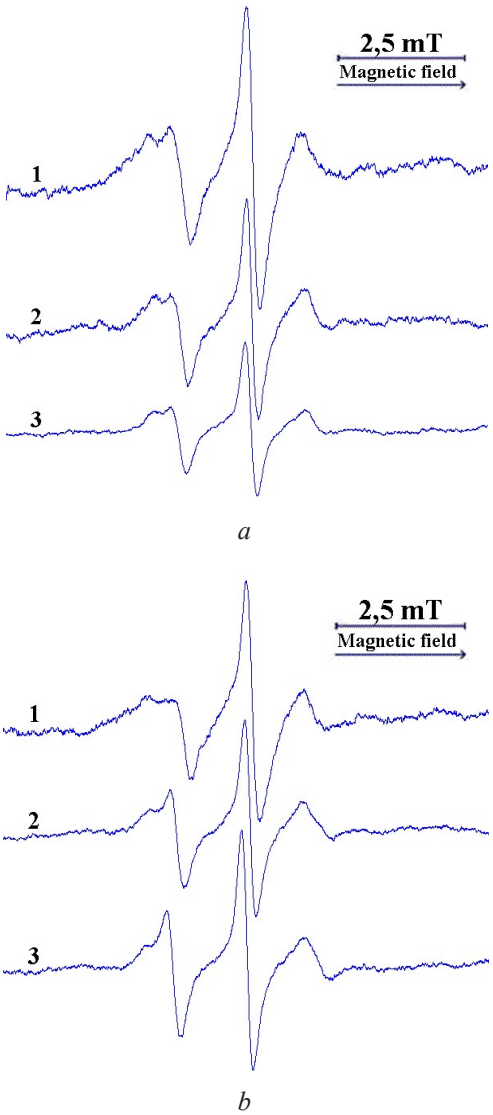



Fig. 8. EPR spectra of probe 3 in: *a* – 20 % P237 solution; *b* – 20 % P407 solution; at: 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

Table 7

Parameters of the EPR spectra of spin probe 3 (5-DSA $NH_4$ salt) in 20 % solution of poloxamers at different temperatures ($t$)

| Object | $t$, °C | $A_N$, mT | $S$ | γ, ° | Spectrum type |
|---|---|---|---|---|---|
| 20 % P188 solution | 25 | 1.52 | 0.09 | 82.0 | triplet |
| | 32 | 1.54 | | | superposition |
| | 37 | 1.55 | 0.33 | 63.1 | anisotropic |
| 20 % P237 solution | 25 | 1.48 | 0.46 | 54.1 | anisotropic |
| | 32 | 1.51 | 0.40 | 58.0 | anisotropic |
| | 37 | 1.53 | 0.39 | 59.0 | anisotropic |
| 20 % P338 solution | 20 | 1.52 | 0.54 | 48.8 | anisotropic |
| | 25 | 1.48 | 0.45 | 55.0 | anisotropic |
| | 32 | 1.48 | 0.42 | 56.9 | anisotropic |
| | 37 | 1.49 | 0.39 | 59.0 | anisotropic |
| 20 % P407 solution | 25 | 1.50 | 0.46 | 54.1 | anisotropic |
| | 32 | 1.50 | 0.41 | 57.5 | anisotropic |
| | 37 | 1.53 | 0.37 | 60.0 | anisotropic |

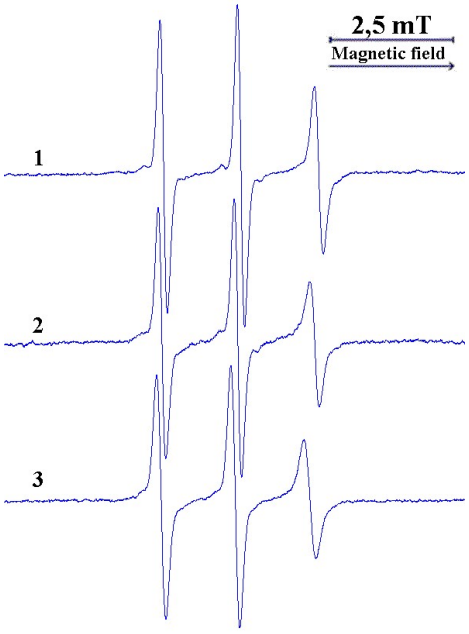
No gels are formed in the 20 % P237 solution at temperatures from 25 °C to 37 °C, but the values of the order parameter of the EPR spectra of probe 3 differ little or not from those when probe 3 is located in gels formed by P338 and P407 (Table 7). Similarly, the values of parameter $S$ in the case of the EPR spectra of probe 3 in the P338 solution, which is a Newtonian liquid at 25 °C, and in the case of the P407 solution, which is a gel at this temperature, differ little.

The doxyl radical of probe 4 (16-DSA) is also localised in the micelle core but at the level of the 16$^{th}$ carbon atom of the alkyl chain of this probe. The EPR spectra of probe 4 are triplets (Fig. 9, 10), indicating a significantly lower ordering of the local surrounding of probe 4 compared to probe 3. The values of the order parameter in the case of the EPR spectra of probe 4 are about 3.8-4.9 times lower (Tables 7, 8). The values of $A_N$ constant in the case of the EPR spectra of probe 4 are also lower (Tables 7 and 8), indicating a more non-polar microenvironment of radicals.

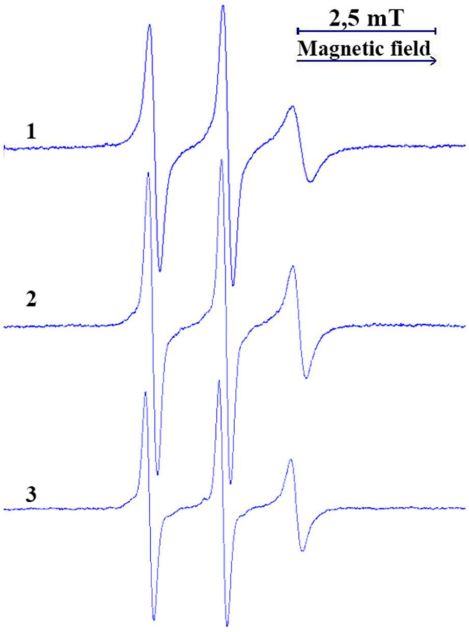
Table 8

Parameters of the EPR spectra of spin probe 4 (16-DSA) in 20 % solution of poloxamers at different temperatures ($t$)

| Object | $t$, °C | $A_N$, mT | $S$ | γ, ° | Spectrum type |
|---|---|---|---|---|---|
| 20 % P188 solution | 25 | 1.52 | 0.07 | 83.4 | triplet |
| | 32 | 1.50 | 0.07 | 83.4 | triplet |
| | 37 | 1.46 | 0.08 | 82.5 | triplet |
| 20 % P237 solution | 25 | 1.44 | 0.11 | 80.0 | triplet |
| | 32 | 1.44 | 0.09 | 81.7 | triplet |
| | 37 | 1.45 | 0.08 | 82.5 | triplet |
| 20 % P338 solution | 20 | 1.39 | 0.16 | 75.4 | triplet |
| | 25 | 1.42 | 0.12 | 78.8 | triplet |
| | 32 | 1.42 | 0.10 | 80.6 | triplet |
| | 37 | 1.43 | 0.09 | 81.7 | triplet |
| 20 % P407 solution | 25 | 1.42 | 0.11 | 80.0 | triplet |
| | 32 | 1.43 | 0.09 | 81.7 | triplet |
| | 37 | 1.43 | 0.08 | 82.5 | triplet |




*a*




*b*

Fig. 9. EPR spectra of probe 4 in: *a* – 20 % P188 solution; *b* – 20 % P338 solution; at: 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

In the case of the EPR spectra of the hydrophobic probe 4, with an increase in temperature of 20 % solutions of poloxamers P237, P338, and P407, there is a decrease in the rotational correlation times ($\tau_{+1}$, $\tau_{-1}$, $\tau_{\pm1}$) and a tendency for the values of the anisotropy parameter (ε) to decrease. At the same time, the values of the $A_N$ constant are approximately the same. In contrast, the rotational correlation times ($\tau_{-1}$, $\tau_{\pm1}$) of probe 4 in 20 % P188 solution increase with increasing temperature; the anisotropy parameter (ε) decreases.

The parameters of the EPR spectra were also determined at different poloxamer contents since increas-

ing the concentration is a factor contributing to the sol→gel transition.

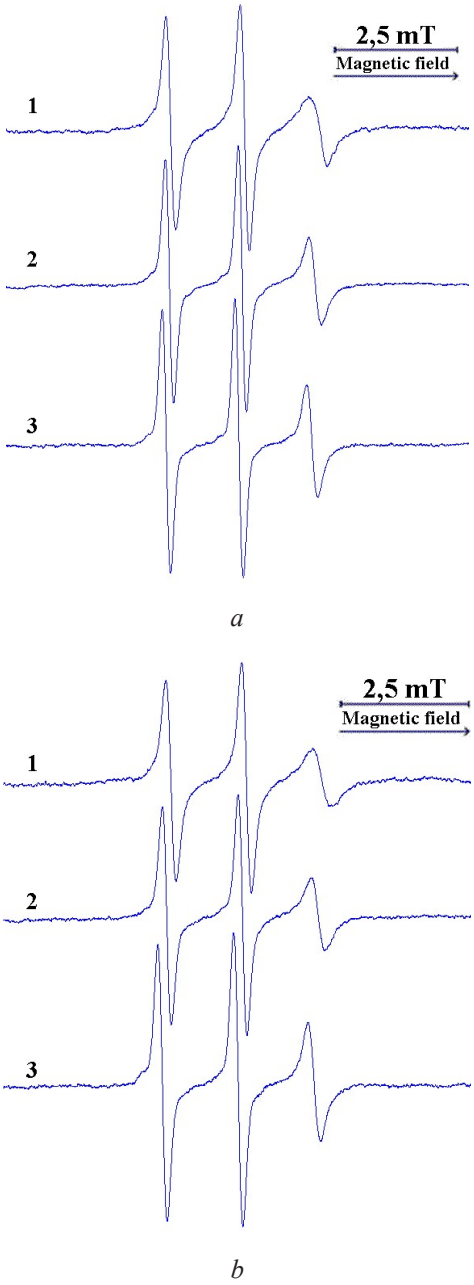



Fig. 10. EPR spectra of probe 4 in: *a* – 20 % P237 solution; *b* – 20 % P407 solution; at: 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

When the P338 concentration is increased from 10 % to 25 %, the parameters of the EPR spectra of spin probe 1 do not change significantly. There is only a tendency for the value of $\tau_{\pm1}$ to increase as the P338 content increases from 10 % to 20 % at 25 °C (Table 10). At P338 contents of 20 % and 25 %, the values of the parameters of the EPR spectra of probe 1 are almost the same. At the same time, at different contents of P338, the rotational correlation times ($\tau_{-1}$, $\tau_{\pm1}$) of spin probe 1 and the values of the $A_N$ constant of the EPR spectra decrease with increasing temperature.

It should be noted that it was not possible to study the dependence of the parameters of the EPR spectra on the concentration of all the poloxamers using probe 1 since the EPR spectra of probe 1 localised in 20 % and 25 % aqueous solutions of P407 are superposition of a triplet with a singlet (Fig. 11, *b*). The singlet is probably due to the partial aggregation of probe 1 molecules in associates of P407, which may be associated with a deterioration in lateral diffusion. The singlet fraction decreases with increasing temperature, probably due to a decrease in the packing density of PPO chains in P407 associates and the creation of better conditions for the uniform distribution of probe 1 molecules.

Table 9

Parameters of the EPR spectra of spin probe 4 (16-DSA) in 20 % solution of poloxamers at different temperatures (*t*)

| Object | *t*, °C | $\tau_{+1}$, ns | $\tau_{-1}$, ns | $\tau_{\pm1}$, ns | ε |
|---|---|---|---|---|---|
| 20 % P188 solution | 25 | 0.35 | 0.16 | 0.32 | 0.13 |
| | 32 | 0.39 | 0.20 | 0.43 | 0.11 |
| | 37 | 0.32 | 0.23 | 0.54 | 0.08 |
| 20 % P237 solution | 25 | 0.69 | 0.44 | 0.97 | 0.09 |
| | 32 | 0.36 | 0.34 | 0.72 | 0.06 |
| | 37 | 0.25 | 0.27 | 0.59 | 0.05 |
| 20 % P338 solution | 20 | 1.43 | 0.71 | 1.42 | 0.11 |
| | 25 | 0.74 | 0.59 | 1.29 | 0.07 |
| | 32 | 0.38 | 0.39 | 0.85 | 0.05 |
| | 37 | 0.29 | 0.30 | 0.68 | 0.05 |
| 20 % P407 solution | 25 | 0.70 | 0.55 | 1.15 | 0.07 |
| | 32 | 0.44 | 0.39 | 0.82 | 0.06 |
| | 37 | 0.27 | 0.29 | 0.64 | 0.06 |

Table 10

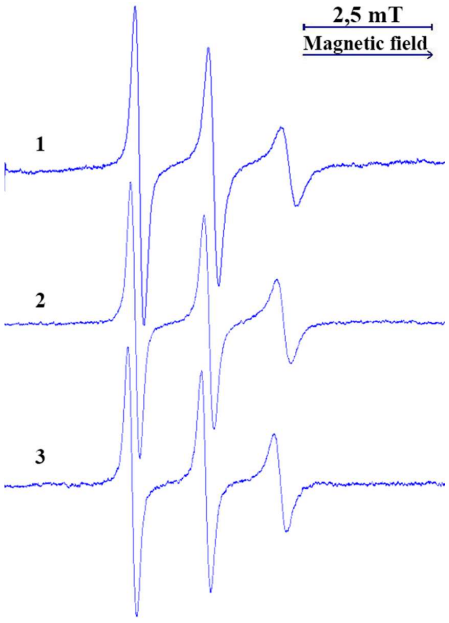
Parameters of the EPR spectra of spin probe 1 in aqueous solutions with different P338 contents (*C*) at different temperatures (*t*)

| *C* P338, % *m/m* | *t*, °C | $A_N$, mT | $\tau_{-1}$, ns | $\tau_{\pm1}$, ns | ε | Spectrum type |
|---|---|---|---|---|---|---|
| 10 % | 25 | 1.63 | 0.44 | 1.04 | –0.15 | triplet |
| | 32 | 1.61 | 0.34 | 0.94 | –0.16 | triplet |
| | 37 | 1.60 | 0.27 | 0.75 | –0.18 | triplet |
| 15 % | 25 | 1.63 | 0.43 | 1.30 | –0.19 | triplet |
| | 32 | 1.61 | 0.35 | 0.95 | –0.18 | triplet |
| | 37 | 1.60 | 0.28 | 0.76 | –0.19 | triplet |
| 20 % | 25 | 1.64 | 0.48 | 1.42 | –0.19 | triplet |
| | 32 | 1.62 | 0.34 | 0.97 | –0.18 | triplet |
| | 37 | 1.61 | 0.27 | 0.84 | –0.18 | triplet |
| 25 % | 25 | 1.63 | 0.48 | 1.41 | –0.16 | triplet |
| | 32 | 1.62 | 0.34 | 1.00 | –0.18 | triplet |
| | 37 | 1.60 | 0.28 | 0.83 | –0.20 | triplet |

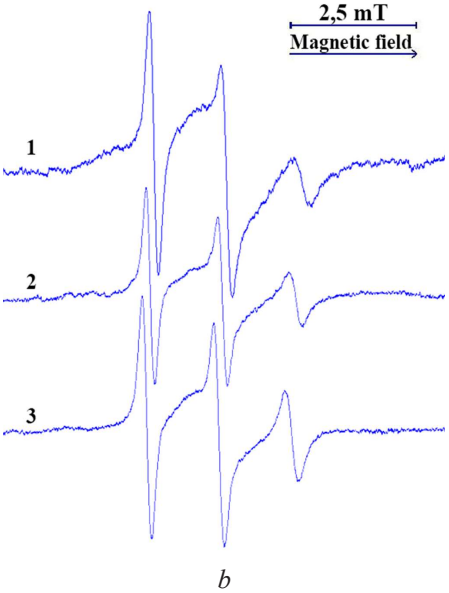
The hydrophilic spin probe 4-OXO-TEMPO (probe 5) was used to study the hydrophilic part of poloxamer associates. In aqueous solutions of non-ionic surfactants, the molecules of the probe 4-OXO-TEMPO are partially dissolved in the aqueous medium and partially localised in the polar part of micelles or lyotropic liquid crystals (hydrated PEO chains). This is evidenced by the difference in the rotational correlation times of the probe 4-OXO-TEMPO in water and aqueous solutions of poloxamers. For example, at 25 °C in water, the values of $\tau_{-1}$

and $\tau_{\pm1}$ of the probe 4-OXO-TEMPO are 0.007 ns and 0.010 ns, respectively, and in 25 % aqueous solution of P407, they are 0.024 ns and 0.037 ns, which are 3.4 and 3.7 times higher, respectively. At the same time, the $A_N$ constant decreases; its value is 1.62 mT in the case of the EPR spectrum of the 4-OXO-TEMPO probe in water, and it is 1.60 mT in 25 % P407solution.

From spectra shown in Fig. 12, it can also be concluded that probe 4-OXO-TEMPO is localised in the polar part of the micelles of non-ionic surfactants.




*a*




*b*

Fig. 11. EPR spectra of lipophilic probe 1 in: *a* – 10 % P407 solution; *b* – 25 % P407 solution; at: 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

In aqueous solutions of non-ionic surfactants, turbidity can occur at elevated temperatures due to dehydration of the PEO chains and separation of the surfactants into a separate phase. This prevents excessive hydrophobic hydration of surfactant alkyl chains by water molecules while reducing their packing density in micelles. At temperatures below the cloud point, the EPR spectrum of the probe 4-OXO-TEMPO in a solution of a non-ionic surfactant is a triplet (Fig. 12). At temperatures above the cloud point, the EPR spectrum becomes a superposition of two signals separated at the high field component (Fig. 12). One signal with $A_N$=1.62 mT is due to the probe molecules dissolved in water and the other signal with $A_N$=1.52 mT is due to the probe molecules localised in the dehydrated PEO chains. This indicates that probe 4-OXO-TEMPO is partially localised in the polar part of the non-ionic surfactant micelles.

The EPR spectra of the spin probe 4-OXO-TEMPO in 25 % aqueous solutions of P338 and P407 are triplets (Fig. 13).

With an increase in temperature from 25 °C to 37 °C, the values of $\tau_{-1}$ and $\tau_{\pm1}$ of the probe 4-OXO-TEMPO in 25 % P338 solution were approximately the same (Table 11). With increasing temperature, there was no tendency for the rotational correlation times of the probe 4-OXO-TEMPO to decrease, as was observed for probes 1, 2, 3 and 4. With an increase in temperature from 25 °C to 37 °C, the value of $\tau_{-1}$ for the probe 4-OXO-TEMPO in 25 % aqueous solution of P407 almost did not change, and the value of $\tau_{\pm1}$ even increased from 0.037 ns to 0.045 ns.

Table 11

Parameters of the EPR spectra of the probe 4-OXO-TEMPO in solutions of poloxamers at different temperatures (*t*)

| Object | *t*, °C | $A_N$, mT | $\tau_{-1}$, ns | $\tau_{\pm1}$, ns | Spectrum type |
|---|---|---|---|---|---|
| 25 % P338 solution | 25 | 1.60 | 0.018 | 0.035 | triplet |
| | 32 | 1.60 | 0.016 | 0.034 | triplet |
| | 37 | 1.60 | 0.018 | 0.034 | triplet |
| 25 % P407 solution | 25 | 1.60 | 0.024 | 0.037 | triplet |
| | 32 | 1.60 | 0.022 | 0.044 | triplet |
| | 37 | 1.60 | 0.024 | 0.045 | triplet |

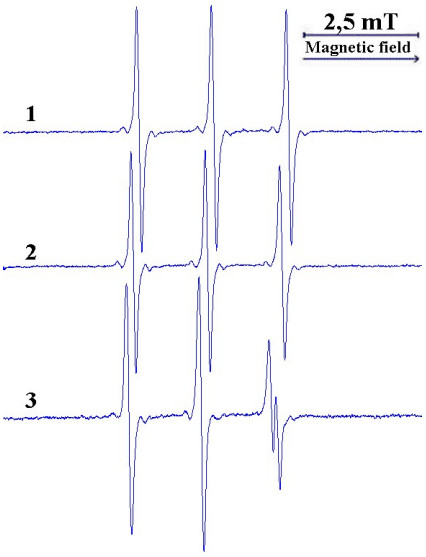



Fig. 12. Representative EPR spectra of the 4-OXO-TEMPO probe in water (1), solution of a non-ionic surfactant (before cloud point) (2), solution of a non-ionic surfactant (after cloud point) (3)

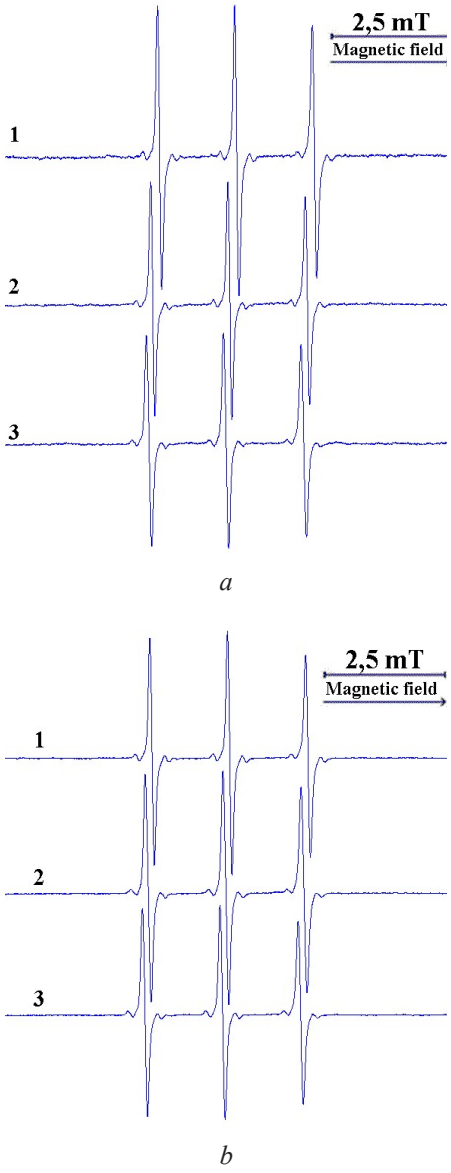



Fig. 13. EPR spectra of probe 5 (4-OXO-TEMPO) in: *a* – 25 % P338 solution; *b* – 25 % P407 solution; at: 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

**5. Discussion of research results**

The rheological properties of aqueous solutions of 4 types of poloxamers (P188, P237, P338 and P407) in the temperature range from 25 °C to 37 °C were studied by rotational viscometry. The values of the rheological parameters of the solutions increase as the concentration of the poloxamers increases from 10 % to 25 %, with an increase in temperature from 25 °C to 37 °C, the dynamic viscosity of a 25 % aqueous solution of P188 decreases, whereas the dynamic viscosity of aqueous solutions of P237, P338 and P407 increases despite the increase in temperature. At certain concentrations and temperatures characteristic of each of these poloxamers, sol→gel transitions occur in the solutions. Gels have a plastic flow behaviour with a yield stress. Three factors are important for gel formation: the type of poloxamer, which is related to its molecular mass and the ratio between the PPO part and the PEO part of the block copolymer; the concentration of the poloxamer in aqueous solution and the temperature of this solution. The greatest ability to form gels is characteristic of P407, followed by P338 and P237. Aqueous solutions of P188 in the concentration range of 10 % to 25 % and temperature range of 25 °C to 37 °C do not form gels.

It should be noted that at 25 °C (the upper limit of the storage temperature for medicinal products), 20 % P338 solution is a liquid, and at 32 °C and 37 °C it is a gel (Fig. 3, *a*). This means that it can be stored, dosed and administered as a liquid that only becomes a gel during contact with a biological object. This is an advantage of 20 % P338 solution over 20 % P407 solution, which is a gel at 25 °C (Fig. 4, *a*).

With increasing temperature, there is a clear significant change, namely a decrease in the rotational correlation times of spin probes 1, 2 and 4 (Tables 4, 6, 9) and in the values of the order parameter calculated from the EPR spectra of probes 3 and 4 (Tables 7, 8) in aqueous solutions of P237, P338 and P407. This indicates a decrease in the orderliness and viscosity of the microenvironment of spin probes 1–4. These changes are due to a decrease in the density and orderliness of the PPO chains in the non-polar part of the poloxamer associates. At 25 °C, the consistency of micelle cores can be described as semi-solid. In the temperature range from 25 °C to 37 °C, the micelle cores formed by the poloxamers melt. At 37 °C, the consistency of the non-polar cores can be described as two-dimensionally liquid and one-dimensionally solid (in the radial direction). It can be assumed that the volume of the micelles/mesophases of poloxamers increases, which should lead to an increase in their volume fraction in solution and promote the formation of gels.

With an increase in the concentration of P338 from 10 % to 25 %, there are no significant changes in the parameters of the EPR spectra of spin probe 1 (Table 10), which could be associated with the sol→gel transitions. Thus, at the P338 concentration of 10 %, the same changes in the parameters of rotational diffusion of probe 1 occur with increasing temperature as at a concentration of 25 %, but at a concentration of 10 %, the volume fraction of micelles is insufficient to create a spatial network of micelles/mesophases to form a gel. A certain content of poloxamer micelles/mesophases in the solution is required for gel formation.

It is possible to observe how the EPR spectra of probes 1, 3 and 4 and the parameters of these spectra change depending on the location of the radicals in the micelles (Fig. 14).

In the case of aqueous solutions of P237, P338 and P407 in which gels are formed, the differences in the rotational parameters of the EPR spectra of probes 1, 3 and 4 in micelles at 25 °C, 32 °C and 37 °C are the same: fast rotation in the case of probe 1 (EPR spectra are triplets) (Table 4, Fig. 5, *b*, 14), the highest value of order parameter in the case of the anisotropic EPR spectra of probe 3 (Table 7, Fig. 7, *b*, 8, 14) and a significantly lower values of order parameter and faster rotation in the case of the EPR spectra of probe 4, which are a triplets (Tables 8, 9, Fig. 9, 10, 14). According to the results of the study, ap-

proximately up to the level of the 5th carbon atom of the alkyl chain of probe 3 in the micelle cores, PPO chain packing is cone-shaped. When the temperature is increased from 25 °C to 37 °C, the differences in the EPR spectra of these spin probes and their parameters are similar. This seems to indicate that as the temperature increases, the micelles retain the spherical shape necessary for the formation of cubic lyotropic liquid crystals.

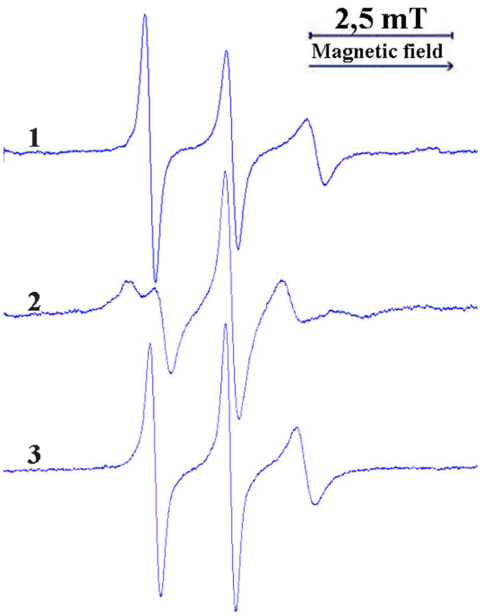



Fig. 14. EPR spectra of probes 1 (1), 3 (2) and 4 (3) in 20 % P338 solution at 25 °C

In the case of aqueous solutions of P188, the types of EPR spectra at 25 °C and 32 °C differ significantly (Fig. 3, *a*, 7, *a*, 9, *a*). In particular, at 25 °C, the EPR spectrum of probe 3 is a triplet characterised by a low order parameter *S*=0.09 (Table 7) and rotational correlation times attributed to the fast rotation range ($\tau_{+1}$=0.98 ns, $\tau_{-1}$=0.45 ns, $\tau_{\pm1}$=0.87 ns). At 32 °C the EPR spectrum of probe 3 is a superposition of two triplet spectra, and only at 37 °C it becomes similar to the anisotropic spectrum (Fig. 7, *a*). At the same time, the order parameter (*S*) is lower than for the EPR spectra of probe 3 in micelles P237, P338 and P407 (Table 7). Differences in the shape of the EPR spectra and spectral parameters probably indicate differences in the shape of the P188 micelles, which is not optimal for the formation of cubic lyotropic liquid crystals under the conditions studied.

A decrease in the packing density of the PPO chains creates the conditions for the penetration of water molecules into the non-polar part of the poloxamer associates and the hydrophobic hydration of the PPO chains. This is probably evidenced by a slight tendency for the values of $A_N$ constant of the EPR spectra of probe 3 (5-DSA NH4 salt) to increase with increasing temperature in solutions of poloxamers P188 and P237. In the case of P338 and P407 micelles, against the background of a decrease in the packing density of the PPO chains in the micelle cores, only a slight increase in values of $A_N$ constant occur at 37 °C. This means that there is no significant penetration of water into the non-polar core at the level of the 5th carbon atom of the alkyl chain of spin probe 3. There must be a mechanism preventing excessive hydrophobic hydration of the PPO chains. With increasing temperature, there is a tendency for the $A_N$ values of the EPR spectra of probe 1 and probe 2, whose nitroxyl radicals are localised in the hydrophilic part of the poloxamer associates, to decrease (Tables 4, 6, 10). This might be due to partial dehydration of the PEO chains and structural rearrangement of the polar part of the micelles.

The hydrophilic spin probe 4-OXO-TEMPO in aqueous solutions of non-ionic surfactants is partly dissolved in water and partly localised in the polar part of the micelles. Despite the increase in temperature, the rotational correlation times of the spin probe 4-OXO-TEMPO in 25 % aqueous solutions of P338 and P407 remain approximately constant or increase (Table 11). According to the research results, it can be assumed that structural rearrangements occur in the polar part of the poloxamer associated with increasing temperature, which probably prevents the hydrophobic hydration of the PPO chains in micelles. It is possible that these structural rearrangements are the result of partial dehydration of the PEO chains.

Therefore, by evaluating the parameters of the EPR spectra of the fatty acids-based spin probes, it can be concluded that with increasing temperature, the packing density and the orderliness of PPO chains in the non-polar part of the poloxamer associates decrease, probably leading to an increase in the volume fraction of the micelles and, cubic lyotropic liquid crystals, respectively, resulting in a sol→gel transition. When the temperature decreases, the opposite processes occur: the rotational correlation times of fatty acid-based spin probes increase sharply, the packing density increases and the volume of poloxamer associates probably decreases. This leads to a gel→sol transition. At temperatures of 15 °C and 20 °C, gels based on poloxamers P407 and P338 are Newtonian liquids, respectively, and their dynamic viscosity decreases with decreasing temperature (Tables 2, 3).

**Study limitations.** This study was conducted with aqueous solutions of poloxamers only at their content of 10 % to 25 % and mainly in the temperature range from 25 °C to 37 °C.

**Prospects for further research.** It is promising to study the effect of hydrophilic nonaqueous solvents on the processes of sol→gel transitions in poloxamer solutions in a wide temperature range and on the properties of the resulting gels.

### 6. Conclusions

The rheological properties of aqueous solutions of poloxamers depend on their type and concentration as well as on the temperature. According to the parameters of the EPR spectra of fatty acid-based spin probes, it was found that with increasing temperature, the packing density and the orderliness of the PPO chains in the non-polar part of the poloxamer associates decrease, probably leading to an increase in the volume of the micelles and causing a sol→gel transition.

**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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**Data availability**

Data will be made available on reasonable request.

**References**

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

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

3. Bodratti, A., Alexandridis, P. (2018). Formulation of Poloxamers for Drug Delivery. Journal of Functional Biomaterials, 9 (1), 11. doi: https://doi.org/10.3390/jfb9010011

4. Bodratti, A. M., Sarkar, B., Alexandridis, P. (2017). Adsorption of poly(ethylene oxide)-containing amphiphilic polymers on solid-liquid interfaces: Fundamentals and applications. Advances in Colloid and Interface Science, 244, 132–163. doi: https://doi.org/10.1016/j.cis.2016.09.003

5. Mortensen, K., Talmon, Y. (1995). Cryo-TEM and SANS Microstructural Study of Pluronic Polymer Solutions. Macromolecules, 28 (26), 8829–8834. doi: https://doi.org/10.1021/ma00130a016

6. Alexandridis, P., lan Hatton, T. (1995). Poly(ethylene oxide) poly(propylene oxide) poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 96 (1-2), 1–46. doi: https://doi.org/10.1016/0927-7757(94)03028-x

7. Alexandridis, P., Athanassiou, V., Fukuda, S., Hatton, T. A. (1994). Surface Activity of Poly(ethylene oxide)-block-Poly(propylene oxide)-block-Poly(ethylene oxide) Copolymers. Langmuir, 10 (8), 2604–2612. doi: https://doi.org/10.1021/la00020a019

8. Alexandridis, P., Holzwarth, J. F., Hatton, T. A. (1994). Micellization of Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide) Triblock Copolymers in Aqueous Solutions: Thermodynamics of Copolymer Association. Macromolecules, 27 (9), 2414–2425. doi: https://doi.org/10.1021/ma00087a009

9. Raval, A., Pillai, S. A., Bahadur, A., Bahadur, P. (2017). Systematic characterization of Pluronic® micelles and their application for solubilization and in vitro release of some hydrophobic anticancer drugs. Journal of Molecular Liquids, 230, 473–481. doi: https://doi.org/10.1016/j.molliq.2017.01.065

10. Cabana, A., Aït-Kadi, A., Juhász, J. (1997). Study of the Gelation Process of Polyethylene Oxidea–Polypropylene Oxideb–Polyethylene OxideaCopolymer (Poloxamer 407) Aqueous Solutions. Journal of Colloid and Interface Science, 190 (2), 307–312. doi: https://doi.org/10.1006/jcis.1997.4880

11. Prud'homme, R. K., Wu, G., Schneider, D. K. (1996). Structure and Rheology Studies of Poly(oxyethylene−oxypropylene−oxyethylene) Aqueous Solution. Langmuir, 12 (20), 4651–4659. doi: https://doi.org/10.1021/la951506b

12. Soliman, K. A., Ullah, K., Shah, A., Jones, D. S., Singh, T. R. R. (2019). Poloxamer-based in situ gelling thermoresponsive systems for ocular drug delivery applications. Drug Discovery Today, 24 (8), 1575–1586. doi: https://doi.org/10.1016/j.drudis.2019.05.036

13. Cook, M. T., Brown, M. B. (2018). Polymeric gels for intravaginal drug delivery. Journal of Controlled Release, 270, 145–157. doi: https://doi.org/10.1016/j.jconrel.2017.12.004

14. Russo, J., Fiegel, J., Brogden, N. K. (2020). Rheological and Drug Delivery Characteristics of Poloxamer-Based Diclofenac Sodium Formulations for Chronic Wound Site Analgesia. Pharmaceutics, 12 (12), 1214. doi: https://doi.org/10.3390/pharmaceutics12121214

15. Russo, E., Villa, C. (2019). Poloxamer Hydrogels for Biomedical Applications. Pharmaceutics, 11 (12), 671. doi: https://doi.org/10.3390/pharmaceutics11120671

16. Zhang, T., Chen, S., Dou, H., Liu, Q., Shu, G., Lin, J. et al. (2021). Novel glucosamine-loaded thermosensitive hydrogels based on poloxamers for osteoarthritis therapy by intra-articular injection. Materials Science and Engineering: C, 118, 111352. doi: https://doi.org/10.1016/j.msec.2020.111352

17. Abdeltawab, H., Svirskis, D., Sharma, M. (2020). Formulation strategies to modulate drug release from poloxamer based in situ gelling systems. Expert Opinion on Drug Delivery, 17 (4), 495–509. doi: https://doi.org/10.1080/17425247.2020.1731469

18. Wanka, G., Hoffmann, H., Ulbricht, W. (1990). The aggregation behavior of poly-(oxyethylene)-poly-(oxypropylene)-poly-(oxyethylene)-block-copolymers in aqueous solution. Colloid & Polymer Science, 268 (2), 101–117. doi: https://doi.org/10.1007/bf01513189

19. Alexandridis, P., Zhou, D., Khan, A. (1996). Lyotropic Liquid Crystallinity in Amphiphilic Block Copolymers: Temperature Effects on Phase Behavior and Structure for Poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) Copolymers of Different Composition. Langmuir, 12 (11), 2690–2700. doi: https://doi.org/10.1021/la951025s

20. Alexandridis, P., Olsson, U., Lindman, B. (1998). A Record Nine Different Phases (Four Cubic, Two Hexagonal, and One Lamellar Lyotropic Liquid Crystalline and Two Micellar Solutions) in a Ternary Isothermal System of an Amphiphilic Block Copolymer and Selective Solvents (Water and Oil). Langmuir, 14 (10), 2627–2638. doi: https://doi.org/10.1021/la971117c

21. Sarkar, B., Venugopal, V., Bodratti, A. M., Tsianou, M., Alexandridis, P. (2013). Nanoparticle surface modification by amphiphilic polymers in aqueous media: Role of polar organic solvents. Journal of Colloid and Interface Science, 397, 1–8. doi: https://doi.org/10.1016/j.jcis.2013.01.034

22. Valero, M., Castiglione, F., Mele, A., da Silva, M. A., Grillo, I., González-Gaitano, G., Dreiss, C. A. (2016). Competitive and Synergistic Interactions between Polymer Micelles, Drugs, and Cyclodextrins: The Importance of Drug Solubilization Locus. Langmuir, 32 (49), 13174–13186. doi: https://doi.org/10.1021/acs.langmuir.6b03367

23. Cook, M. T., Abou-Shamat, M. A., Stair, J. L., Calvo-Castro, J. (2022). Raman spectroscopy coupled to computational approaches towards understanding self-assembly in thermoreversible poloxamer gels. Journal of Molecular Liquids, 351, 118660. doi: https://doi.org/10.1016/j.molliq.2022.118660

24. Berliner, L. (Ed.). (1979). Metod spinovyih metok. Teoriya i primenenie. Moscow: Mir, 635.

25. Georgieva, E. R. (2017). Nanoscale lipid membrane mimetics in spin-labeling and electron paramagnetic resonance spectroscopy studies of protein structure and function. Nanotechnology Reviews, 6 (1), 75–92. doi: https://doi.org/10.1515/ntrev-2016-0080

26. Sahu, I. D., Lorigan, G. A. (2021). Probing Structural Dynamics of Membrane Proteins Using Electron Paramagnetic Resonance Spectroscopic Techniques. Biophysica, 1 (2), 106–125. doi: https://doi.org/10.3390/biophysica1020009

27. Camargos, H. S., Alonso, A. (2013). Electron paramagnetic resonance (EPR) spectral components of spin-labeled lipids in saturated phospholipid bilayers: effect of cholesterol. Química Nova, 36 (6), 815–821. doi: https://doi.org/10.1590/s0100-40422013000600013

28. Catte, A., White, G. F., Wilson, M. R., Oganesyan, V. S. (2018). Direct Prediction of EPR Spectra from Lipid Bilayers: Understanding Structure and Dynamics in Biological Membranes. ChemPhysChem, 19 (17), 2183–2193. doi: https://doi.org/10.1002/cphc.201800386

29. Farafonov, V. S., Lebed, A. V. (2020). Nitroxyl spin probe in ionic micelles: A molecular dynamics study. Kharkiv University Bulletin. Chemical Series, 34 (57), 57–64. doi: https://doi.org/10.26565/2220-637x-2020-34-02

30. 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

31. Bezuglaya, E., Lyapunov, N., Chebanov, V., Liapunov, O. (2022). Study of the formation of micelles and their structure by the spin probe method. ScienceRise: Pharmaceutical Science, 4 (38), 4–18. doi: https://doi.org/10.15587/2519-4852.2022.263054

32. Rusanov, A. I., Shchyokin, A. K. (2016). Micelloobrazovanie v rastvorah poverhnostno-aktivnyh veshchestv. Saint-Peterburg: OOO «Izdatel'stvo «Lan'», 612.

33. Markus, F., Dreher, F., Laschat, S., Baudis, S., Tovar, G. E. M., Southan, A. (2017). Physically and chemically gelling hydrogel formulations based on poly(ethylene glycol) diacrylate and Poloxamer 407. Polymer, 108, 21–28. doi: https://doi.org/10.1016/j.polymer.2016.11.039

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

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

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