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STUDY OF THE EFFECT OF ETHANOL ON THE PROPERTIES OF POLOXAMER 338 SOLUTIONS BY ROTATIONAL VISCOMETRY AND SPIN PROBE METHOD

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The aim. *Study the properties of 20 % solutions of poloxamer 338 (P338) in water and mixed solvents water-ethanol using rotational viscometry and the spin probe method at various temperatures.*

Materials and methods. *20 % m/m solutions of P338 in water and water – ethanol mixtures were the objects of research. The solutions were studied by rotational viscometry at various temperatures; the flow behaviour, lower yield stress (*τ_0*) and dynamic or apparent viscosity (η) were determined. Spin probes based on fatty acids, which differ in molecular structure, solubility, and radical localization, were added to the solutions. Electron paramagnetic resonance (EPR) spectra were obtained to determine their type and parameters.*

Results*. Depending on the content, ethanol affects the rheological properties of 20 % solution of P338. The solution was demonstrated to be able to thermally induce sol→gel transition at 32 °C when ethanol content is 5 % m/m. The rheological parameters of the gel at 32 °C and 37 °C exhibit an increase (in comparison to the gel without ethanol), accompanied by a reduction in the packing density of polypropylene oxide (PPO) chains within the cores of P338 micelles. At an ethanol content of 10 % m/m, the gel formation temperature rises to 40 °C. At ethanol content of 15 % m/m and above, 20 % P338 solutions do not form gels at temperatures between 25 °C and 40 °C. The values of rotational correlation times (τ) and the order parameter (S) of fatty acid-based spin probes were observed to decrease with increasing ethanol content up to 30 % m/m; in the case of the ammonium salt of 5-doxylstearic acid (5-DSA NH4 salt), the anisotropic EPR spectra transform, becoming a superposition of two triplets and subsequently a triplet. P338 solutions retain their ability to undergo thermally induced sol↔gel transitions as long as the EPR spectra of this probe exhibit anisotropy at temperatures ranging from 25 °C to 37 °C. As the concentration of ethanol in the solution increases, the solvation of the cores of P338 micelles by the dispersion medium of the solution also increases.*

Conclusions*. It was demonstrated that ethanol, when added to the 20 % P338 solution, results in changes to the rheological properties of this solution. However, at the ethanol content of 5–10 % m/m, the ability of P338 to thermally induced sol→gel transition remains unaltered. The rheological properties of the 20 % P338 solution exhibit a correlation with the observed change in EPR spectra types for the 5-DSA NH4 salt. As the ethanol content in the solution increases, the solvation of P338 micelle cores by the dispersion medium increases, accompanied by decreased density and orderliness of the PPO chains packing in the micelle cores*

Keywords*: poloxamer 338 (P338), ethanol, solution, gel, viscosity, micelle, spin probe, EPR spectrum, spectrum parameters*

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1. Introduction

It is evident that water is the most commonly used solvent in the pharmaceutical industry [1, 2]. However, in pharmaceutical formulations, water is often combined with hydrophilic, non-aqueous solvents, one of which is ethanol [1, 2]. In certain concentrations, ethanol can improve the solubility of hydrophobic drug substances [3, 4] and ensure the efficacy of the antimicrobial preservative effect in liquid and semi-solid preparations [2, 5].

Due to the hydroxyl group and hydrophobic tail in its molecule, ethanol in aqueous solutions exhibits surface-active properties [1, 2, 6]. Consequently, ethanol can influence the properties of dispersed systems with a liquid dispersion medium and the properties of surfactants, particularly the surface tension and the critical micelle concentration (CMC) [7]. The ethanol concentration at the phase interface is greater than that in the bulk [6]. An increase in the ethanol content to a molar fraction of 0.1 (~22.7 % m/m) results in an accumulation of its molecules and an increase in their orderliness in the surface layer. As the ethanol content increases, the packing density of the ethanol molecules in the surface layer remains approximately constant while their orientation becomes increasingly disordered [8].

Ethanol–water systems are characterized by the so-called volume contraction phenomenon, which is accompanied by an increase in the system's viscosity when ethanol is added to water and when water is added to ethanol [9, 10]. The maximum value for viscosity is observed at the ethanol concentration of 46 % m/m (25 % mol), corresponding to the proportion of one alcohol molecule per three water molecules. According to the calculations, the activation energy for *ethanol-water* systems is at its maximum at an ethanol concentration of 46 % m/m, regardless of temperature. The excess viscosity values decrease with an increase in temperature [9].

The viscous synergy is caused by a change in the structure of water when ethanol is dissolved in it and a subsequent change in the structure of the mixed solvent as the ethanol content increases. On the one hand, the hydrophobic fragment of ethanol fills the voids in the tetrahedral structure of water, which leads to the hydrophobic hydration of the alkyl fragment. Ethanol initially (approximately up to the concentration of 15 % mol (~30 % m/m)) has a structuring effect on the structure of water and then a destructive effect with an increase of its content [11, 12]. On the other hand, weak and strong hydrogen bonds are formed between water and ethanol molecules [13, 14]. At ethanol content up to 20 % mol, the hydroxyl groups of its molecules are more hydrated and oriented towards the bulk of the solution [15]. The formation of hydrogen bonds between alcohol and water is temperature-dependent, resulting in a change in the viscosity of the system [9]. A peculiar equilibrium between hydrophilic and hydrophobic interactions in *water-ethanol* mixtures gives rise to the non-additivity of their properties, including viscosity [9, 12], surface tension [6], density [9, 10], ultrasonic attenuation coefficient [10], as well as in excess values of entropy and enthalpy of mixing [11, 16]. It is important to note that the thermodynamics of mixing is primarily governed by the entropic term; the addition of ethanol to water and water to ethanol results in an increase in the structural orderliness of the mixed solvent [11].

The structure of mixed solvents *water-ethanol* has been the subject of comprehensive research utilizing a range of methods since the 19th century. Numerous studies have been conducted to elucidate the properties of such solutions, employing a range of modern experimental techniques, including infrared absorption spectroscopy, nuclear magnetic resonance spectroscopy, X-ray absorption spectroscopy, Raman spectroscopy, Chirped pulse Fourier-transform microwave spectroscopy, computer simulation, etc. [10, 11, 17].

In a mixed solvent, it is possible to distinguish between concentration ranges regarding the prevailing structural characteristics. These include a water structure, a mixed solvent structure with a predominance of water structure or with a predominance of a non-aqueous solvent structure, and a non-aqueous solvent structure [12, 18]. It can be posited that alterations in the structure of a mixed solvent will affect not only its own physicochemical properties and performance characteristics but also those of various dispersed systems where this solvent is a liquid dispersion medium.

Most scientific studies are dedicated to investigating the solubility of various active pharmaceutical substances in mixtures of water and ethanol. These include sulfadiazine, sulfamerazine and sulfamethazine [3], efinaconazole [4], urea [12], albendazole [19], zonisamide [20], baicalin [21], cefpiramide [22], 5-fluorouracil [23], and many others.

The impact of ethanol and other hydrophilic solvents (glycerol, isopropyl alcohol, etc.) on the formation of micelles and lyotropic liquid crystals in solutions of alkyl-propoxy-ethoxylate surfactants was also studied. In particular, it has been demonstrated that adding ethanol results in an increase in the solubility of these surfactants and an increase in their CMC [24]. B. Sarkar et al. posit that the polypropylene oxide (PPO) segment of surfactants becomes more hydrophilic in the presence of ethanol than in plain water and that the microenvironment in micellar solutions is affected mainly by the co-solvent concentration, not the surfactant degree of ethoxylation. Small-angle X-ray scattering (SAXS) data have demonstrated that ellipsoid micelles are formed in both water and water-ethanol mixtures. Introducing 20 % m/m ethanol reduces the micelle long axis by 10–15 % [24].

It has been demonstrated by R. Ivanova, P. Alexandridis, and B. Lindman that ethanol when interacting with poloxamer 407 associates, can exhibit amphiphilic behaviour and act as a surfactant, which is primarily located at the interface between the polyethylene oxide (PEO) and PPO segments [25]. In addition, the stability of gels based on lyotropic liquid crystals formed by poloxamers was evaluated by studying the phase behaviour and structure in ternary isothermal (25 °C) systems consisting of poloxamer 407, water and one of the solvents: ethanol, propylene glycol, glycerol, polyethylene glycol 400. The SAXS method was employed to ascertain the formed liquid crystals' structure and determine their characteristic sizes. It has been demonstrated that the stability of liquid crystal phases in the studied systems depends on the type of non-aqueous solvent [26].

The ternary phase diagram of the triblock copolymer P123, water, and ethanol has been investigated at a constant temperature of 23 °C by SAXS. The microstructure resulting from the self-assembly of the PEO−PPO−PEO block copolymer varies from micelles in solution to various types of liquid crystalline phases such as cubic, hexagonal, lamellar, etc. [27]. S. S. Soni et al. concluded that the micellar structural parameters depend on the *water-ethanol* ratio and the block copolymer concentration. The micellar core, the aggregation number, and the hard-sphere interaction radius decrease when the ethanol/water ratio in the mixed solvent is increased. It has been demonstrated that the proportion of ethanol present in the core is responsible for the swelling of PPO blocks [27]. It was shown that the larger-sized micelles are formed in the presence of ethanol [28].

Poloxamers have been identified as promising excipients for drug-delivery solutions and gels [29]. Ethanol can perform various functions within its composition, including acting as a co-solvent for active substances, an antimicrobial preservative, a rheology modifier, and a penetration enhancer [30]. In the context of poloxamer solutions studies, it is logical to use a combination of rotational viscometry and spin probe methods. According to the parameters of the EPR spectra of fatty acid-based spin probes, it was found that as temperature increases, the packing density and orderliness of the PPO chains in the non-polar part of the poloxamer associates decrease. This phenomenon is likely to result in an increase in the micelles' volume, which could contribute to the sol→gel transitions [31]. It seems promising to use these methods to investigate the effect of ethanol on the rheological properties of poloxamer solutions and the microstructure of poloxamer associates.

The aim. Study of the properties of 20 % solutions of poloxamer 338 (P338) in water and mixed solvents *water-ethanol* using rotational viscometry and the spin probe method at various temperatures.

2. Planning (methodology) of the research

The study is designed to use P338, which has a solid state and is readily soluble in water and ethanol (95 %) [2]. The research objects are 20 % P338 solutions with various ethanol (96 %) contents from 0 to 30 % m/m. In other words, these are systems whose dispersion medium structure, dependent on the ethanol content, changed towards strengthening the water structure and then towards its destruction [11, 12].

When 20 % aqueous solution of P338 is heated from 25 °C (the upper limit of the storage temperature for medicinal products) to 32 °C (the temperature at which dermatological preparations are applied) or 37 °C (the temperature of application of vaginal and rectal preparations), sol→gel transitions occur [31]. One of the tasks was to investigate the impact of ethanol on these sol-gel transitions.

The rheological properties of 20 % P338 solutions using rotational viscometry were to be studied, with a particular focus on the effects of ethanol content and temperature. It was necessary to distinguish between the conditions under which P338 solutions behave as Newtonian liquids and those under which they are gels.

In order to detect changes in the supramolecular structures formed by P338 in its 20 % solutions, where the dispersion medium is water or mixed solvents *water-ethanol* (with various ethanol contents), the spin probe method was proposed [32–34]. It was planned to evaluate changes in the rheological properties of solutions along with changes in the types and parameters of the electron paramagnetic resonance (EPR) spectra of spin probes.

The method of spin probes is an indirect method that allows the acquisition of information about the object of study from the EPR spectra depending on certain variables [31, 32]. Therefore, it is possible to obtain information about the behaviour of the probe in the phase of its localization, which can be used to assess the state of this phase. It was interesting to establish the relationship between changes in the types and parameters of EPR spectra, as well as rheological properties and sol→gel transitions in P338 solutions depending on the ethanol content and temperature. Four spin probes were proposed for the study. Two of these probes were designed to obtain information about P338 micelles at the interface between their polar and non-polar parts. The other two probes were intended to assess the state of the hydrophobic core at different levels.

The objective of these comprehensive studies was to ascertain the potential for using ethanol in certain concentrations in 20 % P338 solutions, with due consideration given to its impact on thermally induced sol↔gel transitions and the rheological properties of the solutions mentioned above. Furthermore, the study's results were intended to evaluate the relationship between changes in the rheological properties of P338 solutions and changes in the microstructure of its associates depending on the ethanol content.

3. Materials and methods

The research used the following materials: P338 (Kolliphor® P 338), ethanol (96 %) (hereinafter referred to as ethanol), and purified water (hereinafter referred to as water) [1].

The solutions of P338 were the subjects of the study (Table 1).

Table 1

Composition of the tested solutions

Constituent	Content (% m/m) in solution:					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
P338	20	20	20	20	20	20
Ethanol (96 %)	0	5	10	15	20	30
Water	80	75	70	65	60	50

The density (ρ) of ethanol (96 %) was measured at 20 °C using a density meter DMA 500 («Anton Paar GmbH»). The precise concentration of ethanol was determined using alcohol tables [35], and the necessary amount of ethanol and water was calculated accordingly.

P338 was dissolved in water at approximately 12 °C, and ethanol was added. The solutions were stirred and degassed. The P338 solutions were tested at temperatures of 25 °C, 32 °C and 37 °C, with some instances of testing at 40 °C.

Rheological properties were studied by rotational viscometry (2.2.10) [1]. Rheograms (plots of the shear stress (τ_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 characterize 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 (τ_0) of gels [1].

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

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

Electron paramagnetic resonance (EPR) spectroscopy was used for the research [32–34]. 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]);

– probe 2: 1-Piperidinyloxy,4-(hexadecyldimethylammonio)-2,2,6,6-tetramethyl-, iodide (M_r 551.65; CAS [114199-16-5]);

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

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

Probe 1 and probe 4 simulated lipophilic surfactants. Probe 2 and probe 3 simulated cationic and anionic surfactants, respectively. During the solubilization of probe molecules by P338 micelles, the free radicals of probe 1 and probe 2 are localized in the hydrophilic part, and their alkyl chains are localized in the hydrophobic core. The doxyl radicals of probes 3 and 4 are located near the 5th and 16th carbon atoms, respectively, of the alkyl chains localized in the hydrophobic core of the micelles.

The spin probes were added to the studied solutions at 10^{-4} mol/l concentrations. The EPR spectra were recorded using the «ESR Spectrometer CMS8400» («Adani»). The type of EPR spectra (triplet, anisotropic spectrum, superposition spectrum, etc.), 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 equations [33, 34]:

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

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

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

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

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

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

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

The A_N constant, which characterizes the polarity of the radical's environment in the case of triplet spectra, was determined as the distance (in mT) between the central and high-field components [33, 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 [32]:

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

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

The parameter γ, which characterizes the half-amplitude of molecular motion, was determined by the order parameter (S) using the calibration graph [32].

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

4. Research results

4. 1. Study of the effect of ethanol content on the rheological properties of P338 solutions

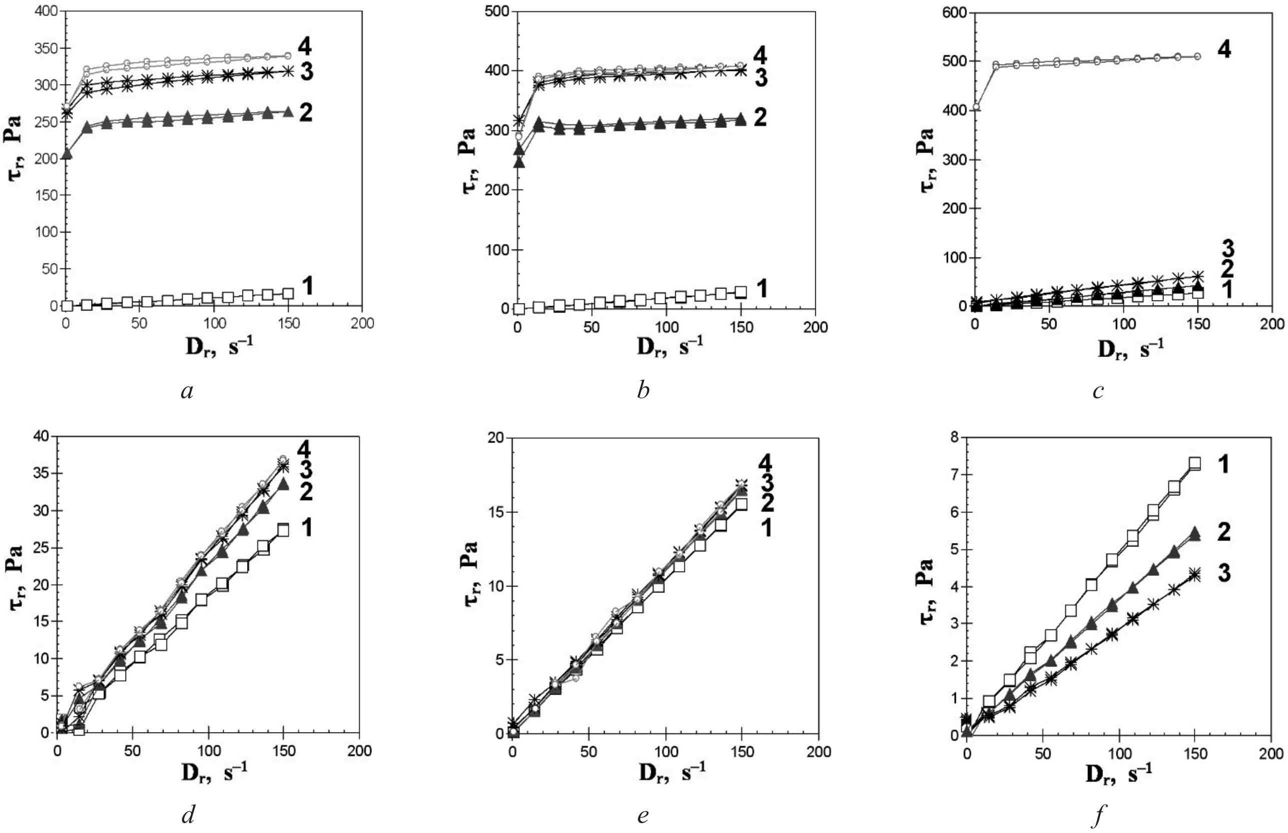
At 25 °C, 20 % solutions of P338 behave as Newtonian liquids regardless of the ethanol content (Fig. 1). The dynamic viscosity of the solutions is dependent on the ethanol content, with the highest value observed at a concentration of 15 % m/m (Table 2).

In the absence of ethanol and at ethanol content of 5 % m/m, 20 % P338 solutions at 32 °C form gels exhibiting plastic flow behaviour, high values of the yield stress and apparent viscosity. Upon heating to 37 °C and 40 °C, the rheological parameters of these gels demonstrate an increase (Fig. 1, Table 2). Cels containing 5 % ethanol exhibit rheological parameters that are approximately 20–30 % higher than those for gels with only water as the dispersion medium (Table 2).

At ethanol content of 10 % m/m at 32 °C and 37 °C, 20 % P338 solution is a non-Newtonian liquid with low values of yield stress and apparent viscosity (Table 2). However, upon heating to 40 °C, the liquid transforms into a gel with plastic flow behaviour (Fig. 1). A comparison of the yield stress and apparent viscosity for gel No. 3 at 40 °C with those for gel No. 1 reveals that the values are approximately twofold higher at 32 °C (Table 2), 1.5–1.6 times higher at 37 °C and 1.2–1.5 times higher at 40 °C. In other words, when 10 % ethanol is added to the P338-based gel, both the rheological parameters of the gel and the temperature of the sol→gel transition increase. This transition occurs at ~40 °C.

No gels are formed at an ethanol content of 15 % m/m in 20 % solutions of P338 at temperatures ranging from 32 °C to 40 °C. Instead, the solution exhibits Newtonian flow behaviour (Fig. 1). It is important to note that the dynamic viscosity of the solution increases with rising temperature (Table 2).

At ethanol content of 20 % m/m, the dynamic viscosity of 20 % P338 solution at temperatures from 25 °C to 40 °C remains relatively constant. However, at an ethanol content of 30 % m/m, the dynamic viscosity of 20 % P338 solution decreases as the temperature increases (Table 2).



a *b* *c*

d *e* *f*

Fig. 1. Rheograms of 20 % P338 solution with varying ethanol contents: *a* – 0 %; *b* – 5 %; *c* – 10 %; *d* – 15 %; *e* – 20 %; *f* – 30 %, at: 1 – 25 °C; 2 – 32 °C; 3 – 37 °C; 4 – 40 °C

Table 2

Rheological parameters of 20 % P338 solutions with varying ethanol contents (*C*) at different temperatures (*t*)

C, % m/m	*t*, °C	τ_0, Pa	η, (Pa·s) at D_r:		
			14.6 s⁻¹	41.6 s⁻¹	82.3 s⁻¹
0 %	25	~0	0.11*		
	32	207.1	16.60**	6.00**	3.09**
	37	267.3	20.60**	7.35**	3.78**
	40	269.0	22.03**	7.90**	4.06**
5 %	25	~0	0.17*		
	32	269.3	21.66**	7.40**	3.80**
	37	316.3	26.20**	9.39**	4.81**
	40	325,5	26.82**	9.57**	4.90**
10 %	25	~0	0.18*		
	32	0.5	0.32**	0.28**	0.27**
	37	8.5	0.85**	0.54**	0.45**
	40	405.2	33.94**	11.97**	6.12**
15 %	25	~0	0.21*		
	32	~0	0.23*		
	37	~0	0.25*		
	40	~0	0.27*		
20 %	25	~0	0.10*		
	32	~0	0.12*		
	37	~0	0.13*		
	40	~0	0.13*		
30 %	25	~0	0.05*		
	32	~0	0.04*		
	37	~0	0.03*		

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

4. 2. Study of 20 % P338 solutions with varying ethanol contents using the spin probe method

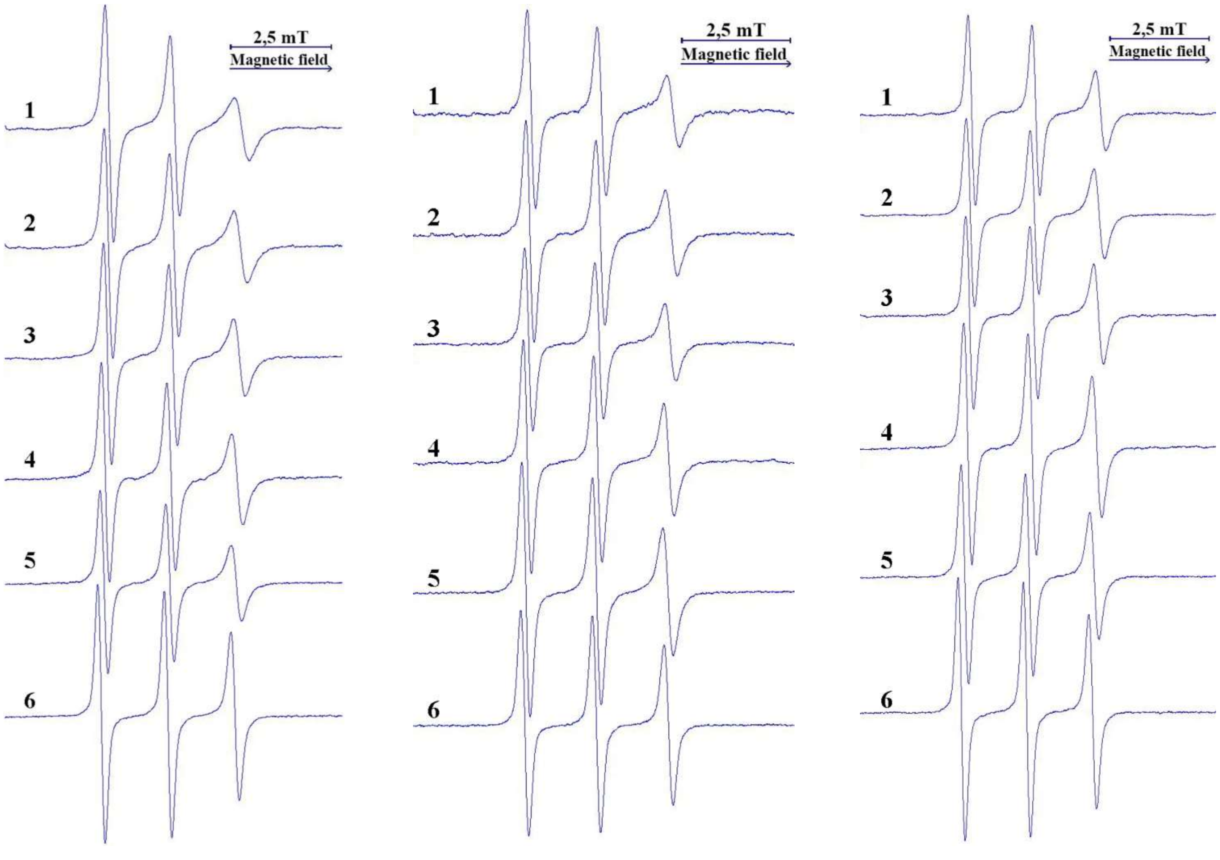
The EPR spectra of lipophilic spin probe 1 in P338 solutions are typical triplets in which the low-field component is more intense than the central component (Fig. 2). This suggests that the molecules of probe 1 rotate very rapidly around the long axis, making it impossible to calculate the value of τ_{+1} using equation (2). The values of the anisotropy parameter (ε) calculated by equation (5) are negative (Table 3).

As the ethanol content increases from 0 to 30 % m/m and the temperature rises from 25 °C to 37 °C, the EPR spectra remain triplet in nature. However, it is evident that the width of all three components exhibits a notable decrease (Fig. 2).

The lipophilic molecules of probe 1 are solubilized by P338 micelles, as evidenced by the EPR spectra, which are triplets; in water, the EPR spectra of probe 1 are singlets [36]. Upon solubilization, the alkyl chains of probe 1 molecules are localized in the lipophilic core of P338 micelles. Nitroxyl radicals are located in the hydrophilic part formed by hydrated polyethylene oxide (PEO) chains (near the interface between PEO and PPO), as evidenced by the values of A_N constant (Table 3). At ethanol content of up to 20 % m/m, the values of A_N constant are 1.60–1.62 mT, increasing to 1.64 mT at ethanol content of 30 % m/m (Table 3).

The rotational correlation times (τ_{-1}, $\tau_{\pm1}$) of probe 1 in P338 micelles are within the range of fast rotations [33, 34]. This indicates that micelles possess two-dimensional liquid consistency at the interface between their polar part and lipophilic core at temperatures from 25 °C to 37 °C. As the ethanol content increases from 0 to 30 % m/m, as well as the temperature rises from 25 °C to 37 °C, the values of τ_{-1} and $\tau_{\pm1}$ decrease (Table 3). This indicates a reduction in the viscosity of the immediate microenvironment of probe 1 molecules [32, 33].

When the ethanol content is 30 % m/m in a 20 % solution of P338, the ethanol concentration in the dispersion medium of this solution is 37.5 % m/m. The A_N values for the EPR spectra of probe 1 at ethanol content of 30 % m/m, at temperatures from 25 °C to 37 °C, are 1.64 mT; these values are nearly identical to the A_N value of 1.65 mT for the EPR spectrum of probe 1 in the mixed solvent *ethanol-water* (37.5:62.5) % m/m (Table 3, 4). However, the EPR spectra of probe 1 in 37.5 % m/m ethanol solution and 20 % P338 solution are different. In the case of the EPR spectrum in the ethanol solution, the low-field component is less intense than the high-field component (Fig. 3). This enables the calculation of τ_{+1} using formula (2). The value of the anisotropy parameter (ε) calculated using equation (5) is positive (Table 4). It can be stated that the molecules of probe 1 are not dissolved in the dispersion medium but rather localized within the P338 associates. The A_N value of 1.64 mT may indicate that ethanol molecules accumulate at the interface between the hydrophilic and lipophilic parts of P338 micelles.



a *b* *c*

Fig. 2. EPR spectra of probe 1 in 20 % P338 solutions with varying ethanol contents: 1 – 0 %; 2 – 5 %; 3 – 10 %; 4 – 15 %; 5 – 20 %; 6 – 30 %, at: *a* – 25 °C; *b* – 32 °C; *c* – 37 °C

Table 3

Parameters of EPR spectra of spin probe 1 in 20 % P338 solutions with varying ethanol contents (*C*) at different temperatures (*t*)

C, % m/m	*t*, °C	A_N, mT	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
0	25	1.62	0.48	1.42	–0.18	triplet
5	25	1.62	0.37	1.22	–0.19	triplet
10	25	1.62	0.36	1.09	–0.18	triplet
15	25	1.62	0.29	0.85	–0.17	triplet
20	25	1.62	0.28	0.83	–0.16	triplet
30	**25**	**1.64**	**0.12**	**0.32**	**–0.11**	**triplet**
0	32	1.61	0.32	0.92	–0.15	triplet
5	32	1.61	0.30	0.84	–0.19	triplet
10	32	1.62	0.22	0.73	–0.18	triplet
15	32	1.62	0.21	0.64	–0.18	triplet
20	32	1.62	0.20	0.56	–0.16	triplet
30	32	1.64	0.10	0.26	–0.12	triplet
0	37	1.62	0.27	0.67	–0.12	triplet
5	37	1.60	0.22	0.66	–0.19	triplet
10	37	1.60	0.18	0.52	–0.18	triplet
15	37	1.60	0.16	0.49	–0.17	triplet
20	37	1.60	0.14	0.39	–0.15	triplet
30	37	1.64	0.09	0.26	–0.12	triplet

The EPR spectra of hydrophilic spin probe 2 in P338 solutions are triplets, with the central component being more intense than the low-field component (Fig. 4). This enables the value of τ_{+1} to be calculated using the equation (2). The values of the anisotropy parameter (ε) calculated by equation (5) are positive (Table 5).

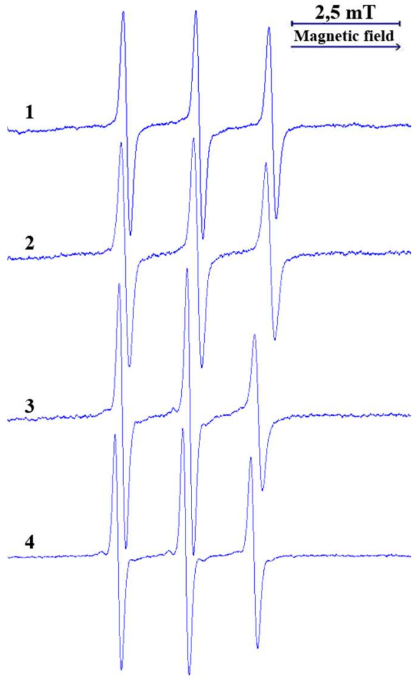
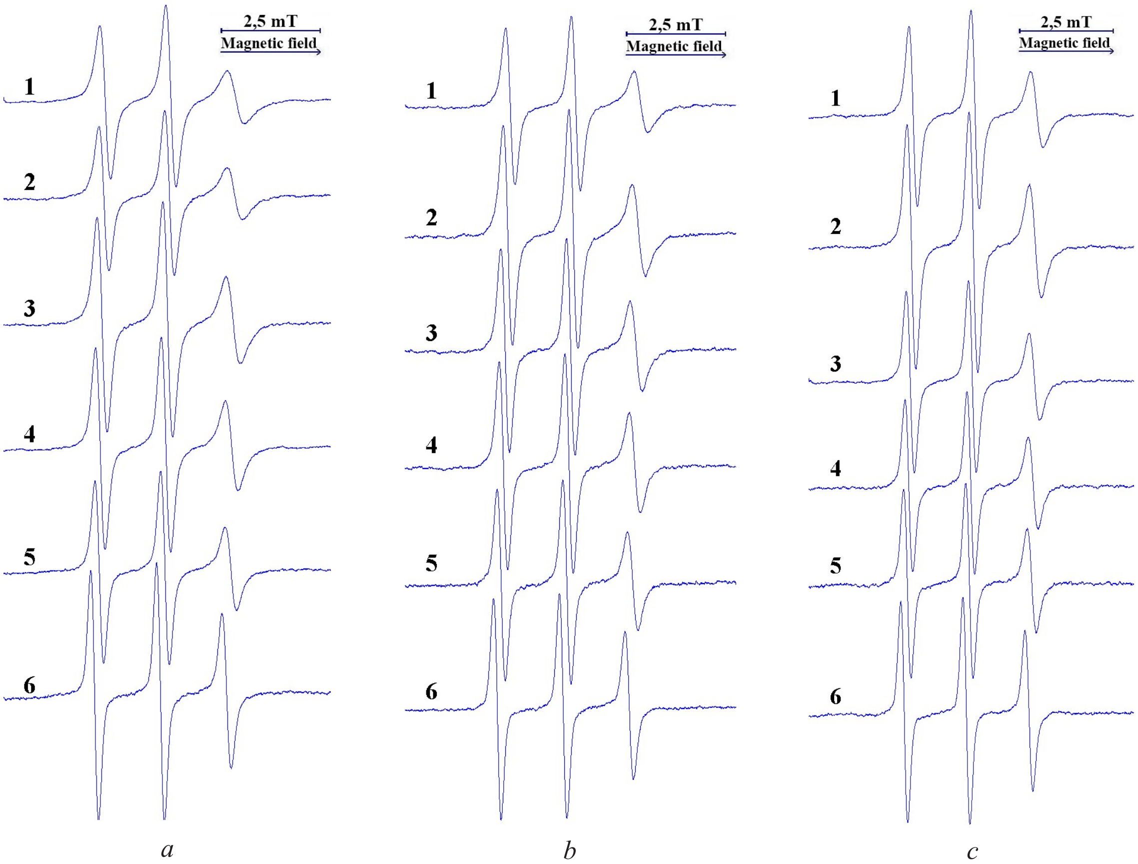


Fig. 3. EPR spectra of spin probes 1 (1), 2 (2), 3 (3) and 4 (4) in mixed solvent *ethanol-water* (37.5:62.5) % m/m at 25 °C



a *b* *c*

Fig. 4. EPR spectra of probe 2 in 20 % P338 solutions with varying ethanol contents: 1 – 0 %; 2 – 5 %; 3 – 10 %; 4 – 15 %; 5 – 20 %; 6 – 30 %, – at: *a* – 25 °C; *b* – 32 °C; *c* – 37 °C

Table 4

Parameters of the EPR spectrum of spin probe 1 in mixed solvent *ethanol-water* (37.5:62.5) % m/m at 25 °C

A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
1.65	**0.11**	**0.05**	**0.09**	**+0.12**	**triplet**

The rotational correlation times (τ_{+1}, τ_{-1}, $\tau_{\pm1}$) of the hydrophilic probe 2 are also within the range of fast rotation; these values decrease with increasing ethanol content from 0 to 30 % m/m and with rising temperature from 25 °C to 37 °C (Table 5). The anisotropy parameter (ε) tends to decrease with increasing ethanol content (Table 5). As the ethanol content increases, the A_N constant also increases, suggesting that the ethanol molecules accumulate at the interface between the hydrophilic and lipophilic parts of P338 micelles. At an ethanol content of 30 % m/m, probe 2 molecules are also localized in P338 associates. This is evidenced by the difference in the rotational parameters derived from the EPR spectra of probe 2 in a 20 % P338 solution containing 30 % m/m ethanol and the spectra of this probe in the 37.5 % m/m ethanol solution (Table 5, 6).

Basically, the analysis of the parameters of the EPR spectra of the hydrophilic probe 2 supports the conclusions drawn from the parameters of the EPR spectra of the lipophilic probe 1 regarding the structure of the P338 micelles at the interface between the polar part and the micelle core.

In the molecule of hydrophilic spin probe 3 (5-DSA, NH_4 salt), the doxyl radical is located approximately at the level of the 5th carbon atom of the alkyl chain. In 20 % aqueous solution of P338, the EPR spectra of this probe are anisotropic at temperatures from 25 °C to 37 °C (Fig. 5).

Table 5

Parameters of EPR spectra of spin probe 2 in 20 % P338 solutions with varying ethanol contents (*C*) at different temperatures (*t*)

C, % m/m	*t*, °C	A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
0	25	1.62	1.29	0.68	1,37	+0.10	triplet
5	25	1.62	0.89	0.57	1,20	+0.09	triplet
10	25	1.62	0.61	0.46	1.08	+0.07	triplet
15	25	1.64	0.42	0.40	0.87	+0.06	triplet
20	25	1.66	0.39	0.37	0.74	+0.06	triplet
30	**25**	**1.66**	**0.20**	**0.18**	**0.42**	**+0.06**	**triplet**
0	32	1.62	0.66	0.45	0.97	+0.08	triplet
5	32	1.62	0.53	0.42	0.89	+0.07	triplet
10	32	1.62	0.36	0.36	0.78	+0.06	triplet
15	32	1.64	0.29	0.29	0.67	+0.06	triplet
20	32	1.64	0.26	0.26	0.55	+0.05	triplet
30	32	1.65	0.08	0.15	0.34	+0.04	triplet
0	37	1.62	0.59	0.40	0.86	+0.08	triplet
5	37	1.62	0.41	0.36	0.84	+0.06	triplet
10	37	1.62	0.33	0.32	0.70	+0.06	triplet
15	37	1.63	0.29	0.25	0.50	+0.06	triplet
20	37	1.64	0.16	0.21	0.48	+0.04	triplet
30	37	1.65	0.12	0.11	0.23	+0.06	triplet

a *b* *c*

Fig. 5. EPR spectra of probe 3 in 20 % P338 solutions with varying ethanol contents: 1 – 0 %; 2 – 5 %; 3 – 10 %; 4 – 15 %; 5 – 20 %; 6 – 30 %, – at: *a* – 25 °C; *b* – 32 °C; *c* – 37 °C

Table 6

Parameters of the EPR spectrum of spin probe 2 in mixed solvent *ethanol-water* (37.5:62.5) % m/m at 25 °C

A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
1.66	**0.12**	**0.08**	**0.17**	**+0.09**	**triplet**

The anisotropic EPR spectra of probe 3 indicate a greater orderliness of the microenvironment of doxyl radicals. It can be observed that the EPR spectra of probe 3 remain anisotropic when the ethanol content in the 20 % P338 solution is 5 % m/m or 10 % m/m (Fig. 5). At 25 °C, the EPR spectrum of probe 3 in a 20 % P338 solution containing 15 % m/m ethanol can be considered anisotropic (Fig. 5, EPR spectrum 4). However, at 32 °C and 37 °C, the EPR spectra for this solution are more likely to resemble a superposition of two spectra. At an ethanol content of 20 % m/m in the 20 % P338 solution, the EPR spectra are superpositions of two triplets. At an ethanol content of 30 % m/m, the EPR spectra are triplets (Fig. 5).

As ethanol content in 20 % P338 solutions increases, there is a tendency for the order parameter (S) to decrease and, accordingly, for the semi-amplitude of molecular motion (parameter ν) to increase. This is accompanied by an increase in the A_N constant of anisotropic EPR spectra. At an ethanol content of 30 % m/m in 20 % P338 solution, the EPR spectrum of spin probe 3 transforms into a triplet, and the S parameter decreases significantly. A decrease in the S parameter is also observed when the temperature is raised from 25 °C to 37 °C (Table 7).

Table 7

Parameters of EPR spectra of spin probe 3 in 20 % P338 solutions with varying ethanol contents (*C*) at different temperatures (*t*)

C, % m/m	*t*, °C	A_N, mT	*S*	ν, °	Spectrum type
0	25	1.47	0.47	53.8	anisotropic
5	25	1.48	0.43	56.3	anisotropic
10	25	1.49	0.43	56.3	anisotropic
15	25	1.49	0.37	60.0	anisotropic
20	25	–	–	–	superposition
30	25	1.52	0.09	81.7	triplet
0	32	1.48	0.42	56.9	anisotropic
5	32	1.49	0.41	57.5	anisotropic
10	32	1.49	0.40	58.0	anisotropic
15	32	–	–	–	transforming into superposition
20	32	–	–	–	superposition
30	32	1.52	0.08	82.5	triplet
0	37	1.48	0.39	57.5	anisotropic
5	37	1.49	0.37	60.0	anisotropic
10	37	1.50	0.33	63.0	anisotropic
15	37	–	–	–	transforming into superposition
20	37	–	–	–	superposition
30	37	1.52	0.07	83.4	triplet

The tendency to increase the A_N constant is probably due to the solvation of the lipophilic core of P338 micelles at the level of the 5th carbon atom of the alkyl chain of probe 3 (Table 7). At ethanol content of 30 % m/m in 20 % P338 solution, the A_N constant of the EPR spectra of probe 3 is only 0.02 mT lower than the A_N constant of the EPR spectra of this probe in 37.5 % m/m aqueous solution of ethanol (Table 8). This may be indicative of the solvation of the cores of P338 micelles by the mixed solvent *ethanol-water*.

Table 8

Parameters of the EPR spectrum of spin probe 3 in 20 % P338 solution containing 30 % m/m ethanol and in mixed solvent *ethanol-water* (37.5:62.5) % m/m at 25 °C and 37 °C

Sample	*t*, °C	A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
P338 20 %, Ethanol 30 % m/m, water ad 100 %	25	1.54	1.36	0.49	0.86	0.15	triplet
	37	1.54	0.63	0.27	0.61	0.13	triplet
Ethanol 37.5 % m/m, water ad 100 %	25	1.56	0.34	0.17	0.36	0.11	triplet
	37	1.56	0.23	0.11	0.23	0.12	triplet

The EPR spectra of the spin probe 4 (16-DSA) in P338 solutions are triplets; the low-field and high-field components of these spectra are less intense than the central component (Fig. 6). As the ethanol content increases from 0 to 30 % m/m and the temperature rises from 25 °C to 37 °C, the EPR spectra of probe 4 remain triplets, with a reduction in the width of all three components (Fig. 6).

In the molecule of spin probe 4, the doxyl radical is located near the 16th carbon atom of the alkyl chain. It was of interest to compare the rotational parameters of probes 3 and 4. Consequently, the parameters for the EPR spectra of probe 4 were calculated using equations (2), (3), (4) and (5) as well as equations (6) and (7) (Tables 9, 11).

The rotational correlation times (τ_{+1}, τ_{-1}, $\tau_{\pm1}$) of the probe 4 indicate rapid rotation, suggesting that the doxyl radicals' microenvironment is in a liquid state. The rotational correlation times decrease significantly as the ethanol content increases from 0 to 30 % m/m. This indicates a notable decrease in the viscosity of the doxyl radicals' microenvironment (Table 9). The decrease in τ values in response to an increase in ethanol content up to 30 % m/m is more pronounced than the reduction in τ values in response to a temperature increase from 25 °C to 37 °C (Table 8). However, at an ethanol content of 5 % m/m, the decrease in τ values is negligible.

The values of the anisotropy parameter (ε) are slightly different at 25 °C, 32 °C and 37 °C. However, the ethanol content up to a concentration of 20 % m/m has a negligible impact on this parameter. When ethanol content reaches 30 % m/m, the ε values increase (Table 9).

The values of A_N constant, determined as the distance (in mT) between the central and high-field components of the EPR spectra of probe 4 [33, 34], tend to increase with increasing ethanol content from 0 to 30 % m/m in 20 % P338 solutions (Table 9). This suggests an intensification of the polarity of the microenvironment surrounding the doxyl radical at the 16th carbon atom of the alkyl chain of spin probe 4. At ethanol content of 30 % m/m, the A_N constant of the EPR spectra of probe 4 in 20 % P338 solution is only 0.02 mT lower than its value in the case of the 37.5 % m/m aqueous solution of ethanol (Tables 9, 10). This probably indicates the solvation of micelle cores by the dispersion medium.

Table 9

Parameters of EPR spectra of spin probe 4 in 20 % P338 solutions with varying ethanol contents (*C*) at different temperatures (*t*)

C, % m/m	*t*, °C	A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
0	25	1.46	0.65	0.49	1.04	0.08	triplet
5	25	1.46	0.54	0.45	0.98	0.07	triplet
10	25	1.47	0.43	0.43	0.94	0.06	triplet
15	25	1.48	0.36	0.33	0.71	0.06	triplet
20	25	1.51	0.32	0.30	0.63	0.07	triplet
30	**25**	**1.52**	**0.30**	**0.16**	**0.34**	**0.10**	**triplet**
0	32	1.47	0.45	0.38	0.83	0.07	triplet
5	32	1.47	0.31	0.33	0.73	0.05	triplet
10	32	1.48	0.29	0.26	0.56	0.06	triplet
15	32	1.49	0.24	0.24	0.52	0.05	triplet
20	32	1.50	0.21	0.21	0.47	0.06	triplet
30	32	1.52	0.18	0.11	0.24	0.10	triplet
0	37	1.47	0.31	0.31	0.80	0.05	triplet
5	37	1.47	0.22	0.26	0.59	0.05	triplet
10	37	1.48	0.21	0.22	0.52	0.05	triplet
15	37	1.48	0.18	0.18	0.40	0.05	triplet
20	37	1.49	0.17	0.17	0.39	0.05	triplet
30	37	1.52	0.20	0.10	0.20	0.11	triplet

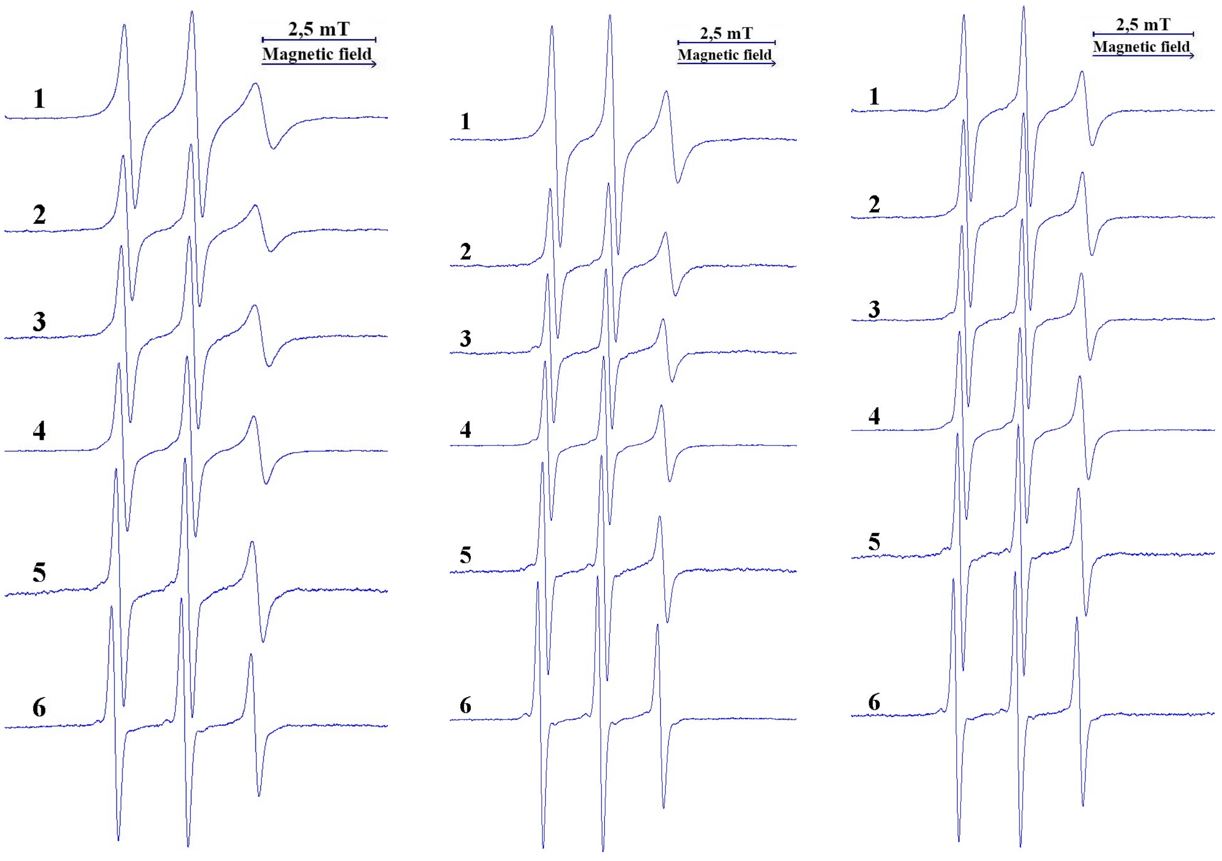
As the ethanol content in 20 % P338 solutions increases, there is a tendency for the order parameter (*S*) to decrease and the semi-amplitude of molecular motion (parameter ν) to increase, as determined from the EPR spectra of spin probe 4 (Table 11). An increase in temperature from 25 °C to 37 °C has a less pronounced effect on the decrease in the order parameter *S* than an increase in the ethanol content to 30 % m/m (Table 11).

The order parameter *S* is several times higher in the case of the EPR spectra of probe 3 (5-DSA, NH_4 salt) in 20 % P338 solutions containing ethanol up to 10 % m/m compared to the EPR spectra of probe 4 (16-DSA). In the case of the EPR spectra of probe 4, the semi-amplitudes of molecular motion (parameter ν) are significantly higher than for the EPR spectra of probe 3 (Table 7, 11). The A_N constant for the EPR spectra of spin probe 4 (calculated using equation (6)) is slightly lower than that in the case of probe 3. This indicates a lower polarity of the doxyl radicals within the micelle core at the level of the 16th carbon atom of probe 4.

Table 10

Parameters of the EPR spectrum of spin probe 4 in mixed solvent *ethanol-water* (37.5:62.5) % m/m at 25 °C

A_N, mT	τ_{+1}, ns	τ_{-1}, ns	$\tau_{\pm1}$, ns	ε	Spectrum type
1.54	**0.20**	**0.07**	**0.14**	**0.16**	triplet



a *b* *c*

Fig. 6. EPR spectra of probe 4 in 20 % P338 solutions with varying ethanol contents: 1 – 0 %; 2 – 5 %; 3 – 10 %; 4 – 15 %; 5 – 20 %; 6 – 30 %, – at: *a* – 25 °C; *b* – 32 °C; *c* – 37 °C

Table 11

Parameters of EPR spectra of spin probe 4 in 20 % P338 solutions with varying ethanol contents (*C*) at different temperatures (*t*)

C, % m/m	*t*, °C	A_N, mT	*S*	ν, °	Spectrum type
0	25	1.40	0.13	78.4	triplet
5	25	1.42	0.11	80.0	triplet
10	25	1.44	0.10	80.6	triplet
15	25	1.44	0.09	81.7	triplet
20	25	1.46	0.08	82.5	triplet
30	25	1.49	0.07	83.4	triplet
0	32	1.43	0.11	80.0	triplet
5	32	1.43	0.09	81.7	triplet
10	32	1.45	0.08	82.5	triplet
15	32	1.45	0.07	83.4	triplet
20	32	1.47	0.07	83.4	triplet
30	32	1.51	0.06	84.9	triplet
0	37	1.43	0.09	81.7	triplet
5	37	1.44	0.08	82.5	triplet
10	37	1.44	0.08	82.5	triplet
15	37	1.45	0.07	83.4	triplet
20	37	1.46	0.06	84.4	triplet
30	37	1.49	0.05	85.0	triplet

5. Discussion of research results

20 % aqueous solution of P338 undergoes a sol→gel transition when the temperature is increased from 25 °C to 32 °C; the rheological parameters of the gel increase as the temperature is raised to 37 °C (Fig. 1, *a*, Table 2) [31]. Adding ethanol to 20 % P338 solution affects its rheological properties at different temperatures. This effect is due to the interaction of ethanol with P338 micelles and depends on the ethanol content.

20 % P338 solution with an ethanol content of 5 % m/m still demonstrates the ability to undergo a thermally induced sol→gel transition. The rheological parameters of the resulting gel at 32 °C and 37 °C are higher than those of the gel with water as the dispersion medium (Fig. 1, *a*, *b*, Table 2).

20 % P338 solution with 10 % m/m ethanol at 32 °C and 37 °C exhibits a non-Newtonian flow behaviour and low values of rheological parameters, with the solution remaining in a liquid state. At 40 °C, a gel with high values of yield stress and apparent viscosity is formed (Fig. 1, *c*, Table 2). Therefore, the presence of 10 % m/m ethanol in a 20 % solution of P338 results in an increase in the temperature of the sol→gel transition.

No gels are formed when the ethanol content in 20 % P338 solutions is increased to 15 % m/m and 20 % m/m in the temperature range from 25 °C to 40 °C. These solutions are liquids with Newtonian flow behaviour (Fig. 1). The dynamic viscosity of the liquids in question decreases with an increase in ethanol content but still increases with increasing temperature (Table 2). At 30 % m/m ethanol content in 20 % P338 solution, the dynamic viscosity of this liquid decreases with increasing temperature (Fig. 1, Table 2).

It is important to consider the impact of ethanol content on the rheological properties of 20 % P338 solutions and the structure of P338 micelles.

As the concentration of poloxamers increases, the volume fraction of micelles also rises. Upon reaching the critical micelle volume fraction (*c*≥0.53), the interaction between micelles occurs, resulting in the formation of lyotropic liquid crystals with cubic packing of spherical micelles in aqueous solution. This process causes a sol→gel transition [37, 38]. It is also possible for lyotropic liquid crystals to adopt a hexagonal or lamellar structure [29, 37]. The findings of the research, conducted using the spin probe method, indicate that as the temperature rises, the packing density of PPO chains within the micelle cores of poloxamers decreases. This results in an increase in the volume fraction of spherical micelles (at a sufficient concentration of poloxamer) [31].

In the case of a 20 % aqueous solution of P338, which is characterized by thermally induced sol↔gel transitions, there is a certain difference in the shape of the EPR spectra and the rotational diffusion parameters of probes 1, 2, 3, and 4, which are localized in micelles, at temperatures from 25 °C to 37 °C. Probes 1 and 2 are in a state of rapid rotation. Their EPR spectra are triplets (Fig. 2, 4, Table 3, 5). The EPR spectra of probe 3 are anisotropic and characterized by high values of order parameter (Fig. 5, Table 7). The EPR spectra of probe 4 are triplets characterized by a significantly lower ordering parameter and indicate a faster rotation (Fig. 6, Table 9, 11). Based on the results of the study, it can be assumed that the wedge-shaped packing of the PPO chains in the micelle cores occurs up to about 5th carbon atoms of the alkyl chain of probe 3 [31]. The same difference in the shape of the EPR spectra and the rotational diffusion parameters of probes 1, 2, 3, and 4 is observed in the case of 20 % P338 solutions containing 5 % m/m and 10 % m/m ethanol, as can be observed in Fig. 7.

With increasing ethanol content in P338 solutions, as well as with heating from 25 °C to 37 °C, the rotational correlation times of spin probes 1, 2, and 4 significantly decrease (Tables 3, 5, 9). However, with the increase in ethanol content, there are no significant alterations in the EPR spectra of spin probes 1, 2, and 4 that could be attributed to changes in the rheological properties of P338 solutions, particularly concerning sol↔gel transitions.

As the ethanol content in the 20 % P338 solution increases, significant changes occur in the shape of the EPR spectra of the spin probe 3. It is important to note that in the case of 20 % P338 solutions, where sol↔gel transitions occur, the EPR spectra remain anisotropic at temperatures from 25 °C to 37 °C (Fig. 8). At ethanol content of 15 % m/m in 20 % P338 solution, the EPR spectrum of probe 3 is anisotropic only at 25 °C (Fig. 5), and no gels are formed at temperatures of 32 °C and above; the solutions remain Newtonian liquids (Fig. 1). At ethanol content of 20 % m/m, the EPR spectra of probe 3 exhibit a superposition of two triplet spectra (Fig. 5), which indicates the localization of doxyl radicals in two phases that differ in polarity. At ethanol content of 30 % m/m, the EPR spectra of probe 3 in a 20 % P338 solution are triplets (Fig. 5); the parameters of these EPR spectra (Table 7) indicate a significant solvation of micelle cores by the dispersion medium.

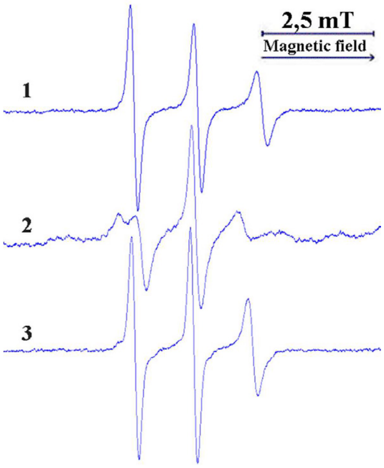


Fig. 7. EPR spectra of spin probes 1 (1), 3 (2) i 4 (3) in 20 % P338 solution containing 5 % m/m ethanol at 37 °C

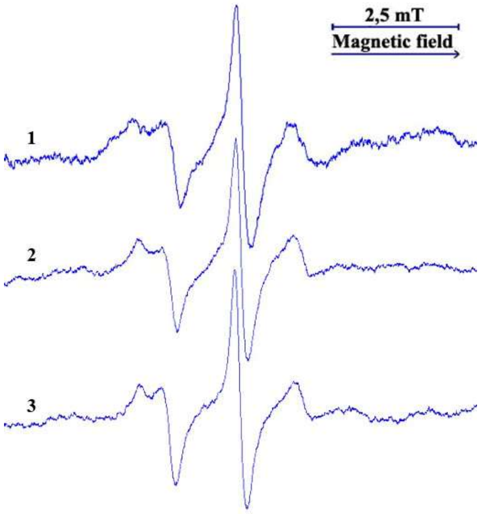


Fig. 8. EPR spectra of spin probe 3 in 20 % P338 solution containing 5 % m/m ethanol at: 1 – 25 °C, 2 – 32 °C, 3 – 37 °C

At 5 % m/m content, ethanol reduces the packing density of PPO chains in the nuclei of P338 micelles; still, it does not significantly reduce the ordering parameter S of the EPR spectra of probe 3 (Table 7). This should result in an increase in the volume fraction of micelles while maintaining their shape, which contributes to an increase in the rheological parameters of 20 % P338 solution, both in the form of a liquid at 25 °C and in the form of gels at 32 °C and 37 °C. At a content of more than 10 % m/m, ethanol causes significant changes in the structure of P338 micelles, as a result of which 20 % P338 solutions lose their ability to thermally induced sol↔gel transitions (Fig. 1).

Study limitations. This work's limitation is the study of the impact of ethanol content on the properties of solutions with only 20 % P338, mainly in the temperature range from 25 °C to 37 °C.

Prospects for further research. It is reasonable that future studies should be conducted on 20 % solutions of P338 with the dispersion medium containing water and other hydrophilic non-aqueous solvents (propylene glycol, glycerol, macrogol 400, etc.) in various proportions. Such studies might also be promising for solutions of other poloxamers that are standardized in the European Pharmacopoeia [1].

6. Conclusions

It was demonstrated that ethanol, when added to the 20 *%* P338 solution, results in changes to the rheological properties of this solution. However, at the ethanol content of 5-10 *% m/m*, the ability of P338 to thermally induced sol→gel transition remains unaltered. The rheological properties of the 20 *%* P338 solution exhibit a correlation with the observed change in EPR spectra types for the 5-DSA NH_4 salt. As the ethanol content in the solution increases, the solvation of P338 micelle cores by the dispersion medium increases, accompanied by decreased density and orderliness of the PPO chains packing in the micelle cores.

Conflict of interests

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

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Data availability

Data will be made available at a reasonable request.

Use of artificial intelligence

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

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