NMR relaxation and self-diffusion in aqueous micellar gels of pluronic F-127

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ABSTRACT

We studied the transverse NMR relaxation of protons of different chemical groups and diffusion of molecules in an aqueous pluronic F-127 system. The system was studied at concentrations of 15, 21 and 28 wt% and in the temperature range of 293–333 K. The dynamic peculiarities in different phases were analyzed based on the NMR relaxation and diffusion data. Our study demonstrated a correlation between phase states, T_2 relaxation times of "solid-like" and "liquid" protons of selected chemical groups of polypropylene oxide (PPO) and polyethylene oxide (PEO) blocks, and diffusion coefficients. Transverse NMR relaxation established that the presence of a "solid-like" component of CH_3 protons of PEO blocks is observed only at temperatures and concentrations corresponding to the rigid-gel phase, due to entanglements between micellar coronas. At all temperatures and concentrations, some CH_3 protons of PPO blocks and some CH_2 protons of PEO blocks show "liquid-like" transverse NMR relaxation. Under the conditions corresponding to formation of the rigid-gel phase of the pluronic, relaxation of the "liquid-like" protons additionally accelerates due to entanglements of micellar coronas. Transition of the system to the rigid-gel phase is accompanied by decrease of diffusion coefficients by a factor of 10–10^4 relative to the diffusivity of free-moving pluronic micelles in the sol phase. Diffusion measurements show that there are pretransition phenomena, which are characteristic for temperatures and concentrations near sol-gel phase boundaries.

1. Introduction

Pluronics are triblock copolymers containing hydrophilic blocks of polyethylene oxide (PEO) and hydrophobic blocks of polypropylene oxide (PPO) such as $(PEO)_a – (PPO)_b – (PEO)_a$ [1]. Under certain circumstances, an aqueous pluronic solution may form a polymer gel, which can absorb water and some biological fluids [2–5]. These gels are actively used as structures to carry medicines to affected organs and tissues [6,7]. The gel state of such a system is formed by a lyotropic liquid crystal [8]. The structure of aqueous pluronic gel F-127 has been studied by a number of techniques. The block copolymer forms micelles, consisting of a relatively compact core and a heavy solvated corona. Gelation occurs due to packing of the micelles. Mortensen [9] suggested that the mechanism of gelation is due to "hard-sphere crystallization" as the micelle concentration approaches the critical volume fraction of 0.53. They concluded that the structure of the gel is a body-centered

cubic packing of spherical micelles. The micelle shells are partially overlapping, and the degree of overlap depends on the polymer concentration. The nearest neighbor distance falls with increasing concentration of the polymer, implying that the degree of overlap of micellar shells increases. The micellar core radius estimated from the intra-particle interference is 4.4 nm [10], while the micelle diameter is ~19 nm [2,11]. The aggregation number of the micelles is independent of polymer concentration. With increasing temperature, the concentration of micelles increases and the degree of hydration of the ethylene oxide units decreases. Under low-temperature conditions, the increase in micellar concentration is the dominant factor, with the result being that the corona overlap increases with increasing temperature. Above 30 °C, almost all of the polymer exists in the micelles and the concentration of the micelles is not a function of temperature. The structure of gel was studied by SANS and rheometry [10]. It has been shown that under low concentrations of pluronic, the micelles are well-separated and the solution is an isotropic Newtonian fluid. Micelle concentration can be controlled by varying either polymer concentration or temperature. At higher concentrations or temperatures, the micelle volume fraction approaches close packing. At higher temperatures, the EO units

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dehydrate and the effective volume fraction again decreases. Polymer gels and lyotropic liquid crystals belong to a class of soft matter. For this class of matter, the properties of solids are combined with the diffusion transport properties of liquids. The same dual character in these media is observed in their NMR relaxation characteristics: some parameters of NMR relaxation conform to the liquid-phase behavior of the nuclear spin system, when dipole-dipole (d-d) interactions between nuclear spins are sufficiently averaged, while other parameters conform to the solid-like behavior of the nuclear spin system (presence of residual d-d interactions) [12,13]. Residual d-d interactions are manifested by splitting of NMR spectra lines, independence of T_2 relaxation time on temperature, and the Gaussian form of decay of transverse magnetization [14]. These phenomena occur because not all possible spatial orientations of the molecules are realized in their movement; as a result, the motion is anisotropic. In polymeric gels, this anisotropy originates from physical interaction between macromolecules. There are a number of studies in which NMR relaxation has been investigated in the gel phase of pluronics [2,3,13,14]. While NMR relaxation is related mainly with local molecular motion, the translational motion of molecules in gels can be studied by pulsed-field-gradient NMR (NMR PFG) [16–18]. Self-diffusion of a triblock-copolymer of PEO and PPO with a molecular mass of 5.1 kDa in aqueous D_2O has been investigated by NMR PFG, with respect to dependence on concentration and temperature with a maximum strength of the gradient 4.8 T/m [16]. Two diffusion coefficients were observed, which were related to the polymer-rich and the polymer-poor phases. No dependence of diffusivity on the diffusion time was observed, therefore, no dynamic exchange processes of the polymer molecules between micellar and non-micellar states took place. The hydrodynamic radii of the micelles were estimated and it was shown that the sizes of the diffusing species depend only to a small extent on the temperature.

Recently, we demonstrated the solid-like behavior of transverse NMR relaxation in an aqueous micellar gel of pluronic F-127 using a low-field ^{1}H NMR apparatus without spectral resolution [13]. A high-field NMR spectrometer permits one to obtain the spectral resolution as well as improved signal-to-noise ratio that allow spin-echo measurements of diffusion of molecules at different concentration and temperature ranges. These conditions were used in our current study to obtain detailed information about dynamics in aqueous solutions of pluronic F-127.

2. Materials and methods

2.1. Sample preparation

The structure of F-127 is shown in Fig. 1. Its molecular weight is ~12.5 kDa, the number of ethylene oxide units is ~200 and the fraction of the ethylene oxide block (PEO block) is ~0.7. Deuterated water (99.9 atom % D) and pluronic F-127 were purchased from Sigma (St. Louis, MO). Aqueous (D_2O) solutions of F-127 with concentrations of 15 wt% (Sample 1), 21 wt% (Sample 2) and 28 wt% (Sample 3) were prepared.

Fig. 2 shows the phase diagram for the aqueous pluronic of F-127, obtained earlier, with a natural abundance of protons [4].

Critical micelle concentration (CMC) of the pluronic F-127 solution was 0.26–0.8 wt% [19]. Pluronic F-127 formed spherical micelles in the studied range of concentrations and temperatures [9,10]. According to this phase diagram, at increasing temperature, Sample 1 (15%)
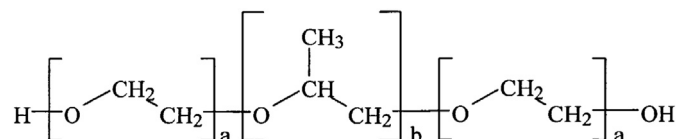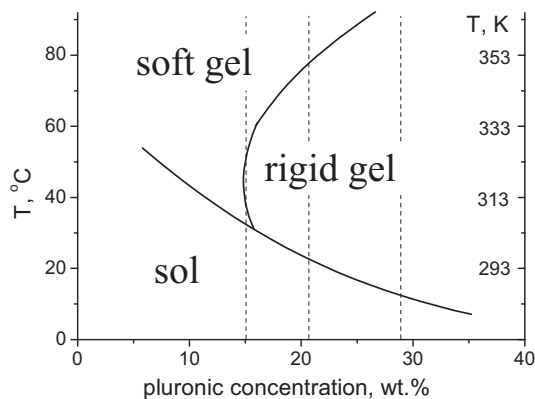

Fig. 2. Phase diagram of aqueous pluronic F-127 [4] contains a sol-phase region, a rigid-gel region (crystals with a face-centered cubic structure) and a soft-gel region (a crystal with body-centered cubic structure). Dashed lines show positions of samples studied: 15 wt% (Sample 1), 21 wt% (Sample 2) and 28 wt% (Sample 3).

successively moves from sol phase (at 31°C) into soft-gel phase, from soft-gel phase into rigid-gel phase (at 40°C) and further from rigid gel-phase into soft gel-phase (at 44°C). Sample 2 (21%) at T = 22°C and Sample 3 (28%) transform from sol phase into rigid-gel phase at T = 14°C and remain in this phase during the whole high-temperature range up to 60°C. It should be noted that using D_2O instead of H_2O leads to a shift of the corresponding transition temperatures to higher values [20] because of changes of entropy and/or enthalpy of the transition, which is due to structural changes in the system [21].

In the rigid-gel phase, the micelle cores formed by the PEO blocks overlap. An illustration of this explaining the mechanism of overlapping micelles—mutual penetration of PEO blocks of neighboring micelles—can be found in the paper of Grant et al. [22]. In the zone of overlap of the coronas of adjacent micelles, PEO-blocks are intertwined. Some of the PEO blocks are not included in the overlap zone of micelles.

2.2. ^{1}H NMR relaxometry and diffusometry

^{1}H NMR measurements were performed using a Bruker Ascend/ Aeon WB 400 (Bruker BioSpin AG, Fällanden, Switzerland) NMR spectrometer with a working frequency of 400.27 MHz for ^{1}H, magnetic field strength of 9.4 T. Only three hundred μl of sample was placed in a standard 5-mm NMR glass tube for measurements to avoid thermal convection in the liquid.

Fig. 3 demonstrates the ^{1}H NMR spectrum of 15 wt% pluronic solution in D_2O. The system forms a soft-gel phase at this concentration and temperature [4] that leads to seeing moderately resolved spectrum
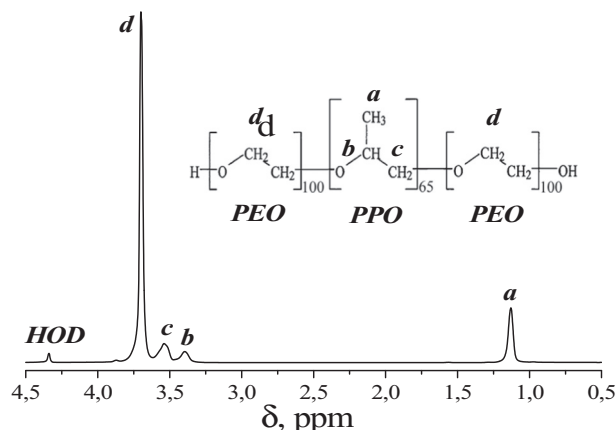

Fig. 1. Chemical structure of Pluronic F-127, where a = 100 and b = 65 denote the number of ethylene oxide and propylene oxide monomers per block.

Fig. 3. ^{1}H NMR spectrum of aqueous (D_2O) 15 wt% pluronic F-127. T = 333 K.

lines. The line assignment was made according to works [6,11,15] that allowed identification of signals of protons of PPO and PEO blocks. The most intense signal, at 3.7 ppm (**d**), relates to -CH$_2$CH$_2$O- groups of the PEO block. Signals of CH-O and CH$_2$-CH- of the PPO block are related to lines in the range of 3.3–3.6 ppm (**b**, **c**). The resonance line of protons of methyl groups of the PPO block is positioned at 1.1 ppm (**a**). The line at ~ 4.4 ppm corresponds mainly to residual water protons. Relative integral intensities of the lines were 0.148 (*a*), 0.048 (*b*), 0.098(*c*) and 0.69 (*d*), respectively. Integral intensity of the HOD line was 0.013. The lines of the ^{1}H NMR spectrum can be used to study NMR relaxation and diffusivity by applying a specified NMR pulse sequence. The resolution of the spectra changes with change in temperature and pluronic content. Of note, lines **b** and **c** become broader (Fig. S1 in the Electronic Supplementary Information (ESI)), consequently we cannot analyze NMR relaxation of the corresponding protons in the whole range of temperatures and concentrations of the study. Hence, we further analyzed the relaxation of only **a** and **d** protons, which characterizes mobility in PPO and in PEO blocks, respectively.

^{1}H NMR transverse relaxation investigations were performed with Carr-Purcell-Meiboom-Gill (CPMG, [90°-τ-180°-τ-echo]) pulse sequence and analyzed using "Bruker" Topspin 3.5 software. Phases of 90° and 180° pulses as well as the phase of the receiver were varied with a certain cycle, and the echo time was fixed to allow elimination of diffusion and *J*-coupling modulation effects [23]. In the case of a single-component molecular liquid characterized by isotropic rotation of molecules in the time-scale of the NMR experiment, expressions for transverse relaxation are presented in the form:

$$A(t) = A(0) \cdot \exp\left(\frac{2t}{T_2}\right). \tag{1}$$

Here, *A* is the signal intensity, 2*t* is the time intervals in the pulse sequence; T_2 is the time of transverse NMR relaxation.

Diffusion measurements were performed using a Diff50 Pulsed-Field-Gradient (PFG) probe. A primary source of information about the diffusivity of a molecule is the diffusion decay (DD) of amplitude of NMR spectral line, obtained by Fourier transformation of a descending half of stimulated-echo (StE), as a function of the amplitude of applied pulsed field gradient. For single-component diffusion, the form of DD can be described as follows [24,25]:

$$A(\tau, \tau_1, g, \delta) \propto \exp\left(-\frac{2\tau}{T_2} - \frac{\tau_1}{T_1}\right) \exp\left(-\gamma^2\delta^2g^2Dt_d\right). \tag{2}$$

Here, τ and τ_1 are the time intervals in the pulse sequence; γ is the gyromagnetic ratio for protons; *g* and δ are the amplitude and the duration of the gradient pulse, respectively; $t_d = (\Delta - \delta/3)$ is the diffusion time; $\Delta = (\tau + \tau_1)$. In the measurements, the duration of the 90° pulse was 7 μs, δ was in the range of 2–6 ms, t_d was in the range of 70–500 ms and the amplitude, *g*, was varied from 0.06 up to 29.73 T·m^{-1}. The recycle delay was 3.5 s. The DD of the most intense line "**d**" in the NMR spectrum of pluronic (Fig. 3) were recorded.

3. Results and discussions

3.1. ^{1}H NMR relaxation

The longitudinal NMR relaxation of protons of the pluronic as a result of averaging by spin diffusion is characterized by an exponential shape, so T_1 turns out to be insensitive to gelation processes [15]. The same was observed in our study. However, the transverse NMR relaxation of protons of the pluronic changes during gelation, in accordance with earlier studies [2,3,13]. Fig. 4 shows decay curves of transverse magnetization of ^{1}H NMR for the sample with a concentration of 21 wt% pluronic, which is typical for the studied system. Decays of transverse magnetization for other concentrations of pluronic used in this
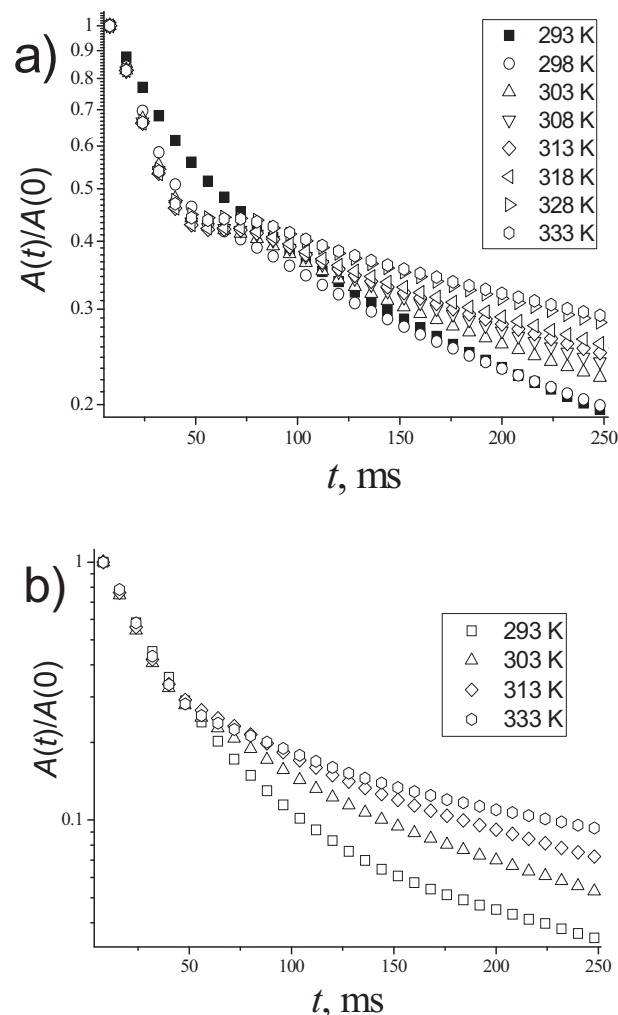

Fig. 4. Decays of transverse magnetization of ^{1}H NMR for the solution with 21 wt% pluronic at different temperatures: a) protons of CH$_2$ groups of PEO, and b) protons of the CH$_3$ group of PPO.

study are shown in Figs. S2–S3 in the ESI. Fig. 4a shows the relaxation of the CH$_2$ groups of the PEO block (3.7 ppm), while Fig. 4b relates to the relaxation of the signal at 1.1 ppm, which corresponds to protons of the chemical group –CH$_3$ of the PPO block.

Fig. 4 shows that T_2 relaxation of protons of each of the chosen chemical groups is not a single exponential. It depends not only on the composition and temperature of the system, as was reported previously [13]; it is also different for protons of different chemical groups of the polymer at the same concentration and temperature. Change in the T_2 relaxation occurs as a result of transforming the system structure and the corresponding modification in the arrangement of PPO and PEO blocks of the pluronic [13]. These data showed that the change in phase state differentially influences the T_2 relaxation and, consequently, the mobility of PPO and PEO blocks of the pluronic. Generally, a complex form of NMR relaxation can be due to a difference in relaxation times of protons of different chemical groups, contributing to the corresponding NMR signal, as well as to anisotropic (hindered) rotation of specified chemical groups [12]. Later, this can be observed in a "Gaussian form" of the T_2 relaxation and usually characterized as a "solid-like" behavior [13]. The initial part of the Gaussian transverse NMR relaxation can be presented in the form [26]:

$$A(t) = A(0) \exp\left(-0.5 \cdot (t/T_2)^2\right).$$

The appearance of the Gaussian form of decay of transverse NMR magnetization and temperature independence of this form occurs in systems with incomplete averaging of the dipole-dipole interaction between nuclear spins because of their anisotropic motion [11,14,21]. The reason for this behavior is evidently due to the net structure formed by the pluronic with fixed nodes in the net. This type of relaxation was observed in aqueous pluronic F-127 solutions and suggested for nodes of the net formed by nuclei of pluronic micelles (PPO blocks) and entanglements of PEO blocks forming coronas at system compositions corresponding to the rigid-gel phase [13]. The component with Gaussian transverse relaxation decay was obtained in most of our experimental data by decomposition of original decays. Decomposition of an original decay was performed by subsequential graphical subtraction of the exponential contributions in the $\lg(A(t)) - t$ coordinates until remaining part was in the form of Gaussian. An example of such decomposition is shown in Fig. S4 in the ESI. Notably, "solid-like" Gaussian decays are observed for protons of the PPO block at all temperatures and concentrations of the study, while they are observed for the PEO block only in certain temperature and composition ranges. A diagram, demonstrating the "solid-like" relaxation of protons of PPO and PEO blocks, is shown in Fig. 5. From the figure, it is seen that the "solid-like" Gaussian relaxation of protons of PEO blocks is observed only in the temperature-concentration range that corresponds to the rigid-gel phase in the phase diagram of the pluronic (Fig. 2).

Generally, the form of T_2 decay for –CH$_3$ PPO protons under all experimental conditions and for CH$_2$ protons of PEO in the presence of their "solid-like" contribution in T_2 relaxation can be expressed as:

$$A(t) = \sum_i p_{ai} \cdot \exp\left(-\frac{t}{T_{2ai}}\right) + p_{b,c} \cdot f_{b,c}(T_{2b,c}), \tag{3a}$$

while CH$_2$ protons of PEO in the absence of their "solid-like" contribution in T_2 relaxation can be expressed in the form:

$$A(t) = \sum_i p_{ai} \cdot \exp\left(-\frac{t}{T_{2ai}}\right) + p_c \cdot \exp\left(-\frac{t}{T_{2c}}\right), \tag{3b}$$

Here, the first contribution relates to the transverse relaxation of protons of PEO and PPO blocks, demonstrating "liquid-like" exponential or multi-exponential transverse relaxation and is characterized by T_{2ai} times of relaxation, and the second contribution ($f_{b,c}$) relates to the Gaussian component of decays, demonstrating "solid-like" relaxation (3a). The subscripts "*b*" and "*c*" refer to PPO (T_{2b}) and PEO (T_{2c}), respectively. p_{ai} and $p_{b,c}$ are proton fractions, $\sum p_{ai} + \sum p_{b,c} = 1$. In the
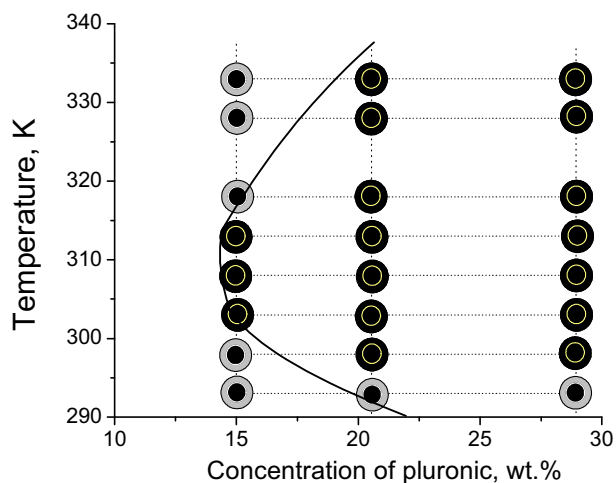

Fig. 5. A diagram demonstrating concentration and temperature ranges of "solid-like" Gaussian transverse NMR relaxation of –CH$_3$ protons in PPO blocks (black central circles) and CH$_2$ protons of PEO blocks (black outer coils) of the pluronic.

temperature-composition regions of the phase diagram corresponding to sol and soft-gel phases, the Gaussian component (3a) transforms to the second component (3b), which is of nearly exponential form, but their relaxation times are slightly longer than those of the Gaussian component.

The form of the transverse ^{1}H NMR relaxation decay when determined by dipole-dipole interaction is related to the correlation time of the local mobility of molecular segments and corresponding protons [12,27]. If the correlation rate of this motion τ_c^{-1} is much smaller than the Larmor precession frequency, the nuclear spins in the external magnetic field experience slightly different dipolar interactions, leading to a distribution of resonance around the mean value ω_L. In this case, the frequency distribution is Gaussian-like, which leads to a specified form of NMR transverse relaxation. When protons are moving faster, local magnetic fields are averaged out and the relaxation process is slowed down. In the motional narrowing state, which is accessed as the τ_c^{-1} in the range of 10^3–10^5 s^{-1}, depending on the strength of the dipolar interactions between spins, the relaxation decay is determined by the homogeneous magnetic field and shows an exponential form of decay. Therefore, transformation of the rigid-gel phase of the pluronic to its sol phase or soft-gel phase leads to a change in the local mobility of CH$_2$ group protons of the PEO blocks from "solid-like" to the conditional of motional narrowing "liquid-like" state.

Complicated multi-exponential decays of ^{1}H NMR relaxation of the "liquid-like" signal were analyzed in our previous study, which used a low-resolution and low-frequency (25 MHz) NMR device [13]. The decays were presented as the sum of two or three exponential contributions. In the current study, with high-resolution and high-sensitive NMR, the signal-to-noise ratios of the NMR signals are much higher and transverse relaxation decays of "liquid-like" components of decays sometimes demonstrate even more complicated forms (Figs. 4, S2–S3 in ESI), which do not completely fit with two- or three-exponential models. Therefore, the most unbiased way to characterize T_2 relaxation in the "liquid-like" part is by the mean value of the corresponding T_2, which is an averaged value over the "liquid-like" protons. Therefore, transverse relaxation decays (3a) and (3b) can be presented in forms:

$$A(t) = p_a \cdot f_a(T_{2a}) + p_{b,c} \cdot f_{b,c}(T_{2b,c})$$

$$A(t) = p_a \cdot f_a(T_{2a}) + p_c \cdot \exp\left(-\frac{t}{T_{2c}}\right) \tag{4b}$$

where $f_a(T_{2a})$ is the exponential or multi-exponential function of relaxation corresponding to the "liquid-like" component. Here, the multi-exponential function of transverse NMR relaxation is the function, which can be formally described as a sum of i exponential functions:

$$A(t) = \sum_i A_i \exp\left(\frac{-2t}{T_{2i}}\right). \tag{5}$$

Averaging of such multi-exponential relaxation leads to the mean relaxation time:

$$(T_2)^{-1} = \sum_i \frac{p_i}{T_{2i}}, \tag{6}$$

where p_i are fractions of the single-relaxation contributions. In practice, an averaged T_2 can be obtained as a derivative of the multi-exponential function $A(t)$ without preliminary separation into single-exponential contributions:

$$(T_2)^{-1} = \frac{\partial(\lg(A(t)))}{\partial t}. \tag{7}$$

Proton populations of "solid-like" fractions p_b and p_c are shown in Fig. 6. Dependences of T_2 for "solid-like" ($T_{2b,c}$) and "liquid-like" (T_{2a}) components of relaxation on temperature for three samples of aqueous
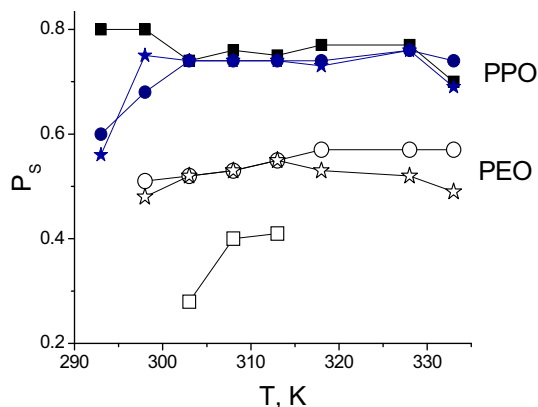
Fig. 6. Temperature dependences of the "solid-like" fraction of transverse NMR relaxation of –CH$_3$ protons in PPO blocks and CH$_2$ protons of PEO blocks of pluronic for aqueous pluronic 15 wt% (squares), 21 wt% (circles) and 28 wt% (stars). Solid symbols correspond to PPO protons, while open symbols correspond to PEO protons.

pluronic with different concentrations used in the experiment are shown in Fig. 7. Separate plots for different concentrations of pluronic are shown in Fig. S5 of ESI.

From Fig. 6, it is seen that the fraction of "solid-like" –CH$_3$ protons of PPO (solid symbols) is near 0.8, while the fraction of such protons of PEO (open symbols) are less than 0.4 for the sample with 0.15 wt% pluronic, and reaches 0.5 for samples with 21 and 28 wt% pluronic. On average, this is close to the fraction of the "fast-relaxing" component observed earlier [13]. Other fractions of protons show "liquid-like" relaxation. Fig. 7 shows that T_2 relaxation times of the "solid-like" protons (solid symbols) are in the range of 16–40 ms and are almost persistent during the temperature increase from 293 to 333 K, which is typical for protons in phases with restricted local mobility of molecules or their segments, such as solid or gel phase [13,14]. Other protons, –CH$_3$ protons of PPO as well as CH$_2$ protons of PEO show "liquid-like" exponential (multi-exponential) relaxation (open symbols), independent of whether first fraction of these other protons demonstrate "solid-like" relaxation (solid symbols). Despite the mobility of the CH$_3$ group usually being higher than that of the CH$_2$ group under the same conditions, relaxation times for –CH$_3$ protons of PPO in the studied system are less than those of CH$_2$ protons of PEO. This testifies to the fact that the mobility of protons is highly related to the state of corresponding segments of the pluronic, thus the mobility in the hydrophilic PEO blocks is higher than that in hydrophobic PPO blocks. It is known that an increase of temperature in the motional narrowing state generally leads to a monotonous increase of T_2 in liquids, because of the increase in their
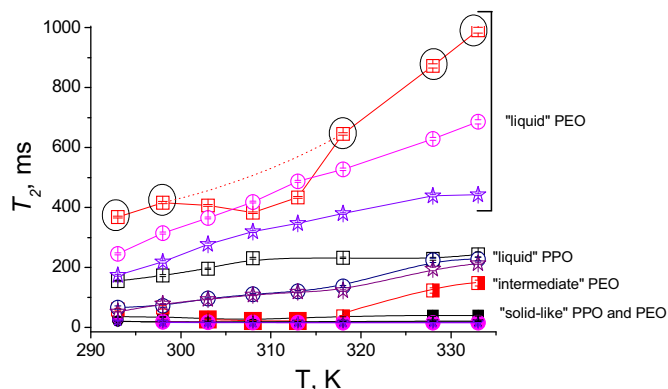
mobility [12,27]. This leads to increased hydration of corresponding parts of PEO segments [10], which is observed for "liquid-like" PEO and PPO protons in the studied system (open symbols). There are also exclusions in such monotonous change. The first concerns the "liquid" PEO in the solution with 15 wt% pluronic (red open squares), where a monotonous increase of T_2 in temperature ranges of 293–298 K and 318–333 K is interrupted with a "laydown" at temperatures of 303–313 K. This temperature range corresponds to transition of the system from the sol phase into the rigid-gel phase region with the appearance of a "solid-like" short T_2 relaxation time of PEO protons (red solid squares), and finally into the soft-gel phase region. Experimental points outside the rigid-gel region are rounded with big open circles. Evidently, the appearance of the rigid-gel phase in which PEO segments are involved leads to additional restriction for the "liquid" protons of PEO segments. The second disruption in the monotonicity of the temperature dependence of T_2 is distinctly observed for "liquid" PPO protons in the system with 15 wt% pluronic (black open squares): T_2 increases in the temperature range of 293–308 K, but it flattens at higher temperatures. This effect is related to the saturation of the local mobility of "liquid" PPO segments entangled between "solid" nodes inside PPO blocks. This effect is predicted by a theory of NMR [14] and agrees with SANS data, which indicates that the degree of overlap of micellar shells reaches a maximum at ~313 K [10].

Arrhenius plots of transverse NMR relaxation rate $1/T_2$ of protons corresponding to "liquid-like" relaxation are shown in Fig. S6 of the ESI. These dependences are not always strictly linear in the Arrhenius plot, particularly in the cases described above, for "liquid-like" relaxation of PEO and PPO in the solution with 15 wt% pluronic (red and black squares). By ignoring these deviations related to phase transformation occurring at increased temperature, we can approximate these dependences by an Arrhenius type of equation:

$$\frac{1}{T_2} = A \cdot \exp\left(\frac{E_A}{RT}\right), \tag{8}$$

where A is a constant independent of temperature, E_A is the apparent activation energy and R is the gas constant. From the picture it was found: E_A for PPO and PEO are 26.6 ± 1.5 kJ/(mol·K) and 23.3 ± 1.4 kJ/(mol·K), respectively. The obtained E_A for PPO correlates with the earlier found activation energy of NMR relaxation time for backbone motions of PPO in PEO-PPO-PEO micelles (~28 kJ/mol) obtained by ^{1}H NMR relaxometry [11] and for segmental motion of the bulk PPO obtained also by ^{1}H NMR (23–28 kJ/mol) and Rayleigh scattering (~25 kJ/mol) [11]. As far as the PEO block is concerned, the rapid local segmental motion in the block has been characterized by activation energy ~24 kJ/mol, similar to the activation energy reported for the PEO chain in alkyl ethers of PEO [2].

3.2. Self-diffusion of pluronic

In the self-diffusion measurements by NMR PFG, only protons with long enough T_2 relaxation times contribute in the echo signal. In our case, the shortest relaxation time for a "solid-like" component is $T_2 \sim$ 16 ms (Fig. 7), which is longer than the shortest interval of τ (2 ms) used in the experiments. Therefore, all phases of the system contribute to the echo, but their contributions may differ from real fractions of protons in these phases. Fig. 8 demonstrates representative diffusion decays obtained for aqueous pluronic in the temperature range of 293–333 K for samples with concentrations of 15 and 21 wt% pluronic; the DD for the 28 wt% pluronic sample can be found in the ESI (Fig. S7).

Diffusion decays (DDs) here demonstrate complicated forms, which depend on the temperature used at each of the studied concentrations. The slope of the decay is proportional to mean translational displacement of protons of molecules or molecular aggregates. It is related to the real or apparent diffusion coefficient of the corresponding species. As seen in the case of 15 wt% pluronic (Fig. 8a), the DDs are close to

Fig. 7. Temperature dependences of "solid-like" and "liquid-like" components of transverse NMR relaxation of –CH$_3$ protons in PPO blocks and CH$_2$ protons of PEO blocks of pluronic for aqueous pluronic 15 wt% (squares), 21 wt% (circles) and 28 wt% (stars). Black symbols correspond to PPO protons, while colored symbols correspond to PEO protons. Experimental points outside the rigid-gel region are rounded with open circles.
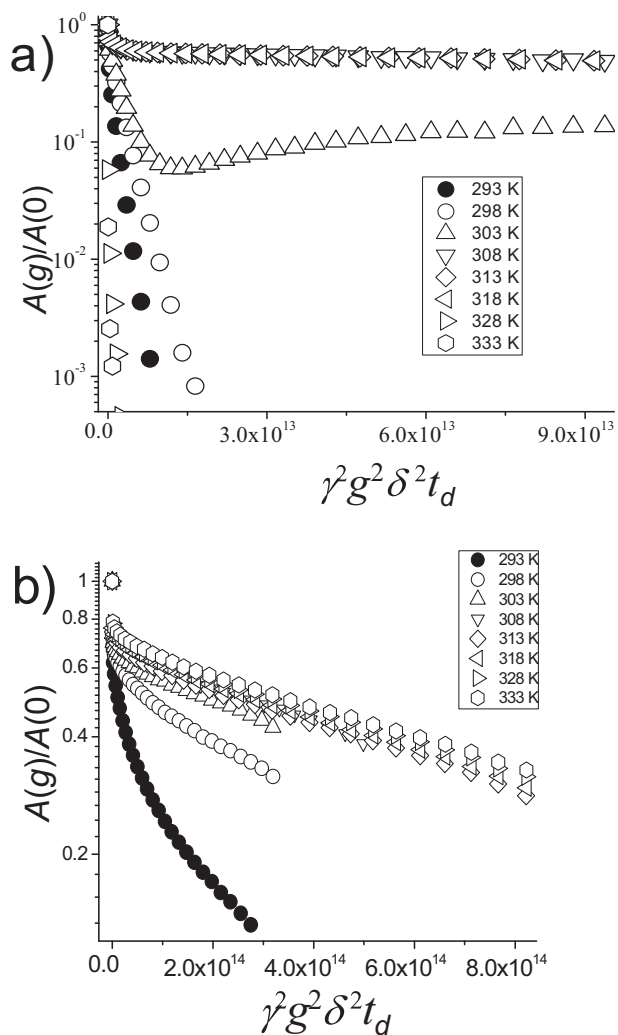
Fig. 8. ^{1}H NMR stimulated-echo diffusion decays obtained for aqueous pluronic at 15 (a) and 21 (b) wt% pluronic and temperatures in the range of 293–333 K.

single exponential forms at low temperatures, 293 and 298 K, where the system forms the sol phase (Fig. 2). Because CMC of the pluronic is very low (0.26–0.8 wt% at 293 K [19]) and decreasing with increase in temperature, freely diffusing pluronic molecules (unimers) do not contribute to the NMR signal in the studied range of concentrations and diffusing species are micelles. For a temperature-activated process of diffusion, we could expect increase of diffusivity with the increase of temperature, however, the slopes of these decays decrease at increased temperature and the average diffusion coefficient (D) decreases from $5.1 \cdot 10^{-12}$ m^2/s to $4.9 \cdot 10^{-12}$ m^2/s. At further increase of temperature to 303 K, the system transforms from sol phase to rigid-gel phase (Fig. 2), which leads to transformation of the usual descending form of DD to an "oscillating" form typical for diffusion of molecules in ordered restricted geometry [28]. The maximum pulsed field gradient is not high enough to obtain the form of the decay in a higher dynamic range, therefore only the initial part of the decay is observed in the experiment. In this case, a part of the pluronic micelles begin to interact each other forming a type of three-dimensional network with very slow diffusivity ($\sim 10^{-15}$ m^2/s), while other part of micelles, which do not interact each other remained confined inside the voids formed by the network. Diffusivity of these micelles becomed spatially restricted. The presence of oscillations of the DD demonstrates that the three-dimensional structure formed by the pluronic network is highly ordered [28]. Therefore, the whole curve of DD became a sum of the oscillating and the slowly-decaying parts. The mechanism by which ordered structure is created

in the pluronic solution is a balance of attractive and repulsive interactions [10]. Positions of minima and maxima on the oscillating DD were determined by a characteristic repeating length L of this ordered structure and by a variable $q = \gamma\delta g$, which is analogous to the wave vector in scattering experiments [28]. Particularly, for spherical geometry of restrictions a unitless parameter $q^* = \gamma\delta gL$ reaches the minimum at ~ 1.5 [29]. From the minimum in the Fig. 8a, we can estimate $L \sim 1.2$ μm.

Further heating of the solution to temperatures of 308–318 K results in completion of formation of the rigid-gel phase where most of the pluronic micelles are bound and experimental measurements show slowly decaying part of the DDs with $D \sim 10^{-15}$ m^2/s. At further heating to temperatures higher than 318 K, the system leaves the rigid-gel region and forms the soft-gel phase (Fig. 2), with some liberation of pluronic micelles, while diffusion coefficients increase to the range 10^{-12} - 10^{-11} m^2/s. From comparison of T_2 relaxation (Fig. 7) and diffusion in the soft-gel phase, it follows that the translational mobility of micelles is rather free as the local mobility of PEO segments.

For samples with concentrations of 21 wt% (and 28 wt%) pluronic, the slopes of the DDs decrease with increasing temperature in the whole temperature range (Fig. 8b). Diffusion decays showed complex forms. Because of the inhomogeneity of the system at higher temperatures and the presence of spatial restrictions in gel phases influencing the form of DDs, detailed analysis of decays is ambiguous. Therefore, we divided experimental DDs into two parts with sufficiently different (one to three decimal order of magnitude) mean apparent diffusion coefficients D_f (fast) and D_s (slow) and analyzed behavior of these diffusion coefficients obtained at different concentrations and temperatures (Fig. 9). As seen from the figure, there are three ranges of the plot, which are related to the presence of three different phases of the aqueous pluronic solution.

In the range of the sol phase, the diffusion of pluronic micelles is relatively free, by definition. According to the Stokes-Einstein equation, the micelle diffusion coefficient in the phase depends on the micelle hydrodynamic radius R:

$$D = \frac{k_B T}{6\pi\eta R} \tag{9}$$

where η is the viscosity of medium. Calculation of D at 293 K with $\eta = 1.25$ mPa·s for deuterated water and micelle radius 19 nm [2,11] gives diffusion coefficient $1.8 \cdot 10^{-11}$ m^2/s, which is comparable with the experimental value $D_f = 1.71 \cdot 10^{-11}$ m^2/s obtained for 15 wt% of pluronic (Fig. 9, solid circles). A trend toward change of the diffusion coefficients
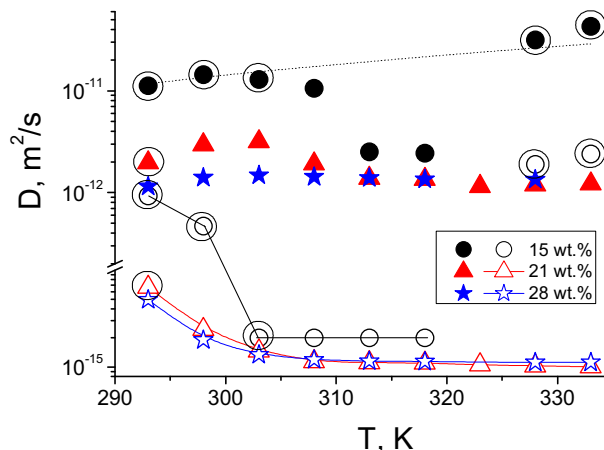

Fig. 9. Diffusion coefficients D_f (solid symbols) and D_s (open symbols) obtained in 15, 21 and 28 wt% pluronic solution by decomposition of diffusion decays. Experimental points outside the rigid-gel region are rounded with big open circles. Dotted line shows trends toward changes in diffusion coefficients of micelles with diameters 19 nm in sol and soft-gel phases, according to the Stokes-Einstein equation, taking into account only thermal energy and the changing dynamic viscosity of water.

according to Eq. (9), taking into account the thermal energy and the changing viscosity of water, but without accounting for gelation of the system, is shown in the figure as a dotted line. D_f obtained for 15 wt% pluronic in the sol phase at 293 K and the soft-gel phase at 328 K follows this trend, demonstrating that the size of the diffusing unit (micelle) does not differ significantly in these phases and the diffusion process of a fraction of micelles in these phases is controlled by temperature, but not phase transformations. Along with this, D_s obtained for 15 wt% pluronic is a factor of $10–10^3$ lesser than D_f and decreases with increase in temperature in the range 293–303 K corresponding to sol phase (Fig. 9, open circles). We suggested that such slow diffusivity was conditioned by the formation of micellar aggregates or/and gradual transition of micelles from sol to rigid-gel phase, as it was suggested by Mortensen [9]. In the temperature range 308–323 K, corresponding to the rigid-gel phase, both D_f and D_s decreased to values $2 \cdot 10^{-12}$ m^2/s and $2 \cdot 10^{-15}$ m^2/s, respectively. Soft-gel phase forms at temperatures 328–333 K that leads to increase of D_f and D_s to values ~$5 \cdot 10^{-11}$ m^2/s and $2 \cdot 10^{-12}$ m^2/s, respectively. The changes in diffusivity are due to the dehydration-rehydration of PEO blocks in the micellar corona, which occurs as a consequence of increased temperature and the interaction between the micellar coronas [10]. It is notable that the change of D_f and D_s were already observed in sol phase and in soft-gel phase, as well as the presence of diffusion coefficients D_s, which were significantly less than micellar diffusion coefficients, can be related to a pre-transition phenomena.

At 293 K and concentrations of 21 and 28 wt%, the system is near the rigid-gel phase area, but still in the sol phase, according to the phase diagram (Fig. 2). The sharp decrease D_f by a factor of 5 and D_s by a factor of 200 at these concentrations relative to the solution with 15 wt% pluronic may be related with the increase in the probability of inter-micellar collisions in the more concentrated solutions or intermolecular entanglements, which is typical for polymers [30], and an increase in corona overlap [9,10]. Indeed, there is continuous, not sharp, dependence of D_s on temperature for these concentrations in the rather broad temperature range of 293–308 K, demonstrating plain, continuous transition occurring in this range. The pre-transition phenomena usually lead to broadening in the spectra of apparent diffusion coefficients [31], which is observed in Fig. 8 as an increase in the non-exponentially of DD in the corresponding temperature and concentration ranges. With further heating of the system, D_s remains independent of temperature and around a factor of 10^4 lower than that expected for diffusion of micelles in the sol phase (dotted line).

4. Conclusions

Our study demonstrated direct correlation between phase states, T_2 relaxation times of "solid-like" and "liquid-like" protons of selected chemical groups of PPO and PEO blocks, and the averaged diffusion coefficients.

1. Transverse NMR relaxation exhibits the presence of a "solid-like" Gaussian component of CH$_3$ protons of PPO blocks at all temperatures and concentrations of our study, which correspond to sol, soft-gel and rigid-gel phase areas of the pluronic F-127 aqueous solutions. At the same time, the Gaussian component of CH$_2$ protons of PEO blocks is observed only at temperatures and concentrations corresponding to the rigid-gel phase, due to entanglements between micelle coronas.

2. Some fraction of CH$_3$ protons of the PPO block and CH$_2$ protons of the PEO block demonstrate "liquid-like" transverse NMR relaxation at all temperatures and concentrations of study.

3. Transverse NMR relaxation rates of CH$_3$ protons of PPO, which form the core of pluronic micelles, are always faster than those of CH$_2$ protons of PEO in micellar coronas. Under the conditions corresponding to formation of the rigid-gel phase of the pluronic, relaxation of the "liquid-like" protons additionally accelerates due to corona entanglements.

4. Transition of the system in the rigid-gel phase is accompanied by a decrease of the pluronic diffusivity by a factor of $10–10^4$ relative to the diffusivity of pluronic micelles in sol and soft-gel phases.

5. Diffusion measurements show that there are pre-transition phenomena, which are characteristic for temperatures and concentrations near the sol-gel phase boundaries.

The study shows that transverse NMR relaxation and NMR diffusometry are helpful for understanding local and translational dynamics of aqueous pluronic solutions, as well as their structure. The system underwent multiple transformations at variations their composition and temperature. Parts of PPO and PEO blocks can "solidify", while micelles can aggregate to form soft-gel or rigid-gel phases resembling solid crystal. However, a certain fraction of hydrophobic as well as hydrophilic areas remain "liquid-like" and attainable for guest molecules such as medicinal molecules.

CRediT authorship contribution statement

Milyausha Shaikhullina: Visualization, Investigation. **Aliya Khaliullina:** Data curation. **Rustam Gimatdinov:** Software, Validation. **Anatoly Butakov:** Writing - original draft. **Vladimir Chernov:** Conceptualization, Methodology. **Andrei Filippov:** Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

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