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NMR study of temperature-induced phase separation and polymer–solvent interactions in poly(vinyl methyl ether)/D_2O/ethanol solutions

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Abstract

The changes in the dynamic structure during temperature-induced phase transition in D_2O/ethanol solutions of poly(vinyl methyl ether) (PVME) were studied using NMR methods. The effect of polymer concentration and ethanol (EtOH) content in D_2O/EtOH mixtures on the appearance and extent of the phase separation was determined. Measurements of ^1H and ^{13}C spin–spin and spin–lattice relaxations showed the presence of two kinds of EtOH molecules: besides the free EtOH expelled from the PVME mesoglobules there are also EtOH molecules bound in PVME mesoglobules. The existence of two different types of EtOH molecules at temperatures above the phase transition was in solutions with polymer concentration 20 wt% manifested by two well-resolved NMR signals (corresponding to free and bound EtOH) in ^{13}C and ^1H NMR spectra. With time the originally bound EtOH is slowly released from globular-like structures. From the point of view of polymer–solvent interactions in the phase-separated PVME solutions both EtOH and water (HDO) molecules show a similar behaviour so indicating that the decisive factor in this behaviour is a polar character of these molecules and hydrogen bonding.

Keywords: Poly(vinyl methyl ether)/D_2O/ethanol solutions; Temperature-induced phase separation; ^1H and ^{13}C NMR spectroscopy

1. Introduction

Poly(vinyl methyl ether) (PVME) is a water-soluble polymer that exhibits phase separation above 308 K as a consequence of a lower critical solution temperature (LCST) behaviour [1–4]. On molecular level, such phase separation is assumed to be a macroscopic manifestation of a coil–globule transition followed by aggregation; this transition is probably associated with competition between hydrogen bonding and hydrophobic interactions [5,6]. The phase transition in aqueous PVME solutions was studied by various physical methods [2–14]. It was found that aqueous PVME solutions exhibit a flat and wide bimodal LCST miscibility gap [4,10]. The effect of additives such as low-molecular weight salts, electrolytes and different alcohols on the LCST of aqueous solutions of PVME has been examined and interpreted in terms of stabilization or destabilization of hydrogen bonding between polymer and water [3,13]. Measurement of the cloud point showed that the addition of methanol or ethanol to aqueous solutions of PVME increases the LCST whereas higher molecular weight alcohols act in solutions as destabilizators and LCST can be shifted to lower values with the increasing alcohol content. Very recently Maeda et al. [11] investigated PVME/alcohol/water ternary systems by micro-Raman spectroscopy. They concluded that association between alcohols and PVME is mainly due to hydrophobic interactions.

Recently, we employed ^1H NMR spectroscopy for study of the temperature-induced phase transition in PVME/D_2O solutions and gels [15]. A similar behaviour was found for linear and crosslinked systems, indicating the formation of rather compact globular-like structures during the phase transition, also called mesoglobules [2,14], which are colloidally stable

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in solution. ^{1}H NMR relaxation measurements revealed that a certain portion of water molecules is bound at elevated temperatures in PVME mesoglobules in semidilute and concentrated solutions ($c = 2$–10 wt%) [16–18]. With time this bound water is slowly released from globular-like structures, the releasing process takes ~24 h. This finding implies that most existing studies of the phase separation in semidilute or concentrated PVME aqueous solutions deal in fact with metastable state where globular-like structures still contain bound water. On the contrary, dehydration of PVME chains is rapid in dilute solutions [18]. A slow exchange and relatively weak hydrogen bonding were found from the position of the separate NMR signal for bound HDO in highly concentrated PVME/D_2O solutions (polymer concentrations $c = 20$–60 wt%) [19]. At the same time, the molar ratio [PVME monomeric unit]/[bound D_2O] ≅ 2.7 is constant in the range of concentrations $c = 20$–60 wt%, i.e., the polymer concentration in the polymer-rich phase (mesoglobules) is 89 wt%, in accord with the recently published phase diagram [10]; a direct connection of the fraction of bound water with the conformational structure of PVME has been suggested [19].

In the present work, we applied ^{1}H and ^{13}C NMR spectroscopy to investigate the temperature-induced phase transition of PVME in D_2O/ethanol (EtOH) mixtures. For this purpose, PVME solutions were prepared in a broad range of polymer concentrations ($c = 0.1$–20 wt%) and volume compositions of D_2O/EtOH mixtures (99/1–80/20).

2. Experimental

2.1. Samples

PVME (purchased from Aldrich, supplied as 50 wt% solution in water; molecular weight determined by GPC in THF: $M_w = 60\,500$, M_w/M_n ≅ 3; tacticity by ^{1}H NMR: 59% of isotactic diads [15]) was used after drying to prepare PVME/D_2O (99.9% of deuterium)/EtOH solutions with polymer concentrations $c = 0.1$, 1, 6, 10, 20 and 30 wt% and volume fractions of ethanol in D_2O/EtOH mixtures 1, 5, 10 and 20 vol% (no apparent milk-white opalescence was observed at elevated temperatures for solutions with EtOH content higher than 20 vol% in water/EtOH mixture). All samples of PVME/D_2O/EtOH solutions in 5-mm NMR tubes were degassed and sealed under argon.

2.2. NMR measurements

High-resolution ^{1}H NMR spectra were recorded with a Bruker Avance 500 spectrometer operating at 500.1 MHz. Typical measurements conditions were as follows: π/2 pulse width 14.25 μs, relaxation delay 10 s, spectral width 5 kHz, acquisition time 1.64 s, 16 scans. ^{13}C spectra were accumulated usually with 100 scans, with relaxation delay 80 s and spectral width 12.5 kHz under full proton decoupling. A ^{13}C pulse duration of 7 μs was applied for the single π/2 pulse used for each scan. The integrated intensities were determined with the spectrometer integration software with an accuracy of ±1% and ±3% for ^{1}H and ^{13}C NMR spectra, respectively. The temperature was maintained constant within ±0.2 °C using BVT 3000 temperature unit.

The ^{1}H spin–spin relaxation times T_2 on the merged EtOH/HDO OH signal and on EtOH CH_2 and CH_3 signals were measured using the CPMG [20] pulse sequence $90°_x - (t_d - 180°_y - t_d)_n$-acquisition, with $t_d = 5$ ms; the total time of T_2 relaxation was an array of 12 values. In T_2 measurements at temperatures above the LCST we also used $t_d = 0.5$ ms and the total time of T_2 relaxation was an array of 32 values. Every experiment was done with 8 or 16 scans and relaxation delay between scans was 80 s. For selected sample the dependence of ^{1}H T_2 on the interval t_d in the range of $t_d = 0.2$–5 ms was also measured. The ^{13}C T_2 relaxation times of ethanol groups were measured using CPMG sequence with ^{1}H π pulse added to remove cross-correlation between chemical shift anisotropy and dipole–dipole interactions [21]. The interval $t_d = 5$ or 0.5 ms and relaxation delay 80 s were used in ^{13}C T_2 measurements (the relaxation delay was adequately long to allow a complete recovery of ^{13}C magnetization). The ^{13}C spin–lattice relaxation times T_1 of ethanol were measured using an inversion recovery pulse sequence $180° - \tau_D - 90°$ with 8 scans separated by a relaxation delay of 80 s. All obtained ^{1}H and ^{13}C T_2 and T_1 relaxation curves had the monoexponential character and the fitting process always enabled us to determine the single value of the respective relaxation time.

3. Results and discussion

3.1. Temperature dependences of ^{1}H NMR spectra – phase-separated fraction

All studied solutions were cloudy at temperatures above the LCST and no precipitation (sedimentation) was observed even after long time (~days). This indicates that rather large particles (mesoglobules) are formed in studied solutions with a size around hundred nanometers [14,22]; this is also in accord with results of our preliminary small-angle neutron scattering measurements. An example of high-resolution ^{1}H NMR spectra of PVME/D_2O/EtOH solution ($c = 6$ wt%, EtOH fraction in D_2O/EtOH mixture = 5 vol%) measured at two slightly different temperatures (310 K and 313 K) is shown in Fig. 1. The assignment of resonances to various types of protons of PVME and ethanol is shown directly in a spectrum measured at 310 K; the strong line on the left is a merged signal of ethanol/HDO OH protons. The splitting of CH, CH_3 and CH_2 resonances of PVME is due to tacticity [15]. The most important effect observed in the spectrum measured at a higher temperature (313 K) is a marked decrease in the integrated intensity of all PVME lines. This is due to the fact that at temperatures above the LCST the mobility of most PVME units is reduced to such an extent that the corresponding lines become too broad to be detected in high-resolution spectra. A narrow component with unrestricted mobility (with much smaller integrated intensity) that is directly detected in high-resolution NMR spectra corresponds to PVME units in the dilute (polymer-pure) phase; from our former results it follows that it corresponds mainly

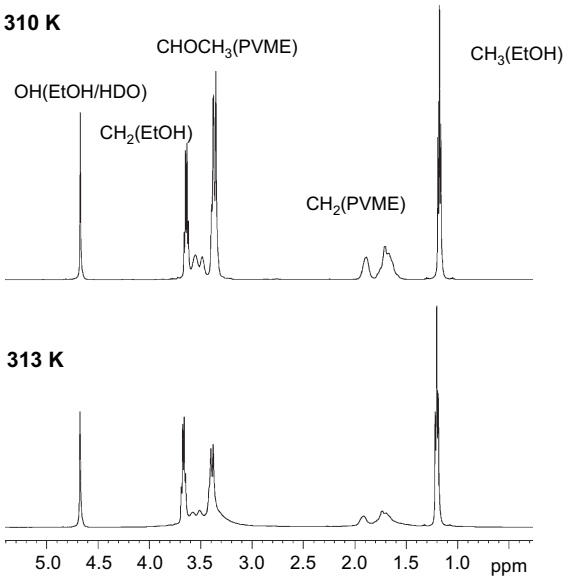

Fig. 1. ^{1}H NMR spectra of PVME/D_2O/EtOH solution ($c = 6$ wt%, 5 vol% of EtOH in D_2O/EtOH mixture) measured at 310 K and 313 K.

to low-molecular weight fraction of PVME where the chains are too short to exhibit a cooperative coil–globule transition [18]. The depicted changes of the NMR spectra have been previously observed for D_2O solutions of PVME [15–19,23] and acrylamide-based polymers ([23] and references therein). They confirm that reaching LCST results in marked line broadening of a major part of PVME units, evidently due to the phase separation and formation of rather compact mesoglobules. No reduction of integrated intensities at temperatures above LCST was observed for EtOH signals. The integrated intensities of EtOH monotonously decrease with absolute temperature, as expected, so confirming that all EtOH molecules are directly detected in ^{1}H NMR spectra in the whole range of temperatures.

For further analysis, in accord with our previous publications [15–19,23], we shall define the phase-separated PVME fraction as fraction of PVME units in concentrated, polymer-rich phase; the mobility of these PVME units is significantly lower in comparison with that at temperatures below the LCST transition. We have determined the values of fraction p of phase-separated PVME units (units in mesoglobules) from the following relation

$$p = 1 - (I/I_0) \quad (1)$$

where I is the integrated intensity of the given polymer line in a partly phase-separated system and I_0 is the integrated intensity of this line if no phase separation occurs [15,16,23]. For I_0 we took values based on integrated intensities below the phase transition, using the fact that integrated intensities should decrease with absolute temperature as $1/T$. The integrated intensities were measured $\sim$20 min after the corresponding temperature was reached (by heating).

In the analysis of the phase transition behaviour, first, the influence of the polymer concentration c on the transition region was investigated. Temperature dependences of the fraction p of PVME, as obtained from integrated intensities of $CHOCH_3$ protons of PVME, are shown for EtOH content in D_2O/EtOH mixture, 10 vol%, and various polymer concentrations in Fig. 2. From this figure it follows that the transition in more concentrated solutions ($c = 6$ and 20 wt%) sets in at a temperature lower by $\approx$5 K in comparison with the dilute solution ($c = 0.1$ wt%). This shift of the transition is probably a consequence of the preferred polymer–polymer contacts at higher concentrations, allowing hydrophobic interactions to predominate at lower temperatures. Lower polymer concentrations show more gradual character of the transition and at the same time, the fraction at temperatures above the transition is only $p \approx 0.5$; thus approximately 50% of polymer units have unrestricted mobility even at temperatures above the transition.

The effect of ethanol content in D_2O/EtOH mixture on the phase transition in PVME solutions is demonstrated in Fig. 3 where temperature dependences of the fraction p corresponding to solutions with polymer concentration $c = 1$ wt% and various EtOH content are plotted. To complete these plots, the temperature dependence of the fraction p for PVME/D_2O solution (without ethanol) [15] is also included in Fig. 3. This figure shows clearly that the phase transition shape depends on the ethanol content. The transition region is shifted towards higher temperatures with the increasing amount of EtOH in D_2O/EtOH mixture, and at the same time the transition extent (fraction p) markedly decreases. For example, the transition of the solution with the highest EtOH content (20 vol%) sets in at temperature higher by 15 K in comparison with that of PVME in D_2O. Simultaneously, for this sample the width of the transition region is approximately 30 K and the value of the phase-separated fraction p above the transition is roughly 0.5. The shift of the transition temperature with increasing ethanol content in PVME/D_2O/EtOH solutions is in accord with previous measurements of the cloud points in

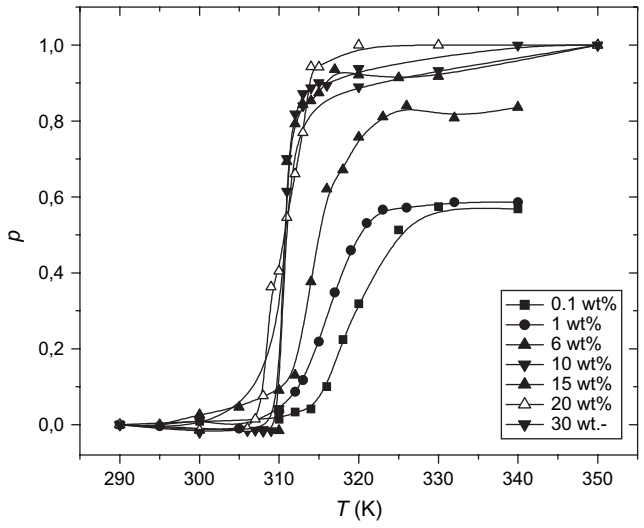

Fig. 2. Temperature dependences of phase-separated fraction p as determined from integrated intensities of $CHOCH_3$ band in ^{1}H NMR spectra of PVME/D_2O/EtOH solutions with 10 vol% of EtOH in D_2O/EtOH mixture and various polymer concentrations.

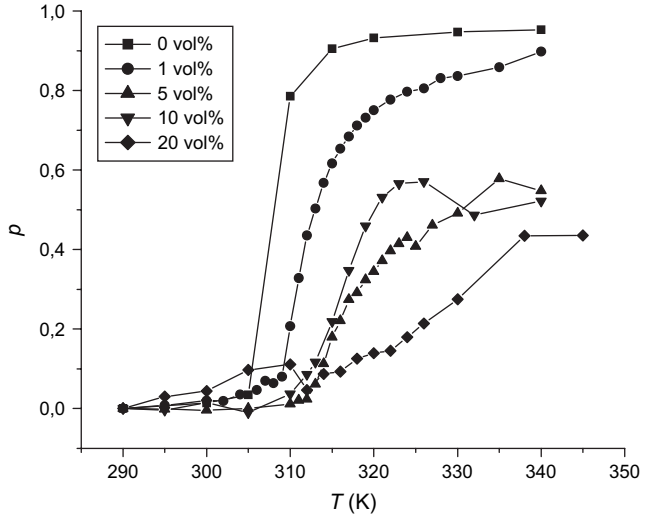

Fig. 3. Temperature dependences of phase-separated fraction p as determined from integrated intensities of $CHOCH_3$ band in ^1H NMR spectra of PVME/D_2O/EtOH solutions with $c = 1$ wt% and various contents of EtOH in D_2O/EtOH mixtures.

PVME/water/alcohol solutions [3,11]. The assumption of the positive role of ethanol (which is a better solvent) in stabilization of polymer–solvent interactions is supported by the found decrease of the fraction p above the transition with increasing ethanol content (Fig. 3). The ethanol molecules probably prevent hydrophobic polymer–polymer interactions and therefore the transition is shifted towards higher temperatures and becomes less "perfect" (the transition is broader and phase-separated fraction p above the transition is smaller).

3.2. ^1H NMR spectra and ^1H spin–spin relaxation times T_2 of EtOH in PVME/D_2O/EtOH solutions

In ^1H NMR spectra of the studied PVME solutions in D_2O/EtOH mixtures (containing 1–20 vol%, i.e., 0.3–7.2 mol% of EtOH) there is a merged single line of EtOH and HDO OH protons (cf. Fig. 1). In this respect the situation is the same as in water/EtOH mixtures of the same composition [24]. Taking into account the fact that the used D_2O contains 99.9% of deuterium then it follows that while for the mixture EtOH/D_2O containing 1 vol% of EtOH, the molar ratio EtOH/HDO = 3/2 and the contribution of both species to the merged OH peak is roughly comparable, for mixtures EtOH/D_2O containing 5, 10 and 20 vol% of EtOH the respective molar ratios are EtOH/HDO = 8/1, 24/1 and 36/1. This means that especially for PVME solutions in EtOH/D_2O mixtures containing 10 or 20 vol% of EtOH the OH protons of EtOH predominantly contribute to the merged EtOH/HDO peak and contribution from HDO to this peak can be neglected. This is confirmed by the integrated intensities of EtOH signals. Thus, e.g., for PVME solution ($c = 20$ wt%) in D_2O/EtOH mixture containing 20 vol% of EtOH, the ratio of integrated intensities of OH, CH_2 and CH_3 signals of EtOH related to 1 proton is 1:0.95:0.94. We have found that slight deviation from the expected ratio 1:0.97:0.97 is due to the presence of small amount (1 mol%) of H_2O in the used EtOH. Therefore for D_2O/EtOH mixtures containing 20 vol% of EtOH, the contribution of EtOH, H_2O and D_2O (HDO) to the merged OH peak is in the ratio EtOH/H_2O/D_2O = 32/0.6/1; we took here into account the fact that while EtOH and D_2O (i.e., HDO) contribute to the merged OH signal by 1 proton, H_2O contributes to this signal by 2 protons. For D_2O/EtOH mixtures containing 10 vol% of EtOH, we have found from integrated intensities of EtOH signals that this ratio is EtOH/H_2O/D_2O = 22/0.4/1.

Measurements of ^1H spin–spin relaxation times T_2 of EtOH molecules in PVME/D_2O/EtOH systems should provide us information about their mobility, and consequently about the contacts between EtOH molecules and polymer chains. A comparison of T_2 relaxation behaviour of EtOH with that previously found for HDO in PVME/D_2O solutions [17–19,23] should enable us to compare the behaviour of both the types of small molecules during temperature-induced phase transition. To observe the effect of the polymer concentration on dynamic behaviour of EtOH we have chosen three samples with EtOH content in EtOH/D_2O mixture being 10 vol%, and polymer concentrations $c = 0.1$, 6 and 20 wt%. Table 1 shows the values of ^1H spin–spin relaxation times T_2 of EtOH as obtained for these samples at temperatures 310 K (below the transition) and 325 K (above the transition).

As it follows from Table 1, for dilute solution ($c = 0.1$ wt%) T_2 values of all proton types of EtOH do not change as the solution undergoes the phase transition. This result is in accord with the behaviour of water (HDO) as it was previously found from T_2 measurements in PVME/D_2O solution of the same polymer concentration [17–19]. The behaviour found for T_2 values of EtOH in the solution with $c = 6$ wt% is quite different (cf. Table 1). At elevated temperature above the transition (325 K) the relaxation times T_2 of all EtOH groups are 1–2 orders of magnitude shorter than those at 310 K. This behaviour that again for HDO has been previously observed for PVME/D_2O solutions with $c = 2$–10 wt% [17–19] shows that in PVME/D_2O/EtOH solutions with $c = 6$ wt% at temperature above the transition there is a portion of EtOH molecules bound in mesoglobules. The exponential character of T_2 relaxation curves and the fact that there is always a single line of the respective proton group in the NMR spectrum indicate for EtOH molecules a fast exchange between bound and free sites regarding T_2 values (the residence time of the bound

Table 1
^1H spin–spin relaxation times T_2 of EtOH for PVME solutions in D_2O/EtOH mixtures containing 10 vol% of EtOH

c (wt%)	T_2 (s)[a]					
	OH[b]		CH_2		CH_3	
	310 K	325 K	310 K	325 K	310 K	325 K
0.1	0.9	0.9	6.7	6.7	5.6	5.5
6	2.5	0.16	4.2	0.06	4.3	0.12
20	0.8	2.2[c], 0.02[d]	3.2	6.4	5.0	7.8

[a] Estimated experimental error is ±5%.
[b] Contribution of HDO to merged EtOH/HDO OH peak is negligible.
[c] "Free" EtOH, peak at 4.65 ppm (cf. Fig. 5).
[d] "Bound" EtOH, peak at 4.4 ppm (cf. Fig. 5).

states is ≤0.01 s, i.e., at least 1 order of magnitude shorter than the observed T_2, cf. Table 1 and Ref. [18]).

There are two most important possible sources for the short T_2 values of EtOH observed for PVME solution ($c = 6$ wt%) in D_2O/EtOH mixture at temperature above the LCST transition: (i) a lower, spatially restricted mobility, similar to the phase-separated PVME and (ii) chemical exchange. The dependence of measured T_2 values on the time interval t_d in CPMG pulse sequence is often used for the characterization of microsecond–millisecond chemical exchange [25,26]. Fig. 4 shows such dependence as obtained for OH protons of EtOH, for PVME solution ($c = 6$ wt%) in D_2O/EtOH mixture containing 10 vol% of EtOH. From this figure it follows that contribution of chemical exchange to the spin–spin relaxation rate $(T_2)^{-1}$ is important. Solid curve in Fig. 4 shows the best fit as obtained using the following equation [26]

$$(T_2)^{-1} = \left(p_A p_B \Delta^2 \omega^2 / k_{ex}\right)\left\{1 - [\tanh(k_{ex} t_d)/k_{ex} t_d]\right\} + (R_2)^0 \quad (2)$$

with $k_{ex} = 2500\ s^{-1}$ and $(R_2)^0 = 0.296\ s^{-1}$. Here k_{ex} is the rate constant for exchange process, $(R_2)^0$ is the spin–spin relaxation rate in the absence of the exchange assumed to be the same in states A and B, p_A and p_B are populations of the states, Δ is the chemical shift difference between the states and ω is the resonance frequency. In Eq. (2) we assumed that the exchange is fast and that the exchange time $\tau_{ex} = 1/k_{ex}$ is much longer than the motional correlation times τ_c causing other relaxation mechanisms. To verify the second assumption we calculated the correlation time for the bound EtOH assuming that it will be the same as the correlation time for the motion of globules of phase-separated PVME as a whole. For previously studied phase-separated poly(*N*,*N*-diethylacrylamide)/D_2O solutions a good agreement was obtained for the size of globular-like particles obtained from the small-angle neutron scattering [27] and from the correlation time obtained from NMR experiments [28,29], and then using a relation [30]

$$\tau_c = 4\pi\eta a^3 / 3kT \quad (3)$$

where η is the solvent viscosity and a the radius of globular-like particles. In this case $a = 18$ and 15 nm, as determined from the small-angle neutron scattering and NMR, respectively; $\tau_c = 2$ μs. Our preliminary small-angle neutron scattering measurements on PVME/D_2O solutions indicate that PVME globular-like structures are somewhat larger with $a \sim 50$ nm. From Eq. (3) it follows that $\tau_c \approx 70$ μs; this value is still almost 1 order of magnitude shorter in comparison with the exchange time $\tau_{ex} = 0.4$ ms as determined from the dependence in Fig. 4.

While for PVME/D_2O/EtOH solutions with $c \leq 10$ wt% there was only one merged signal of the EtOH/HDO OH protons, for higher concentrations $c \geq 15$ wt% and temperatures above the LCST transition a new signal of EtOH/HDO OH protons was detected with ~0.25 ppm smaller chemical shift in comparison with the main EtOH/HDO OH peak. In Fig. 5 where the ^{1}H NMR spectrum of the PVME/D_2O/EtOH solution with $c = 20$ wt% and 20 vol% of EtOH in D_2O/EtOH mixture is shown, a new EtOH OH signal (we neglect the contribution of HDO to this signal) is marked by asterisk. This new EtOH OH signal appears only at temperatures above the phase transition and evidently corresponds to EtOH molecules bound in globular-like structures. For EtOH in highly concentrated PVME/D_2O/EtOH solutions therefore there is a slow exchange between bound and free sites, similar to what we have found for bound and free water in highly concentrated PVME/D_2O solutions [19]. Here the term "slow exchange" includes also the situation that there is no exchange at

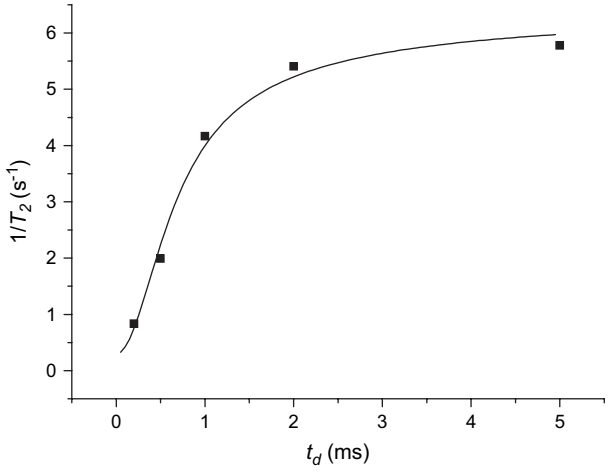

Fig. 4. Dependence of spin–spin relaxation rate $(T_2)^{-1}$ on the interval t_d in CPMG sequence as obtained for EtOH OH protons in PVME/D_2O/EtOH solution ($c = 6$ wt%, 10 vol% of EtOH in D_2O/EtOH mixture) kept at 325 K. Solid curve is a fit according to Eq. (2) with $k_{ex} = 2500\ s^{-1}$ and $(R_2)^0 = 0.296\ s^{-1}$.

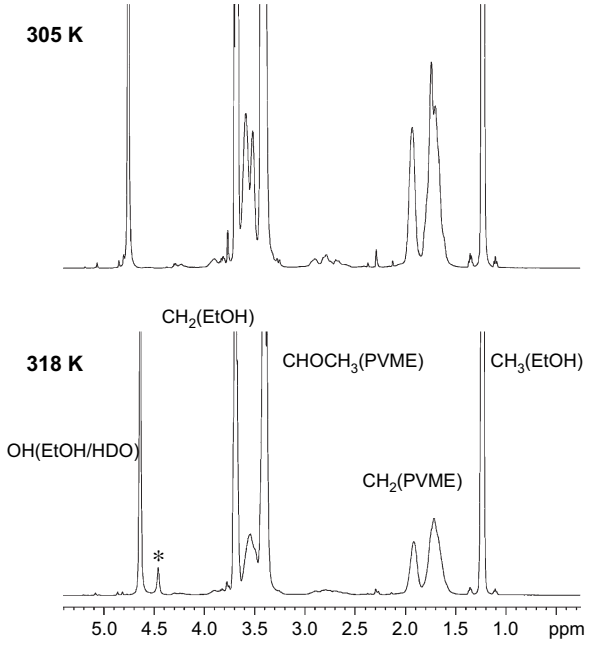

Fig. 5. ^{1}H NMR spectra of PVME/D_2O/EtOH solution ($c = 20$ wt%, 20 vol% of EtOH in D_2O/EtOH mixture) measured at 305 K and 318 K. OH line of the bound EtOH is marked by asterisk.

all (the residence time is infinity). From the condition [31] $1/\tau \ll \Delta\nu$, where τ is the residence time and $\Delta\nu$ is the difference of the respective chemical shifts in hertz, it follows that for the residence time of bound EtOH molecules it holds $\tau \gg 8$ ms. From integrated intensities of lines corresponding to OH protons in ''free'' and bound EtOH/HDO (cf. Fig. 5), the relative amount of EtOH/HDO molecules bound in PVME mesoglobules can be determined (Table 2). From Table 2 it follows that the relative amount of bound EtOH/HDO as a function of EtOH fraction in D_2O/EtOH mixture is virtually constant. Table 2 also contains chemical shifts of the OH line of bound EtOH/HDO. It is well known that hydrogen bonding leads to larger chemical shifts in ^{1}H NMR spectra [31,32]. The fact that the chemical shift of the bound EtOH/HDO OH protons is always smaller in comparison with the main OH signal of ''free'' EtOH/HDO indicates that for the EtOH/HDO (with predominant contribution of EtOH for D_2O/EtOH mixtures with EtOH fractions $\geq$5 vol%) bound in globular-like structures the hydrogen bonding is somewhat weaker in comparison with that existing in neat D_2O/EtOH mixtures. Increasing amount of EtOH in D_2O/EtOH mixture results in higher values of the chemical shift of OH protons of bound EtOH/HDO (cf. Table 2) as a consequence of the strengthening of the hydrogen-bonding structure. The same trend was also found in neat water/EtOH mixtures with EtOH fraction $\leq$20 vol% where the presence of small quantity of EtOH promotes the formation of new water–ethanol hydrogen bonds and/or incremental water–water association [24].

The assignment of two separated OH lines in concentrated PVME/D_2O/EtOH solutions to ''free'' and bound EtOH (or EtOH/HDO for solutions with small EtOH fraction in D_2O/EtOH mixture) is corroborated by measurements of spin–spin relaxation times T_2 on both signals (cf. Table 1, $c =$ 20 wt%). Significantly longer T_2 values as obtained at 325 K for the signal of the ''free'' EtOH in comparison with the values measured at 310 K are evidently due to the fact that at higher temperature the respective EtOH molecules do not interact with PVME, and therefore they are really free while at temperature 310 K a significant part of EtOH molecules interacts with polymer forming, e.g., hydrogen bonds and their motions are consequently somewhat restricted; a contribution from the chemical exchange can be also important in the latter case. On the other hand, due to spatially restricted mobility, T_2 value of OH protons of EtOH bound in globular-like structures is 2 orders of magnitude shorter in comparison with free EtOH (cf. Table 1). Also for CH_2 and CH_3 protons of EtOH, T_2 values at 325 K were significantly longer in comparison with those at 310 K, indicating that also for these proton types the separate lines of ''free'' and bound EtOH can exist for concentrated PVME/D_2O/EtOH solutions (cf. Table 1). However, for CH_2 and CH_3 EtOH groups the lines of the bound EtOH were not detected; they might be overlapped by signals of $CHOCH_3$ and CH_2 protons of PVME segments in the dilute phase (cf. Fig. 5).

Table 2
Fraction of bound EtOH/HDO and chemical shift of bound EtOH/HDO OH protons in PVME/D_2O/EtOH solutions ($c = 20$ wt%) at 315 K

Content of EtOH in D_2O/EtOH mixture (vol%)	Fraction of bound EtOH/HDO[a] (%)	Chemical shift of OH protons in bound EtOH/HDO[a] (ppm)
1	10	4.26
5	8	4.30
10	8	4.41
20	8	4.56

[a] For contents of EtOH in D_2O/EtOH mixture $\geq$ 5 vol% a contribution of HDO to the merged EtOH/HDO OH signal is negligible.

We were interested in knowing whether the amount of EtOH bound in PVME mesoglobules formed in concentrated aqueous solutions is changing with time or not. Sample was kept at temperature above the phase transition (325 K in our case) and time dependence of the spin–spin relaxation time T_2 of EtOH OH protons was measured for this purpose. As it follows from Fig. 6 where such time dependence is shown for PVME/D_2O/EtOH solution with $c = 6$ wt% and EtOH fraction in D_2O/EtOH mixture 10 vol%, T_2 values do not change during 17 h. However, after 40 h the T_2 significantly increased and then remains constant; the respective T_2 is even larger than the value observed at temperature below the transition (cf. Table 1). This result shows that EtOH molecules originally bound in globular-like structures are with time very slowly released from these structures. The same process was previously found for bound water (HDO) in semidilute or concentrated PVME/D_2O solutions but release of EtOH as demonstrated in Fig. 6 is slower as compared with the dehydration process in PVME/D_2O solution of the same polymer concentration [18]. We also followed the relative intensity of the separate OH resonance of bound EtOH in PVME/D_2O/EtOH solution ($c = 20$ wt%, EtOH fraction 10 vol%) and we have found that after 4 h at 325 K the relative intensity of this line decreased to one half. From the same experiment at 315 K it follows that the relative intensity of the separate OH resonance of bound EtOH decreased after 4 h to one fifth, i.e., the fraction of bound EtOH dropped from original 8% to 1.6%. These results show that at these conditions the releasing process is much faster.

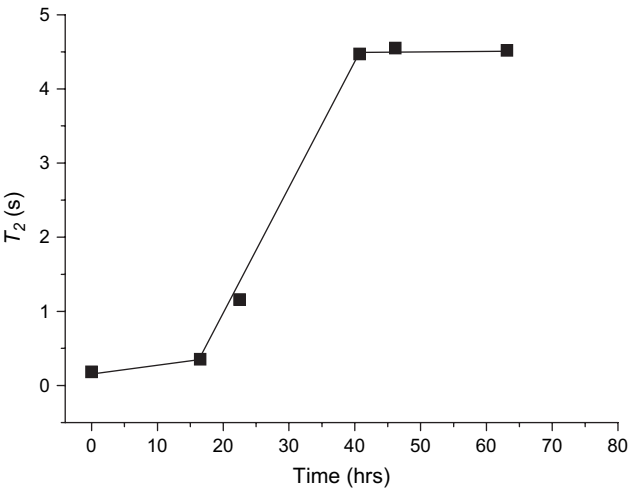

Fig. 6. Time dependence of spin–spin relaxation time T_2 of EtOH OH protons in PVME/D_2O/EtOH solution ($c = 6$ wt%, 10 vol% of EtOH in D_2O/EtOH mixture) kept at 325 K.

3.3. ^{13}C NMR spectra and ^{13}C spin–spin (T_2) and spin–lattice (T_1) relaxation times of EtOH in PVME/D_2O/EtOH solutions

^{13}C NMR spectra of PVME/D_2O/EtOH solution with polymer concentration $c = 6$ wt% and EtOH content in D_2O/EtOH mixture 10 vol%, measured at 310 K and 320 K, i.e., below and above the LCST transitions, respectively, are shown in Fig. 7. The assignment of observed resonances to PVME and EtOH carbon types is marked in the spectrum measured at 310 K. The pronounced reduction of intensities of polymer bands can be observed at 320 K due to the formation of rather compact mesoglobules, similarly as in ^1H NMR spectra (cf. Fig. 1). The intensities of EtOH signals are virtually unaffected by the transition; therefore we applied ^{13}C NMR relaxation measurements on these signals to study dynamics of EtOH molecules during the transition process.

Tables 3 and 4 summarize ^{13}C spin–spin relaxation times T_2 and spin–lattice relaxation times T_1 as obtained for CH_2 and CH_3 ethanol carbons in PVME/D_2O/EtOH solutions with $c = 0.1$ and 6 wt% and ethanol fraction in D_2O/EtOH mixture 10 vol%. In all cases the relaxation curves were single exponential. One can see that even at temperature below the phase transition (310 K), T_2 values are somewhat shorter than the respective T_1 values; we assume that this rather unusual behaviour for small molecules is due to small spatial anisotropy in their motion as consequence of their interactions (by hydrogen bonding) with PVME segments (cf. Ref. [19] and further text below). In accord with results of ^1H T_2 measurements shown in previous paragraph, completely different behaviour of EtOH can be seen for these two polymer concentrations from

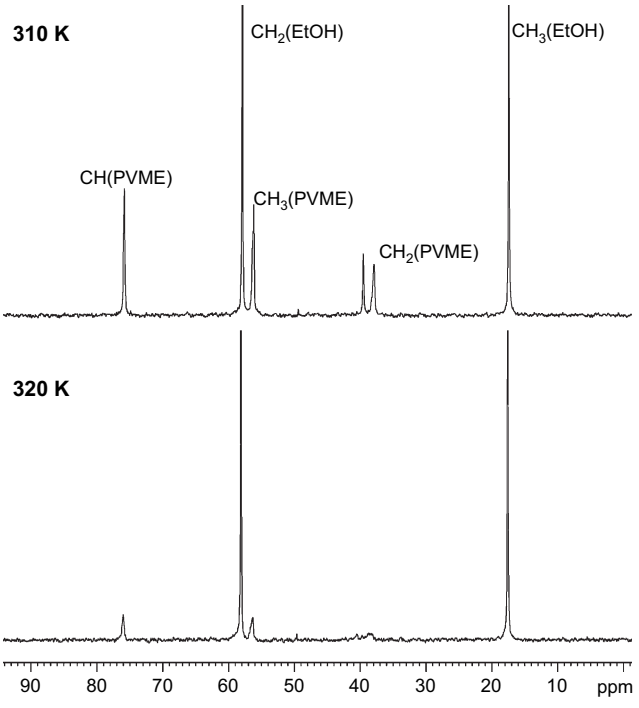

Fig. 7. ^{13}C NMR spectra of PVME/D_2O/EtOH solution ($c = 6$ wt%, 10 vol% of EtOH in D_2O/EtOH mixture) measured at 310 K and 320 K.

Table 3
^{13}C spin–spin relaxation times T_2 of EtOH for PVME solutions in D_2O/EtOH mixtures containing 10 vol% of EtOH

c (wt%)	T_2 (s)			
	CH_2		CH_3	
	310 K	325 K	310 K	325 K
0.1	4.3	6.6	9.0	10.2
6	8.5	3.0	7.0	1.2

Table 4
^{13}C spin–lattice relaxation times T_1 of EtOH for PVME solutions in D_2O/EtOH mixtures containing 10 vol% of EtOH

c (wt%)	T_1 (s)			
	CH_2		CH_3	
	310 K	325 K	310 K	325 K
0.1	13.3	17.0	9.0	12.4
6	12.6	15.4	9.0	11.8

Table 3. While for $c = 0.1$ wt%, ethanol T_2 values at 325 K are somewhat longer than those at 310 K, as expected for higher temperature, for $c = 6$ wt% a marked reduction of T_2 values was found for the phase-separated system. This confirms that in 6 wt% solution a certain portion of EtOH molecules is bound in globular-like structures with fast exchange between bound and free sites; this supports the sponge-like character of the globular-like structures as suggested in the recent study [18]. On the contrary, from Table 4 it follows that T_1 values are insensitive to the phase transition, even for $c = 6$ wt% the values at 325 K are always somewhat longer than those at 310 K. Situation here is similar as it was previously found for HDO molecules in PVME/D_2O solution of the same polymer concentration where also a different sensitivity of T_1 and T_2 relaxation times to the LCST transition was established and interpreted in such a way that while the rates of the motion of bound and free HDO molecules are virtually the same, the motion of bound HDO is spatially restricted and anisotropic. The internuclear vector cannot reach all orientations and the resulting existence of near-static dipolar interactions predominantly affects T_2 relaxation time; nevertheless, spatial restriction of the motion of bound HDO is rather small [19]. Such interpretation probably holds also for the bound EtOH. Moreover, as discussed in Section 3.2, a contribution from the chemical exchange to the total spin–spin relaxation rate can be also important.

^{13}C NMR spectra of the PVME/D_2O/EtOH solution with the highest polymer concentration ($c = 20$ wt%) and EtOH fraction in the mixed solvent 5 vol%, again measured at 310 K and 325 K, are shown in Fig. 8. The most significant new feature in the spectrum measured at temperature above the phase transition (325 K) is the existence of two additional peaks that appear in the vicinity of the original EtOH resonances. In accord with ^1H NMR spectra discussed above (cf. Fig. 5) these new signals obviously belong to CH_2 and CH_3 carbons of EtOH molecules bound (probably forming hydrogen bonds with oxygen atoms of PVME units) in

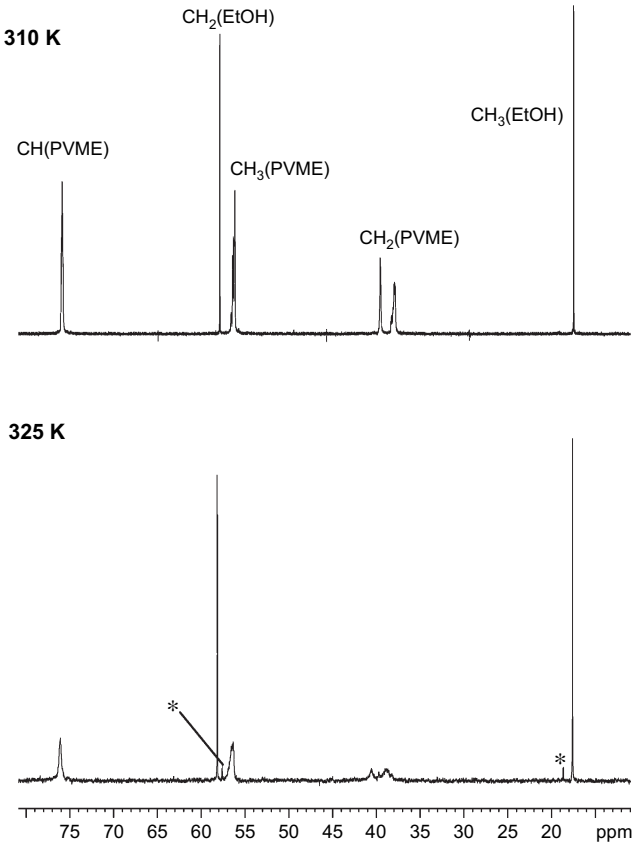

Fig. 8. ^{13}C NMR spectra of PVME/D_2O/EtOH solution ($c = 20$ wt%, 5 vol% of EtOH in D_2O/EtOH mixture) measured at 310 K and 325 K. Lines of the bound EtOH CH_2 and CH_3 carbons are marked by asterisks.

PVME globular-like structures. Contrary to the system with polymer concentration $c = 6$ wt% where the exchange between bound and free ethanol molecules was fast on the NMR time-scale, for 20 wt% solution the existence of separate resonances for bound and free EtOH molecules shows a slow exchange between both states; the lifetime of the bound EtOH molecules has to be much larger than 10 ms (the difference between chemical shifts of bound and free ethanol molecules is approx. 100 Hz on the frequency scale), again in accord with ^1H NMR results. From integrated intensities of the lines of bound and free EtOH in ^{13}C NMR spectra we obtained that the ratio of ethanol molecules in the bound and free states is 0.06:0.94, i.e., the relative amount of bound EtOH as obtained from ^{13}C NMR spectra is in rather good agreement with the respective value obtained from ^1H NMR spectra (cf. Table 2). It is interesting that while CH_3 line of bound EtOH is shifted 1.2 ppm downfield from the CH_3 line of the free EtOH, the CH_2 line of bound EtOH is shifted 0.8 ppm upfield from the respective line of free EtOH, i.e., CH_2 carbons in bound EtOH are more shielded in comparison with free EtOH. In accord with results obtained from ^1H NMR spectra, higher shielding of CH_2 carbons in bound EtOH can be probably attributed to a weakening of the hydrogen bonding in comparison with free EtOH, similarly as described for carboxylic acid derivatives (anhydrides, esters, amides, etc.) [33].

For PVME/D_2O/EtOH solution ($c = 20$ wt%) the study of dynamics of the ethanol molecules in bound and free states was performed using the measurements of ^{13}C spin–spin and spin–lattice relaxation times T_2 and T_1, respectively. The values of the relaxation times as obtained for ethanol carbons in PVME/D_2O/EtOH solutions with $c = 20$ wt% and various ethanol fraction in D_2O/EtOH mixture are shown in Tables 5 and 6. From Table 5 it follows that at temperature above the transition, EtOH bound in mesoglobules is characterized by the T_2 values significantly shorter than those corresponding to free ethanol, though the observed differences are smaller than those obtained from ^1H T_2 measurements (cf. Table 1, $c = 20$ wt%). Simultaneously, the values obtained at temperature below the transition are located between the T_2 relaxation times of the free and bound ethanol as found for the systems above the transition. This is in accord with an idea of solvent (in our case ethanol) molecules which are initially (at temperatures below the transition) in contact with polymer chains, probably through hydrogen bonding, and their motion is consequently slightly restricted. However, as the solution passes the LCST transition, ethanol occurs either in the free state, without any restriction in its mobility, or in the bound state, where it is bound in relatively immobilized PVME globular-like structures. In contrast to solution with $c = 6$ wt%, Table 6 shows that for $c = 20$ wt% also T_1 values are for bound EtOH significantly shorter in comparison with free EtOH indicating that in this case the rates of the motion of bound and free EtOH are somewhat different. At the same time, T_1 values at temperature below the transition are in-between T_1 values of free and bound EtOH in the phase-separated system. Taking into account the fact that the spatial anisotropy of the motion of bound EtOH is similar as previously found for the bound HDO, i.e., rather small in both cases [19], then assuming a model of rigid sphere isotropic rotation and the relaxation due predominantly to dipolar interactions of carbons with directly attached protons, we calculated the values of the correlation times τ_c using the following expressions [30]

$$(T_1)^{-1} = (n/10)(\mu_0/4\pi)^2\gamma_C^2\gamma_H^2\hbar^2 r^{-6} \times [J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad (4)$$

$$J(\omega) = \tau_c/\left(1 + \omega^2\tau_c^2\right) \quad (5)$$

Table 5
^{13}C spin–spin relaxation times T_2 of EtOH in PVME/D_2O/EtOH solutions with $c = 20$ wt%

EtOH content in D_2O/EtOH mixture (vol%)	T_2 (s)			
	CH_2		CH_3	
	310 K	325 K	310 K	325 K
5	8.7	12.9[a]	6.3	8.8[a]
		2.4[b]		3.5[b]
10	7.1	11.6[a]	5.7	8.1[a]
		1.1[b]		3.0[b]
20	7.2	11.7[a]	6.1	8.4[a]
		5.1[b]		4.7[b]

[a] From the signal of free EtOH, cf. text.
[b] From the signal of bound EtOH, cf. text.

Table 6
^{13}C spin–lattice relaxation times T_1 and correlation times τ_c (values in brackets) of EtOH in PVME/D_2O/EtOH solutions with $c = 20$ wt%

EtOH content in D_2O/EtOH mixture (vol%)	T_1 (s) [τ_c (ps)]					
	CH_2			CH_3		
	310 K	325 K		310 K	325 K	
		Free[a]	Bound[b]		Free[a]	Bound[b]
5	11.9 [2.0]	17.6 [1.4]	3.4 [7.2]	8.5 [1.9]	12.4 [1.3]	5.8 [2.8]
10	9.8 [2.5]	17.1 [1.4]	8.0 [3.0]	7.5 [2.2]	12.1 [1.3]	8.0 [2.1]
20	9.6 [2.5]	17.3 [1.4]	6.7 [3.7]	7.6 [2.1]	12.1 [1.3]	6.9 [2.4]

[a] Determined from the respective signal of "free" EtOH.
[b] Determined from the respective signal of "bound" EtOH.

where n is the number of attached protons, ω_H and ω_C are resonance frequencies of protons and carbons, respectively, and other constants have their usual meaning. The obtained values of the correlation times are given in Table 6 in brackets. From Table 6 it follows that τ_c values are for the bound EtOH on average 2–3 times longer in comparison with free EtOH, indicating that the motion of EtOH bound in mesoglobules formed in 20 wt% PVME/D_2O/EtOH solutions is slowed-down. For PVME/D_2O/EtOH solutions at 325 K the τ_c values of the free EtOH are virtually the same as we have found at the same temperature for EtOH in neat D_2O/EtOH mixtures (without PVME) of the same composition ($\tau_c = 1.3$ ps both for CH_2 and CH_3 carbons), so supporting the idea that at 325 K molecules of free EtOH do not interact with PVME chains. Similar to T_1 and T_2 values, correlation times of EtOH at 310 K are in-between the τ_c values as obtained for free and bound EtOH at 325 K.

4. Conclusions

In this work the temperature-induced phase separation in PVME/D_2O/EtOH solutions was investigated by NMR spectroscopy. The measurement of the temperature dependences of PVME integrated intensities permitted to determine the influence of polymer concentration and ethanol content in D_2O/EtOH mixture on the phase transition. It was found that the transition region was shifted to lower temperatures with increasing polymer concentration, probably due to the preferred polymer–polymer contacts in more concentrated solutions. For lower polymer concentrations ($c = 0.1$ and 1 wt%), the broadening of the transition temperature region was observed and at the same time the fraction of PVME units involved in globular-like structures was only 0.5. The effect of the stabilization of hydrogen bonding in the presence of ethanol in solution was manifested by marked shift of the transition region to higher temperatures and by decrease of the phase-separated fraction p with increasing ethanol content in D_2O/EtOH mixture.

^1H and ^{13}C NMR relaxation behaviour of EtOH molecules above the phase transition showed a distinct dependence on the polymer concentration. EtOH molecules in the most dilute solution ($c = 0.1$ wt%) were all probably expelled from polymer structures at elevated temperatures, whereas a certain portion of ethanol molecules remained bound in PVME mesoglobules in the solution with $c = 6$ wt%, as revealed from ^1H and ^{13}C spin–spin relaxation times T_2 of EtOH. While there is fast exchange between bound and free sites in solutions with $c \leq 10$ wt%, slow exchange regime follows from the existence of separate signals of bound and free EtOH as found for PVME/D_2O/EtOH solutions with $c = 20$ wt% in ^1H NMR spectra (resonance of OH protons) and especially in ^{13}C NMR spectra (resonances of CH_2 and CH_3 carbons). The lifetime of the bound EtOH molecules is much larger than 10 ms (the difference between ^{13}C chemical shifts of bound and free EtOH is approx. 100 Hz on the frequency scale). From the chemical shifts of OH protons it follows that the hydrogen bonding is for bound EtOH somewhat smaller in comparison with that existing in neat D_2O/EtOH mixtures. While for solutions with $c = 6$ wt%, the ^{13}C spin–lattice relaxation times T_1 of bound EtOH were virtually the same as for free EtOH indicating that the rate of the motion of bound ethanol molecules is not changed, for solutions with $c = 20$ wt% ^{13}C T_1 values of bound EtOH were significantly shorter in comparison with free EtOH. For the latter case, the correlation times of the bound EtOH were 2–3 times longer in comparison with free EtOH, indicating that the motion of EtOH bound in globular-like structures is slowed-down. Much larger differences between bound and free EtOH molecules were found from T_2 measurements (both ^1H and ^{13}C), where T_2 values were for bound EtOH up to 2 orders of magnitude shorter in comparison with free EtOH molecules. The main sources of these large differences are evidently that the motion of bound EtOH is spatially restricted and anisotropic (molecules cannot reach all orientations) and for the solutions with $c \leq 10$ wt%, where a fast exchange regime exists, also a contribution from the chemical exchange. With time the originally bound EtOH is very slowly released from phase-separated mesoglobules. A similar behaviour as described above for EtOH molecules from ^1H and ^{13}C NMR spectra and ^1H and ^{13}C relaxation measurements was previously found for water (HDO) molecules in PVME/D_2O solutions [17–19] so indicating that the decisive factor in this behaviour is in both cases a polar character of these molecules (dipole moments of water and EtOH are very similar) and hydrogen bonding.

Acknowledgments

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