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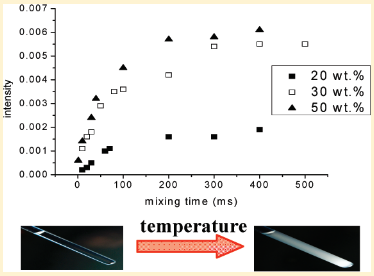
Behavior of Water during Temperature-Induced Phase Separation in Poly(vinyl methyl ether) Aqueous Solutions. NMR and Optical Microscopy Study

Jiří Spěváček,*[,†] Lenka Hanyková,[‡] and Jan Labuta[§]

[†]Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovsky sq. 2, 162 06 Prague 6, Czech Republic,
[‡]Faculty of Mathematics and Physics, Charles University, V Holešovičkách 2, 180 00 Prague 8, Czech Republic
[§]Supermolecules Group, WPI Center for Materials Nanoarchitectonics, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

ABSTRACT: The behavior of water during the temperature-induced phase separation in PVME/D_2O solutions was investigated by ^1H NMR methods and optical microscopy. A fast exchange between water (HDO) bound in mesoglobules and free water was found from ^1H NMR spin—spin relaxation measurements at temperature above the LCST transition for semidilute PVME/D_2O solutions where the exchange time is 1.2 ms for c = 6 wt %. In contrast, there is a slow exchange between bound and free water in highly concentrated PVME solutions and the residence time of the bound water 2 s was found for concentrations c = 20—50 wt % using one-dimensional NOE ^1H NMR experiment. A 3 order of magnitude slower exchange in highly concentrated PVME/D_2O solutions is in accord with our optical microscopy findings that globular-like structures are here approximately 20 times larger than in PVME solution with c = 6 wt %.



INTRODUCTION

It is well-known that some acrylamide-based polymers and other polymers with amphiphilic character, including poly(vinyl methyl ether) (PVME), exhibit in aqueous solutions a lower critical solution temperature (LCST). These polymers are soluble at lower temperatures but heating above the LCST results in phase separation that, especially for polymer concentrations $c \geq$ 1 wt %, manifests itself by milk-white turbidity of solutions.[1,2] On the molecular level, both phase separation in solutions and similar volume phase transition (collapse) in cross-linked hydrogels are assumed to be a macroscopic manifestation of a coil—globule transition followed by further aggregation and formation of so-called mesoglobules.[2] The transition is probably associated with a changed balance between various types of interactions. Their thermosensitivity makes these polymers interesting for miscellaneous biomedical and technological applications, e.g., as drug release polymers.[1,3] A similarity to the LCST behavior of elastin-like polypeptides[4] and to thermal denaturation of proteins in aqueous solution also makes them interesting from an academic point of view. Of various methods used in investigations of phase separation behavior, NMR spectroscopy plays an important role providing information on phase-separated globular structures and interactions in these systems.[5]

For PVME aqueous solutions the LCST is around 308 K,[6] i.e., at least 60 K above the temperature of the glass transition, T_g, of PVME in bulk.[7] This is in contrast to acrylamide-based polymers in aqueous solutions where the LCST is well below the T_g of the respective polymer in bulk. The phase separation in aqueous PVME solutions was studied by several physical methods, mainly by cloud point measurements, DSC, and vibrational spectroscopy.[6,8—21] It was found that aqueous PVME solutions exhibit a flat and wide bimodal LCST miscibility gap.[8] The phase diagram and very complex phase behavior were revealed for PVME aqueous solutions from modulated temperature DSC measurements.[12,14,15] Infrared spectra have shown that most of the methyl groups of PVME are dehydrated above the LCST, whereas there is only partial dehydration of the ether groups above the LCST.[11] Moreover, a two-step mechanism of the phase separation process was also shown by infrared spectroscopy.[13,16,17]

In the past decade we used ^1H NMR spectroscopy to investigate changes in the structure and dynamics during temperature-induced phase separation in PVME/D_2O solutions.[22—29] The phase separation is manifested in NMR spectra by marked line broadening for a major part of PVME units, indicating the formation of more compact globular-like structures.[5,22,23] The minority mobile component, which does not take part in the phase transition, mostly consists of a low-molecular-weight fraction of PVME.[25] Information on behavior of water was obtained mainly from measurements of spin—spin relaxation times T_2 of both PVME and water (HDO) protons.[24—28] In the present study we extended our ^1H NMR investigations concerning the behavior of water during the phase separation in PVME/D_2O solutions combining T_2 measurements on HDO protons in

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semidilute solutions and one-dimensional exchange NMR experiments in concentrated solutions with optical microscopy.

EXPERIMENTAL SECTION

Samples. PVME (purchased from Aldrich, supplied as 50 wt % aqueous solution; molecular weight determined by SEC in THF, M_w = 60 500; $M_w/M_n \cong 3$; tacticity by ^{1}H NMR, 59% of isotactic diads[23]) was used after drying to prepare PVME/D_2O (99.9% of deuterium) solutions of desired concentration in the range c = 6−50 wt %. All PVME/D_2O samples in 5 mm NMR tubes were degassed and sealed under argon; sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal NMR standard.

NMR Measurements. ^{1}H NMR spectra were recorded with a Bruker Avance 500 spectrometer operating at 500.1 MHz. The ^{1}H spin−spin relaxation times T_2 of HDO were measured using the same instrument and the CPMG[30] pulse sequence $90°_x-(t_d-180°_y-t_d)_n-$acquisition with t_d = 5 ms, relaxation delay 100 s, and 8 scans. The total time for T_2 relaxation was an array of ∼30 values. The relative error for T_2 values did not exceed ±5%. All obtained T_2 relaxation curves had monoexponential character. A modified double pulsed-field-gradient spin−echo (DPFGSE) NOE pulse sequence[31] was used in one-dimensional exchange ^{1}H NMR experiment to detect a slow exchange in concentrated PVME/D_2O solutions. This sequence was combined with a spin−echo T_2 filter to suppress the broad signal from PVME protons;[26] the Gaussian-shaped pulses[32] were used for selective excitation of the main HDO signal. In these measurements we used mixing times in the range 1−500 ms, relaxation delay 100 s, and 32 scans.

Optical Microscopy. Photomicrographs were obtained under a nitrogen atmosphere using an Olympus BX51 microscope equipped with Olympus MP5Mc/OL digital camera and Linkam THMS600 hot stage and Linkam TMS94 controller/LNP94 cooling system. Sample was placed between a support glass slide and a coverslip inserted in the hot stage. Digital image processing was performed using the ImageJ software (Image Processing and Analysis in Java).

RESULTS AND DISCUSSION

In our previous studies of PVME/D_2O solutions we have shown that for polymer concentrations in the range c = 2−10 wt % ^{1}H spin−spin relaxation times T_2 of HDO molecules at temperatures above the LCST phase transition (309.5 K) are 1 order of magnitude shorter than those at temperature below the phase transition (305 K). This shows that at temperatures above the transition there is a portion of HDO bound in globular-like structures.[25,26] In all cases there was a single line of HDO in the ^{1}H NMR spectrum and the T_2 relaxation curves were exponential, indicating a fast exchange between bound and free sites. Interestingly enough, at temperatures above the phase transition T_2 values of HDO slowly increase with time, showing that originally bound water is very slowly released from globular-like structures, and the character of mesoglobules is changed from the spongelike to a rather compact one. This dehydration process is also manifested by decreasing values of a predominant short T_2 component of CH_2 protons of PVME with time when sample is kept at temperatures above the LCST transition.[25]

There are two most important possible sources of the short T_2 values of HDO observed for PVME/D_2O solutions with c = 2−10 wt % at temperatures above the LCST transition: (i) a lower, spatially restricted mobility; (ii) chemical exchange. Chemical exchange is known to affect transverse relaxation rates measured in spin−echo experiments. The dependence of measured T_2 values on the time interval t_d in CPMG pulse sequence is often used for characterization of microsecond−millisecond chemical exchange.[33,34] Figure 1 shows such dependence as obtained for HDO protons in PVME/D_2O solution (c = 6 wt %). From this figure it follows that contribution of chemical exchange to the spin−spin relaxation rate $(T_2)^{-1}$ is important. Solid curve in this figure shows the best fit as obtained using the equation[34]

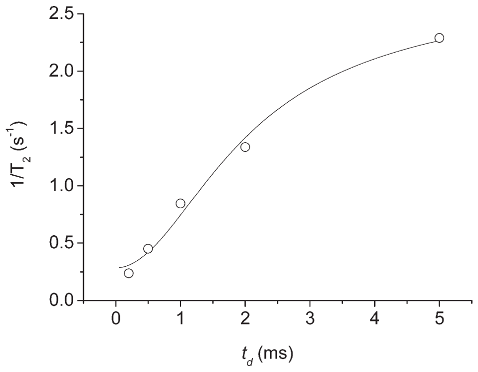


Figure 1. Dependence of spin−spin relaxation rate $(T_2)^{-1}$ on the interval t_d in CPMG sequence as obtained for HDO protons in PVME/D_2O solution (c = 6 wt %) at 309.5 K. Solid curve is a best fit according eq 1.

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

with $k_{ex} = 816\ s^{-1}$, $(R_2)^0 = 0.284\ s^{-1}$, $K = p_F p_B(\Delta\omega)^2 = 2140\ s^{-2}$. 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 F (free) and B (bound), p_F and p_B are populations of the free and bound HDO, respectively, and $\Delta\omega = 2\pi\Delta\nu$, where $\Delta\nu$ is the difference of resonance frequencies of the free and bound HDO in hertz. For PVME/D_2O solution (c = 6 wt %), therefore, the exchange time or the residence time of the bound HDO $\tau_{ex} = 1/k_{ex}$ is τ_{ex} = 1.2 ms. Similar values of the exchange time were found for ethanol in PVME/D_2O/ethanol solution (c = 6 wt %) at 325 K and for HDO in D_2O solutions of poly(*N*-isopropylmethacrylamide) (PIPMAm) (c = 20 wt %) at 320 K where in both cases τ_{ex} = 0.4 ms.[27,29] We assume that slightly faster exchange as found for these systems where the LCST transition appears at higher temperature in comparison with PVME/D_2O solution is mainly due to the higher temperature. For PVME/D_2O solutions the values of the short T_2 component of PVME CH_2 protons are constant ($T_2 \cong$ 0.75 ms) in the range of polymer concentrations c = 6 − 50 wt %.[28] Therefore also for c = 6 wt % one can assume that molar ratio [PVME monomer unit]/[bound D_2O] ≅ 2.7, similarly as established for highly concentrated solutions (c = 20−60 wt %).[26] Taking this into account, we obtain that for c = 6 wt % the fraction of the bound HDO, p_B = 0.007, and subsequently from the value of the constant $K = 2140\ s^{-2}$ it follows that the difference of resonance frequencies of the free and bound HDO is $\Delta\nu$ = 88 Hz, i.e., 0.18 ppm.

In contrast to semidilute solutions, two separate NMR signals of the free and bound HDO (difference of chemical shifts 0.74 ppm) were detected for highly concentrated PVME/D_2O solutions (c = 20−60 wt %).[26] This shows that in these systems there is a slow exchange on the NMR scale and for the residence time of the bound HDO it holds $\tau_B \gg 2.7$ ms. This includes also the case that there is virtually no exchange ($\tau_B \rightarrow \infty$). At the same

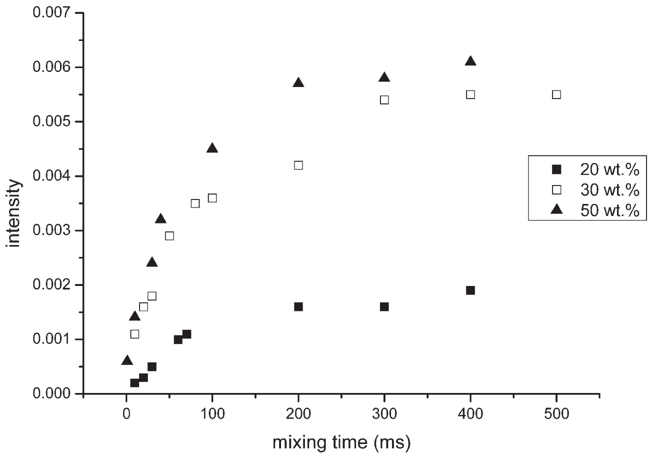


Figure 2. Dependences of the intensity of the signal of the bound HDO as a function of the mixing time in 1D DPFGSE NOE ^{1}H NMR experiment with selective excitation of the signal of the free HDO for PVME/D_2O solutions with c = 20 wt % (■), 30 wt % (□), and 50 wt % (▲) at 309.5 K.

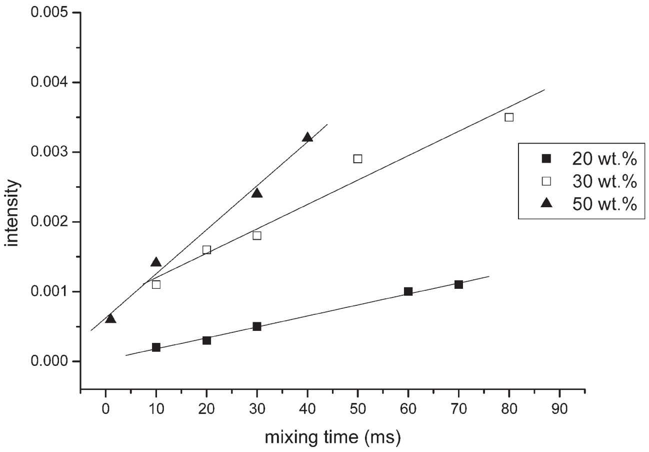


Figure 3. Initial linear portions of the curves shown in Figure 2: c = 20 wt % (■), 30 wt % (□), and 50 wt % (▲).

Table 1. Rate Constants $k_{F\rightarrow B}$ and $k_{B\rightarrow F}$ of the Exchange between Bound and Free Water and Residence Times of the Bound Water τ_B for PVME/D_2O Solutions of Various Concentrations at 309.5 K

c (wt %)	b^a	$k_{F\rightarrow B}$ (s^{-1})	$k_{B\rightarrow F}$ (s^{-1})	τ_B (s)
20	0.035	0.016	0.441	2.3
30	0.057	0.035	0.579	1.7
50	0.116	0.063	0.480	2.1

[a] Fraction of bound HDO, cf. ref 26.

time the fractions of bound HDO were unchanged even for 70 h. To detect a slow exchange in highly concentrated PVME/D_2O solutions (c = 20−50 wt %), we applied a one-dimensional exchange ^{1}H NMR experiment with selective excitation of the main HDO signal and detection of the changes in the intensity of the signal of the bound HDO as a function of the mixing time in the modified DPFGSE NOE pulse sequence (cf. Experimental Section). The dependences of the intensity of the signal of the bound HDO in the difference spectrum on the mixing time as obtained for three polymer concentrations are shown in Figure 2. From the initial linear portions of these curves which are shown in Figure 3 we obtained the rate constants $k_{F\rightarrow B}$ of the exchange process; here subscripts F and B correspond to the free and bound HDO, respectively. The $k_{F\rightarrow B}$ values are shown in Table 1. Taking into account that in the equilibrium, the following relation holds

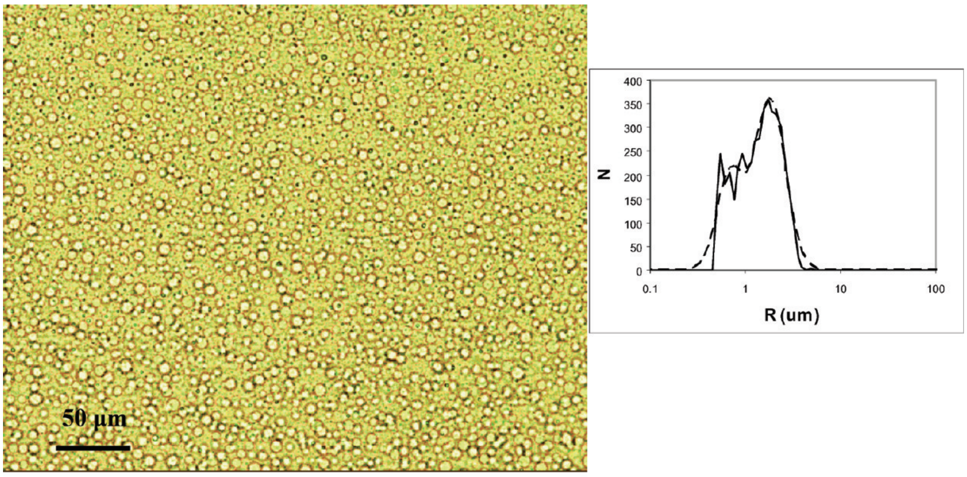
$$k_{F\rightarrow B}(1-b) = k_{B\rightarrow F}b \tag{2}$$

where b is the fraction of the bound HDO, we can obtain the values of the rate constant $k_{B\rightarrow F}$ and the values of the residence time of the bound HDO, $\tau_B = 1/k_{B\rightarrow F}$; these values are also shown in Table 1. From Table 1 it follows that while $k_{F\rightarrow B}$ values increase with increasing polymer concentration, $k_{B\rightarrow F}$ and τ_B values are virtually independent of polymer concentration in the range c = 20−50 wt % with $\tau_B \cong 2$ s, i.e., a 3 order of magnitude larger value than the residence time of bound HDO in semidilute PVME/D_2O solution with c = 6 wt %.

To explain this substantial difference in the exchange rate between semidilute PVME solutions on the one hand and highly concentrated PVME solutions on the other hand, we assumed that exchange between free and bound water is associated with diffusion process of water molecules through mesoglobules. Taking into account a classical diffusion equation[35]

$$\langle x^2 \rangle = 2Dt \tag{3}$$

i.e., the direct proportionality between diffusion time and mean square distance, we assumed that the observed difference in the exchange rate might be mainly in connection with different size of globular-like structures in both cases. The evidence supporting this hypothesis was obtained by optical microscopy. Figure 4a shows a photomicrograph for PVME/D_2O solution (c = 6 wt %) at 309 K, i.e., just above the LCST transition. Formation of rather isolated spherical mesoglobules can be clearly seen in this figure. Figure 4b shows the size distribution of mesoglobules (number of mesoglobules within the visual field N vs their radius R) obtained by digital image processing. The main maximum at R = 1.75 μm and weaker maximum around R = 0.75 μm can be seen in Figure 4b. The smoothed bimodal distribution (dashed line in Figure 4b), therefore, shows the mean sizes (diameters) of mesoglobules as 3.5 and 1.5 μm. Figure 5 shows a photomicrograph for PVME/D_2O solution with c = 20 wt %, again at 309 K. Pictures similar to those shown in Figure 5 were obtained also for PVME solutions of higher concentration (c = 30 and 50 wt %). Figure 5 shows formation of very large agglomerates with dimensions that are approximately 20 times larger than mesoglobules in PVME solution with c = 6 wt %. Taking into account eq 3, we can therefore expect that exchange between bound and free water in highly concentrated PVME/D_2O solutions ($c \geq 20$ wt %) can be ∼400 times slower in comparison with PVME/D_2O solution with c = 6 wt %. In this analysis we assumed that water is bound mainly inside mesoglobules and that diffusion coefficient D is a constant because, as already discussed above, constant values of the dominating short T_2 component of PVME CH_2 protons in the range of polymer concentrations c = 6 − 50 wt % suggest that also polymer concentration in mesoglobules, c = 89 wt %, does not vary in this concentration range.[26,28] A part of the bound water might also exist as water bound on the surface of mesoglobules (two types of bound water were discriminated and assigned to water bound inside mesoglobules and on their surface for PIPMAm/D_2O solution[29]) with a faster exchange with free water in comparison with water bound inside mesoglobules.



(a) (b)

Figure 4. (a) Photomicrograph of PVME/D_2O solution (c = 6 wt %) at 309 K. (b) Size distribution of mesoglobules (their number within the visual field N vs radius R) (solid line) and smoothed bimodal distribution (dashed line) for PVME/D_2O solution (c = 6 wt %) at 309 K.

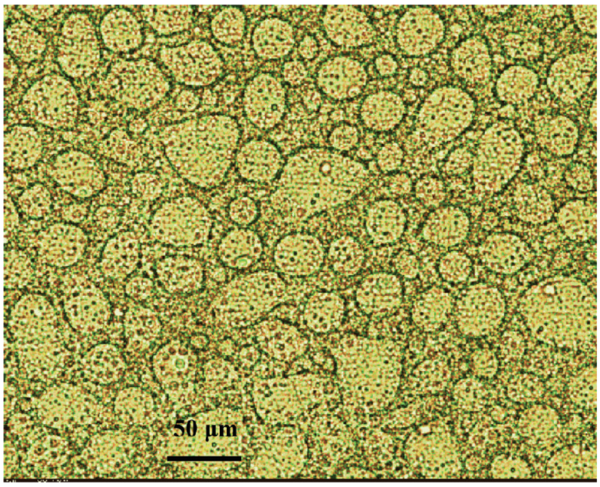


Figure 5. Photomicrograph of PVME/D_2O solution (c = 20 wt %) at 309 K.

Taking into account that the relative population of the surface bound water should decrease with increasing size of the mesoglobules, this might be an additional factor that can contribute to the total observed ratio 1667 in the rates of chemical exchange in semidilute and highly concentrated PVME/D_2O solutions (1667 = 2000 ms/1.2 ms). The finding that observed differences in the rate of the exchange process can be mainly in connection with different sizes of globular-like structures is also corroborated by the fact that a fast exchange between bound and free HDO was found for highly concentrated PIPMAm/D_2O solution (c = 30 wt %)[36] where optical microscopy showed us formation of mesoglobules with the mean size ~2 μm.

CONCLUSION

In the present study we used ^{1}H NMR methods to investigate the behavior of water during the temperature-induced phase separation in PVME/D_2O solutions. For semidilute PVME solutions (c = 2−10 wt %) there is a fast exchange between water bound in mesoglobules and free water. The exchange time τ_{ex} = 1.2 ms was found for PVME/D_2O solution (c = 6 wt %) from dependences of spin−spin relaxation rate on the time interval t_d in CPMG pulse sequence. For highly concentrated PVME/D_2O solutions (c = 20−60 wt %) the existence of the separate signal of the bound HDO with ~0.74 ppm smaller chemical shift in comparison with the main HDO signal suggests a slow exchange process.[26] Using one-dimensional NOE ^{1}H NMR experiment, we found that in the range c = 20−50 wt % the residence time of the bound water is $\tau_B \cong 2$ s. Assuming that exchange between free and bound water is associated with diffusion process, a 3 order of magnitude slower exchange in highly concentrated PVME/D_2O solutions is in accord with our optical microscopy findings that globular-like structures are here approximately 20 times larger than in PVME solutions with c = 6 wt %.

AUTHOR INFORMATION

Corresponding Author

*Tel: +420 296809380. Fax: +420 296809410. E-mail spevacek@imc.cas.cz.

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