Phase Separation in Aqueous Polymer Solutions as Studied by NMR Methods

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Summary: Some possibilities of ^1H NMR spectroscopy in investigations of structural-dynamic changes and polymer-solvent interactions during the temperature-induced phase transitions in aqueous polymer solutions are described. Results obtained recently on D_2O solutions of poly(vinyl methyl ether) (PVME), poly(*N*-isopropylmethacrylamide) (PIPMAm), negatively charged copolymers of *N*-isopropylmethacrylamide and sodium methacrylate, and PIPMAm/PVME mixtures are discussed. A markedly different rate of dehydration process in dilute solutions on the one hand, and in semidilute and concentrated solutions on the other hand, was revealed from ^1H spin-spin relaxation measurements.

Keywords: aqueous polymer solutions; ionized copolymers; NMR; poly(N-isopropylmethacrylamide); poly(vinyl methyl ether); temperature-induced phase separation

Introduction

It is well known that some acrylamide-based polymers, including poly*(N*-isopropylacrylamide) (PIPAAm), poly(*N*-isopropylmethacrylamide) (PIPMAm) and poly(*N*,*N*-diethylacrylamide) (PDEAAm), and some other polymers like poly(vinyl methyl ether) (PVME) exhibit interesting behaviour in aqueous solutions, showing a lower critical solution temperature (LCST). They are soluble at low temperatures but heating above the LCST results in phase separation. On molecular level, both phase separation in solutions and similar collapse transition in crosslinked hydrogels, are assumed to be a macroscopic manifestation of a coil-globule transition often followed by aggregation, as shown for PIPAAm and PDEAAm in water by light scattering and small angle neutron scattering.[1-5] The transition is probably associated with competition between hydrogen bonding and hydrophobic interactions.[6,7]

Their thermosensitivity makes these systems interesting for possible biomedical and technological applications, especially if the polymers are chemically crosslinked (in the form

DOI: 10.1002/masy.200550401

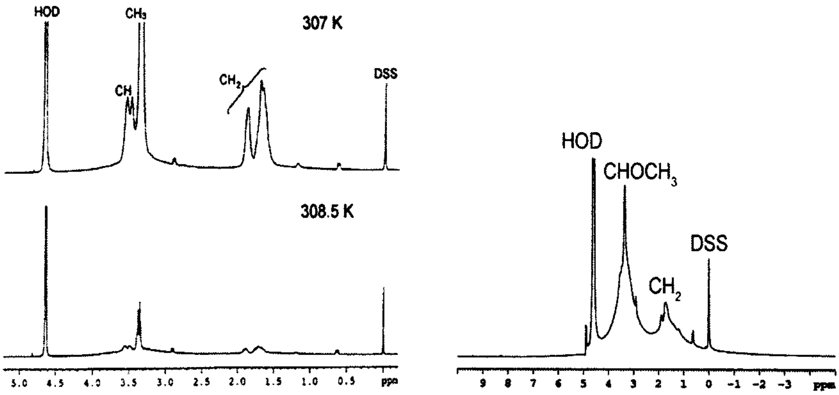
of hydrogels). Stimuli-responsive hydrogels have potential application in the creation of "intelligent" material systems, e.g., as drug delivery systems. A certain similarity to thermal denaturation of proteins in aqueous solutions also makes them interesting from an academic point of view.

Though phase transitions especially in PIPAAm aqueous solutions were extensively studied by various methods, the application of NMR spectroscopy to investigate the phase separation in these systems was rather seldom.[8-14] The present paper provides an overview of our recent ^{1}H NMR studies dealing with temperature-induced phase transitions in D_2O solutions of PVME and PIPMAm, ionized random copolymers of *N*-isopropylmethacrylamide and sodium methacrylate (MNa), and PIPMAm/PVME mixtures.[15-21] From the methodical point of view, we combined an approach based on measurements of temperature dependences of high-resolution ^{1}H NMR spectra (mainly integrated intensities) with measurements of ^{1}H NMR relaxation times.

Coil-globule Transition and its Manifestation in ^{1}H NMR Spectra

High-resolution ^{1}H NMR spectra of PVME/D_2O solution (c = 4 wt %; PVME was purchased from Aldrich; molecular weight determined by SEC in THF: M_w = 60 500g/mol, $M_w/M_n \cong 3$; tacticity by ^{1}H NMR: 59% of isotactic diads) measured at two slightly different temperatures (307 and 308.5 K) and under identical instrument conditions are shown in Figure 1a. The assignment of resonances to various types of protons of PVME and to residual water (HDO) is shown in spectrum measured at 307 K. While an ordinary spectrum of the polymer in solution was recorded at 307 K, the most significant effect observed at slightly higher temperature but just above the LCST (308.5 K) is a marked decrease in the integrated intensity of all PVME lines. This is evidently due to the fact that at temperatures above the LCST the mobility of most PVME units is reduced to such an extent that corresponding lines become too broad to be detected in high-resolution spectra. All studied PVME/D_2O solutions exhibit a milk-white opalescence at 308.5 K and higher temperatures, so corroborating that a marked line broadening of a major part of PVME units is due to phase separation and formation of compact globular-like structures.

For PVME aqueous solutions the LCST is around 308 K, i.e., well above the temperature of the glass transition of PVME in bulk where values in the range T_g = 191-251 K are



(a) (b)

Figure 1. 500.1 MHz ^1H NMR spectra of PVME/D_2O solutions, c = 4 wt% (a) and 2 wt% (b): (a) spectra at 307 and 308.5 K measured under the same instrumental conditions; (b) spectrum at 312 K measured with spectral width 15 kHz.[16,21]

reported.[22] This means that for this polymer the segmental mobility in globular structures is still relatively high and makes possible to detect directly the broad lines corresponding to PVME units in globular structures by using liquid-state NMR spectrometer. Figure 1b shows ^1H NMR spectrum of PVME/D_2O solutions (c = 2 wt %) measured at 312 K with larger spectral width and higher amplification. A typical two-component line shape can be seen. Most of PVME units contribute to the broad component (linewidths 408 and 734 Hz for $CHOCH_3$ and CH_2 protons, respectively) on which narrow lines of PVME units retaining a high mobility are superimposed. In contrast to PVME, for acrylamide-based polymers (PIPAAm, PDEAAm, PIPMAm) in aqueous solutions the LCST is well below the respective T_g (e.g., for PIPAAm and PIPMAm in bulk, T_g = 403 and 449 K, respectively,[22,23] while LCST for respective aqueous solutions are around 307 and 316 K). Therefore due to much lower mobility of acrylamide-based polymer segments forming globular structures, the corresponding linewidths of the broad component are significantly larger. Linewidths ~ 3.7 and 3.0 kHz were reported for phase-separated segments of PDEAAm and collapsed segments of chemically crosslinked PIPAAm, respectively.[9,13,14]. ^1H magic angle spinning NMR spectra and results of MW4 multipulse relaxation measurements ($T_{2\mathrm{eff}}$) have shown that the linewidh 3.7 kHz found for PDEAAm/D_2O solutions and gels is probably associated with isotropic Brownian tumbling of globules as a whole.[13,14]

From a comparison of absolute integrated intensities of NMR lines (measured under conditions allowing to detect only the narrow component, cf. Fig. 1a), the fraction p of phase-separated units (units in globular-like structures) can be determined using the relation[14-17,19,20]

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

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. For I_0 we took values based on integrated intensities below the phase transition, using the expected $1/T$ temperature dependence. Figure 2 shows temperature dependences of the fraction p of phase-separated units of PIPMAm as obtained from integrated intensities of CH protons and several concentrations of D_2O solutions[20]; the same dependences were also obtained from analysis of integrated intensities of other proton groups. From Figure 2 it follows that there is no concentration dependence of the transition temperatures in D_2O solutions of neat PIPMAm.

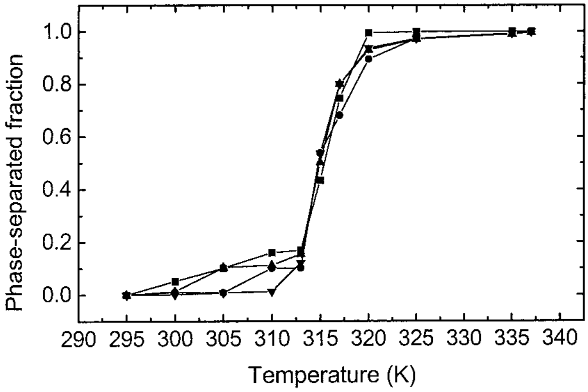


Figure 2. Temperature dependences of the phase-separated fraction p for PIPMAm/D_2O solutions; c = 0.1 wt% (■), 1wt% (●), 5 wt% (▲), 10 wt% (▼).

Origin of Nonseparated Portion in PVME/D_2O Solutions

Figure 2 also shows that at temperatures above the phase transition the fraction p of phase-separated segments in PIPMAm/D_2O solutions is virtually equal to 1, i.e., virtually no narrow lines were detected in these cases. Similar behaviour was found also for D_2O solutions of PIPAAm or PDEAAm.[14,24] For PVME/D_2O solutions the fraction of phase-separated PVME segments is $p = 0.85$.[16] As the most probable explanation for 15% of PVME segments that contribute to the minority narrow (mobile) component and do not participate in phase separation we suggested that they probably are from a low-molecular-weight fraction which one can expect for polymer with rather large polydispersity ($M_w/M_n \cong 3$), as in our case. To support this hypothesis, the following experiment was done:[21] PVME/H_2O solution was centrifugated for ~ 10 min at 313 K and after seclusion of the phase-separated part the remaining solution was dried and subsequently analyzed by SEC with THF as a mobile phase. The respective SEC chromatogram is shown in Fig. 3. From this figure it follows that really only low-molecular-weight fraction of PVME is present in water solution after removal of the phase-separated polymer. There are several peaks visible on SEC curve, and comparison with

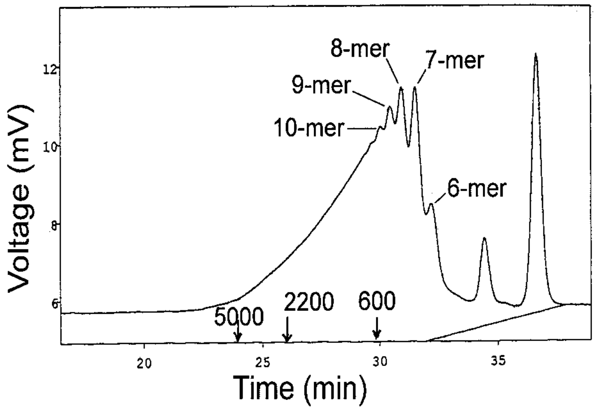


Figure 3. SEC curve (in THF) of the PVME remaining in aqueous solution after seclusion the phase separated part by centrifugation at 313 K and 15 000 rpm (polystyrene standards are shown by arrows).[21]

polystyrene standards shows that PVME oligomers (6-10 mers) are prevailing in the analyzed sample. One can assume that a certain minimum chain length is a prerequisite for the phase separation as a consequence of the cooperative character of the respective interactions. On the basis of Figure 3, one can assume that for PVME in aqueous solution such minimum chain length amounts on average ~10 monomeric units; however, this figure also shows that some chains with degree of polymerization up to ~50 are not phase-separated at 313 K.

Effects of Ionization

The effect of ionization on the thermotropic phase transition is demonstrated in Figure 4, where the temperature dependences of the phase-separated fraction are shown for ionized copolymers P(IPMAm/MNa) in D_2O solutions (c = 1 and 0.1 wt%) and various ionic comonomer mole fraction (i = 0-10 mole%).[19] MNa units, that are dissociated in aqueous solution ($-CH_2C(CH_3)(COO^-)-$), introduce negative charges on polymer chains. From Figure 4a (c = 1 wt%) it follows that the transition region shifts towards higher temperatures with increasing concentration of negative charges on the chain and simultaneously the fraction of PIPMAm segments involved in globular structures is reduced. Very similar behavior was found also for c = 10 wt%[19], as well as for hydrogels of chemically crosslinked P(DEAAm/MNa)[13]. In all cases the increasing content of hydrophilic MNa units increases

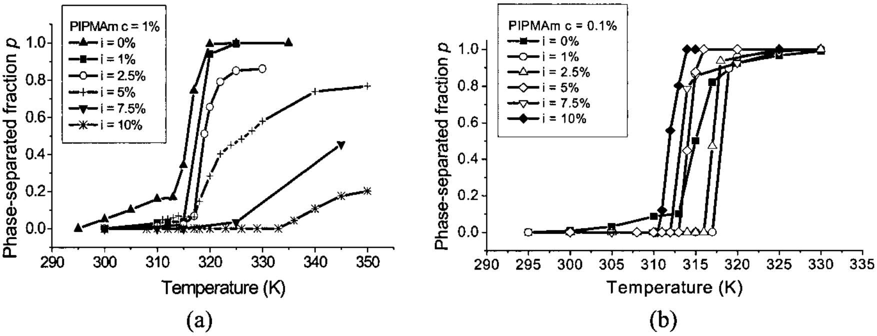


Figure 4. Temperature dependences of the phase-separated fraction p for P(IPMAm/MNa) copolymers in D_2O with polymer concentration 1 wt% (a) and 0.1 wt% (b) and various mole fractions of ionic comonomer i.[19]

the mobility of polymer segments and higher temperature is necessary to allow hydrophobic interactions to predominate. A completely different behavior was found with dilute solutions (c = 0.1 wt%) of P(IPMAm/MNa) copolymers (Fig. 4b). In this case the transition is always rather sharp and interestingly enough, while for i = 1 mole% the transition is shifted towards higher temperature in comparison with the neat PIPMAm, further increasing of ionic comonomer mole fraction i gradually shifts the transition towards lower temperatures, even below the dependence for the neat PIPMAm. We assume that this complex behaviour is probably associated with the fact that while for dilute solutions the globular structures are formed mainly by individual macromolecules, for semi-dilute and concentrated solutions intermolecular interactions are important. From Fig. 4 it also follows that the temperature interval and character of the transition can be "tuned" by changing the polymer concentration c and ionic comonomer mole fraction i; this fact might be important for possible practical applications.

Phase Transitions in D_2O Solutions of Polymer Mixtures

^{1}H NMR spectroscopy was used to investigate thermotropic phase transitions in D_2O solutions of PIPMAm/PVME mixtures.[20] In all studied solutions (c = 0.1 - 5 wt%) two phase transitions were detected at temperatures roughly corresponding to different LCST of PIPMAm and PVME. While the PVME transition (appearing at lower temperatures) is not affected by the presence of the PIPMAm component in the mixture, the PIPMAAm transition (appearing at higher temperatures) is markedly affected by the presence of the phase-separated PVME component. This is among others demonstrated by the finding that the PIPMAm transition in PIPMAm/PVME mixtures is affected by the concentration of the solution (Fig. 5). While there is no concentration dependence of the transition temperatures for D_2O solutions of neat PIPMAm (cf. Fig. 2), Figure 5 shows that increasing concentration of PIPMAm/PVME mixture leads to the shift of the PIPMAm transition region toward lower temperatures. This shift can be attributed to more frequent PIPMAm-PVME contacts at higher concentrations (some PIPMAm segments might be ketch in PVME globular-like structures). Moreover, for solutions with c = 5 wt%, the PIPMAm transition temperatures depend on the composition of PIPMAm/PVME mixture. Similar behavior as described here for

PIPMAm/PVME mixtures was found also for D_2O solutions of PIPMAm/PIPAAm mixtures.[24]

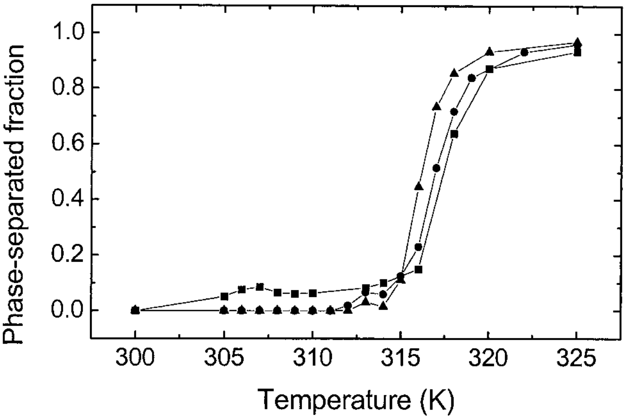


Figure 5. Temperature dependences of the phase-separated fraction p for PIPMAm component in D_2O solutions of mixture PIPMAm/PVME = 1/1 and polymer concentrations c = 0.1 wt% (■), 1 wt% (●), 5 wt% (▲).

In contrast to mixtures of two homopolymers, only single phase transition was found for P(IPMAm/IPAAm) random copolymers by cloud point and ^1H NMR measurements.[24,25] At the same time, the phase-transition temperatures strongly depend on the composition of the copolymer. ^1H NMR measurements revealed a certain departure from the linear dependence of the transition temperatures on the copolymer composition for a sample with 75 mole% of IPMAm units.[24]

Polymer-water Interactions as Revealed from Measurements of Spin-spin Relaxation

Measurements of ^1H spin-spin relaxation time T_2 in PVME/D_2O solutions (with spectra measured in analogous way to that in Figure 1b; T_2 values were measured using the CPMG[26] pulse sequence $90°_x$-(t_d-$180°_y$-t_d)$_n$-acquisition) have shown that a very short component (T_2 < 1 ms) dominates the spin-spin relaxation of PVME protons at temperature above the transition

(309.5 K).[18,21] Intensities of the shortest T_2 component do not depend on the concentration of the solution and amount on average ~75%. This value agrees well with the phase-separated fraction p = 0.85 as determined from integrated intensities of high-resolution ^{1}H NMR spectra[16] and confirms that shortest T_2 component corresponds to PVME segments forming globular-like structures. While T_2 values of the shortest component of $CHOCH_3$ protons virtually do not depend on the polymer concentration, for CH_2 protons the very short component decreases with decreasing concentration of the solution (Figure 6). This shows that globular-like structures are more compact in dilute solution in comparison with semidilute or concentrated solutions, where globules probably contain a certain amount of water. The result that T_2 values of the short component of $CHOCH_3$ protons (with dominant contribution of CH_3 protons) do not depend on the concentration of the solution is in accord with results obtained by infrared spectroscopy[27] that at temperatures above the LCST most CH_3 groups of PVME are dehydrated.

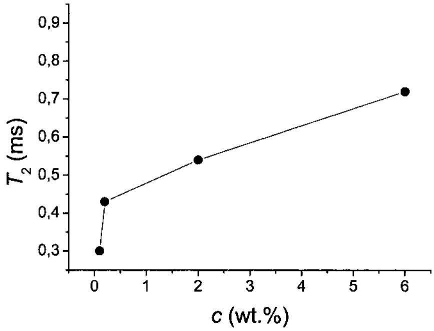


Figure 6. Concentration dependence of a very short component of spin-spin relaxation time T_2 as determined for CH_2 protons in PVME/D_2O solutions at 500.1 MHz and 309.5 K.[18]

To characterize polymer-water interactions in PVME/D_2O solutions, ^{1}H spin-spin relaxation times T_2 of residual HDO molecules were measured.[18,21] The T_2 values of HDO molecules in solutions of various polymer concentration and measured at temperatures below (305 K) and above (309.5 K) the transition are shown in Table 1. While for dilute solutions (c = 0.1 and 0.2 wt%) T_2 values measured at 305 and 309.5 K did not differ too much, a significant difference was found for higher concentrations where T_2 values at 309.5 K were 1 order of

Table 1. ^{1}H spin-spin relaxation times T_2 of HDO molecules in PVME/D_2O solutions at 500.1 MHz and two temperatures (305 K and 309.5 K).

c (wt.-%)	T_2 (s)	
	305 K	309.5 K
0.1	4.2	3.1
0.2	5.4	4.3
2	8.7	1.5
6	4.8	0.44
10	4.5	0.74

magnitude shorter than those at 305 K. This shows that in semidilute and concentrated PVME solutions at temperature above the transition there is a portion of HDO molecules that exhibit a lower (spatially restricted) mobility, similarly to the phase-separated PVME. Evidently, this portion corresponds to HDO molecules bound in globular-like structures. The exponential character of T_2 relaxation curves (for HDO) indicates a fast exchange between bound and free sites regarding T_2 values (~1 s); i.e., the lifetime of the bound HDO molecules is < 0.1 s. In such case the observed relaxation time $T_{2\mathrm{obs}}$ is given as

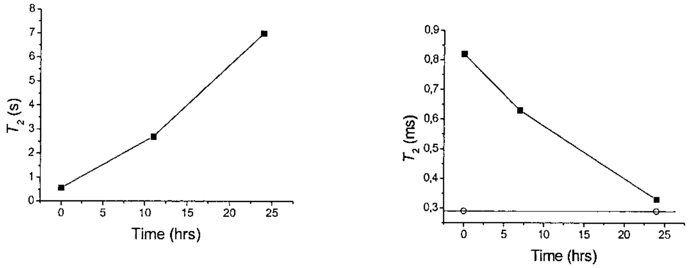
$$(T_{2\mathrm{obs}})^{-1} = (1 - f)(T_{2\mathrm{F}})^{-1} + f\,(T_{2\mathrm{B}})^{-1} \qquad (2)$$

where subscripts F and B correspond to free and bound states, respectively, and f is the fraction of bound HDO molecules. In accord with results of T_2 measurements for PVME protons (Fig. 6), it follows from Table 1 that in dilute solutions the fraction of bound HDO molecules is almost negligible. A certain portion of water molecules bound at elevated temperatures in PVME globular structures in semidilute and concentrated solutions was revealed also from measurements of selective and nonselective spin-lattice relaxation times T_1 of residual HDO molecules.[15,18,21]

From temperature dependences of T_2 of HDO in concentrated D_2O solutions of PIPMAm/PVME mixtures, it follows that a certain portion of water is bound both in globular-like structures of PVME and PIPMAm component; a major part of water is bound in globular-like structures of predominant polymer component in the mixture.[20]

Time Dependences of the Spin-spin Relaxation Times T_2: Process of Dehydration

We were interested in knowing whether the amount of water bound in PVME globular structures formed in semidilute and concentrated aqueous solutions is changing with time or not. Figure 7 shows the time dependence of relaxation time T_2 of HDO molecules and time dependences of a very short component of relaxation time T_2 as determined for CH_2 protons of PVME, both measured at 309.5 K.[21] While for c = 0.1 wt %, T_2 values of HDO obtained after 24 h only slightly differ from the respective value in Table 1, for c = 6 wt% it follows from Figure 7a that T_2 values of HDO very slowly increase with time, reaching after 24 h a similar value as observed at temperature below the transition (cf. Table 1). Simultaneously, for c = 6 wt % values of a very short T_2 component of CH_2 protons of PVME (Figure 7b) slowly decrease with time, reaching after 24 h a similar value as found for dilute solution (c = 0.1 wt %). Even after very long time (~ day) we did not observe any sedimentation of the phase-separated part in the studied sample. Therefore, these results evidence that water, originally bound in globular-like structures existing in semidilute and concentrated solutions, is with time very slowly released from these structures. On the contrary, dehydration is rapid in dilute solutions.



(a) (b)

Figure 7. Time dependences of spin-spin relaxation times T_2 for HDO protons (a) and a very short component of CH_2 protons of PVME (b) in PVME/D_2O solutions at 500.1 MHz and 309.5 K; c = 0.1 wt % (○), 6 wt% (■).[21]

T_2 measurements of HDO in D_2O solution (c = 5 wt%) of PIPMAm/PVME mixture have shown that dehydration process is about 4 times slower for more rigid globular-like structures of PIPMAm component (at temperature well below the T_g of PIPMAm in bulk) in comparison with globules of the flexible PVME component (at temperatures well above the respective T_g).[21]

Conclusion

Some possibilities of ^{1}H NMR spectroscopy in investigations of structural-dynamic changes and polymer-solvent interactions during the temperature-induced phase transitions in aqueous polymer solutions were shown on examples of several systems studied by us recently. The temperature dependences of the phase-separated fraction p, that can be obtained from integrated intensities in high-resolution ^{1}H NMR spectra, allowed us to quantitatively characterize phase separation in D_2O solutions of negatively charged P(IPMAm/MNa) copolymers or PIPMAm/PVME mixtures. In combination with SEC these measurements have shown that in PVME/D_2O solutions the minority mobile component, which does not take part in the phase transition, mostly consists of low-molecular-weight fraction of PVME. The sponge-like structure containing pores where molecules of water can be accommodated, was suggested for globules existing in semi-dilute and concentrated PVME/D_2O solutions from ^{1}H spin-spin relaxation measurements.[21] With time the bound water is slowly squeezed out by polymer segments, pores disappear, and globules become rather compact. From results obtained on D_2O solutions of PIPMAm/PVME mixtures it follows that the dehydration process is significantly slower for more rigid globular-like structures of PIPMAm in comparison with globules of the flexible PVME. In contrast to this behaviour, in dilute solutions globular-like structures are rather compact and dehydrated already immediately after phase transition (they are mostly formed by single macromolecules).

Acknowledgment

The autor would like to thank his coworkers Lenka Hanyková, Larisa Starovoytova, Michal Ilavský and Petr Holler for their important contributions to the work described here. Support by the Grant Agency of the Academy of Sciences of the Czech Republic (project IAA4050209) is gratefully acknowledged.

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