



^1H NMR study of temperature-induced phase separation in solutions of poly(*N*-isopropylmethacrylamide-*co*-acrylamide) copolymers

Hana Kouřilová^a, Julie Šťastná^a, Lenka Hanyková^a, Zdeňka Sedláková^b, Jiří Spěváček^{b,*}

^a Faculty of Mathematics and Physics, Charles University, V Holešovičkách 2, 180 00 Prague 8, Czech Republic

^b Institute of Macromolecular Chemistry AS CR, v.v.i., 162 06 Prague 6, Czech Republic

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ABSTRACT

^1H NMR spectroscopy was applied to investigate temperature-induced phase separation in solutions of poly(*N*-isopropylmethacrylamide-*co*-acrylamide) [P(IPMAm/AAm)] random copolymers in D_2O , D_2O /ethanol and D_2O /acetone. The NMR relaxation behaviour of water (HDO) was also examined. The effects of P(IPMAm/AAm) composition and the ethanol or acetone content in the mixed solvents on the temperature, width and extent of the phase transition as well as on the mobility of polymer segments and water molecules were characterized. For D_2O solutions of the copolymers prepared with the AAm fraction in the polymerization mixture not exceeding 25 mol% ^1H NMR spectra show dynamic heterogeneity of copolymer chains in mesoglobules where AAm sequences and surrounding short IPMAm sequences are hydrated and mobile, while sufficiently long IPMAm sequences are dehydrated and their mobility is strongly reduced. The obtained results are consistent with the idea that P(IPMAm/AAm) copolymer mesoglobules are rather porous and disordered.

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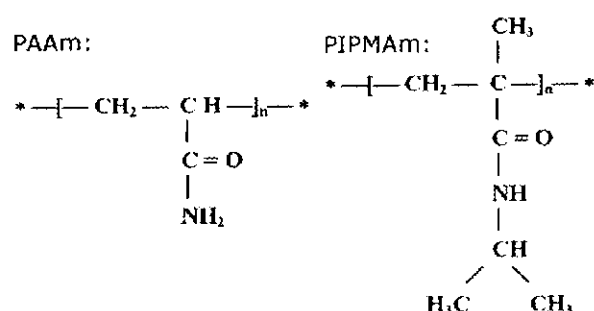
1. Introduction

It is well known that acrylamide (AAm) polymers and other polymers with amphiphilic character (e.g., poly(vinyl methyl ether) (PVME)) exhibit in aqueous solutions a lower critical solution temperature (LCST). These polymers are soluble at lower temperatures but heating of their solutions above the LCST results in phase separation [1–6]. This reversible process is probably associated with a changed balance between various types of interactions, mainly hydrogen bonds and hydrophobic interactions. Below the LCST hydrogen bonds between polymer and water are present and the polymer chains are flexible. Above the LCST transition most water is excluded from polymer structures and polymer chains are packed in rather compact globular structures (mesoglobules) [1] where hydrophobic polymer–polymer interactions prevail. Phase separation, especially at polymer concentrations $c \geq 1$ wt%, manifests itself by milk-white turbidity of solutions. Their thermosensitivity,

or their general ability to respond to various external stimuli, makes these polymers interesting for potential biomedical and technological applications, especially in the case of crosslinked polymer hydrogels, e.g., as drug release polymers [2,7,8]. Such polymers are also interesting from the academic point of view due to their behaviour analogous to that of elastin-like polypeptides at the LCST [9] and a similarity between phase separation of synthetic polymers and thermal denaturation of proteins. Of various methods used in investigations of phase-separation behaviour, NMR spectroscopy plays an important role providing information on phase-separated globular structures and interactions in these systems [10].

Although the critical solution phenomena were most often studied in poly(*N*-isopropylacrylamide) (PIPAAm) solutions, phase separation in aqueous solutions of poly(*N*-isopropylmethacrylamide) (PIPMAm) was also studied by various methods [3,4,11–18], including ^1H NMR spectroscopy [10,19–26]. The presence of the α -methyl group in the PIPMAm monomer units (Scheme 1) was found to increase the LCST compared with that of PIPAAM [3,12,13,21]. NMR study of aqueous PIPMAm solutions

* Corresponding author. Tel.: +420 296809380; fax: +420 296809410.
E-mail address: spevacek@imc.cas.cz (J. Spěváček).



Scheme 1. Structural formulae of polyacrylamide (PAAm) and poly(*N*-isopropylmethacrylamide) (PIPMAm).

showed that the LCST does not depend on polymer concentration in the range $c = 0.1$ – 10 wt% [20]. The temperature-induced phase separation in D_2O solutions of PIPMAm/PIPMAm mixtures and random copolymers was also investigated [13,21]; it was found out that in copolymer solutions only a single phase transition occurs while in polymer mixture solutions, two distinct transitions were observed. For D_2O solutions of PIPMAm and PIPMAm/PVME mixtures ($c = 5$ wt%), ^1H NMR relaxation and diffusion coefficient measurements revealed that a certain portion of water is bound in phase-separated mesoglobules at elevated temperatures [10,20,23,25,27]. Two types of bound water molecules were assigned to water bound inside mesoglobules and on their surface [25]. From ^1H NMR relaxation measurements it also follows that after a rather long induction period (~ 30 h for PIPMAm) the originally bound water is slowly released from globular-like structures with time [23,27]. Recently we also used NMR spectroscopy in investigation of the consolvency phenomenon in PIPMAm solutions. This polymer is soluble in water and ethanol at room temperature but little soluble or insoluble in their mixtures with a certain mixture composition [25,26].

In addition to temperature, phase separation can also be induced by changing other parameters and conditions such as pH, electric field, light, the presence of biochemicals, ions, etc. Phase separation in aqueous solutions of polyacrylamide (PAAm) cannot be induced by a temperature change but this polymer undergoes phase separation in the mixed solvents as a result of a change in solvent composition. Tanaka was the first who observed the coil-globule transition and the volume phase transition (collapse) with linear and crosslinked PAAm, respectively, in water/acetone mixtures [28]. While linear PAAm is completely soluble in water at

room temperature, in a mixed solvent of water and ethanol it is soluble only up to the ethanol concentration ~ 60 vol% [29].

The power of NMR spectroscopy in investigation of temperature-induced phase separation in copolymer solutions consists in its ability to provide quantitative information on the behaviour at LCST of both monomer units in a binary copolymer separately. In the present work we used ^1H NMR spectroscopy to investigate the phase separation in solutions of random poly(*N*-isopropylmethacrylamide-co-acrylamide) [P(IPMAm/AAm)] copolymers. Solutions of the copolymer in D_2O and in mixed solvents D_2O /ethanol (EtOH) and D_2O /acetone were studied. Though the LCST behaviour of P(IPMAm/AAm) copolymers in aqueous solutions was investigated by cloud point and turbidity measurements [30,31], no similar studies of P(IPMAm/AAm) copolymers have been published so far.

2. Experimental

2.1. Samples

N-isopropylmethacrylamide (IPMAm, Aldrich) and acrylamide (AAm, Fluka) were used to prepare PIPMAm homopolymer and P(IPMAm/AAm) copolymers by radical polymerization. 4,4'-Azobis(4-cyanopentanoic acid) was used as initiator and polymerization was carried out in an ethanol/water mixture (85/15 by volume) at 333 K; the weight fraction of the sum of the monomers in the mixture was 0.25, AAm mole fractions in the reaction mixture were 0.05, 0.15, 0.25, 0.40, 0.50, 0.70 and 0.85. After polymerization, diethyl ether and ethanol were used to precipitate copolymers and to wash off residual monomers and oligomers. Molecular weights M_w and M_n were determined by GPC with Superos 6 (Amersham Bio Sciences). M_w and polydispersity (M_w/M_n) values are shown in Table 1. Only the polymers investigated by ^1H NMR spectroscopy are shown in this table; copolymers which were prepared from the reaction mixtures containing more than 40 mol% of AAm did not show temperature-induced phase separation. M_w values of the investigated polymers ranged from 136 000 to 800 000, polydispersities were 1.2–2.1 (copolymers) and 3.7 (PIPMAm homopolymer). Table 1 also shows the real composition of the copolymers as determined from ^1H NMR spectra at temperatures below the phase transition. Signal B corresponding to CH protons of IPMAm units and signal H corresponding to CH_2 protons of IPMAm units as well as to CH and CH_2 protons of AAm units (cf. Fig. 1) were used to

Table 1
Molecular characteristics of investigated samples of PIPMAm, PAAm and P(IPMAm/AAm) copolymers.

Composition of reaction mixture (IPMAm/AAm monomer ratio)	Molecular weight M_w (g mol^{-1})	Polydispersity M_w/M_n	Real composition of the copolymer determined from ^1H NMR spectra (IPMAm/AAm molar ratio)
100/0	136 300	3.67	–
95/5	799 900	1.17	99/1
85/15	730 300	1.27	94/6
75/25	132 400	2.09	84/16
60/40	502 100	1.51	71/29
0/100	147 300	2.57	–

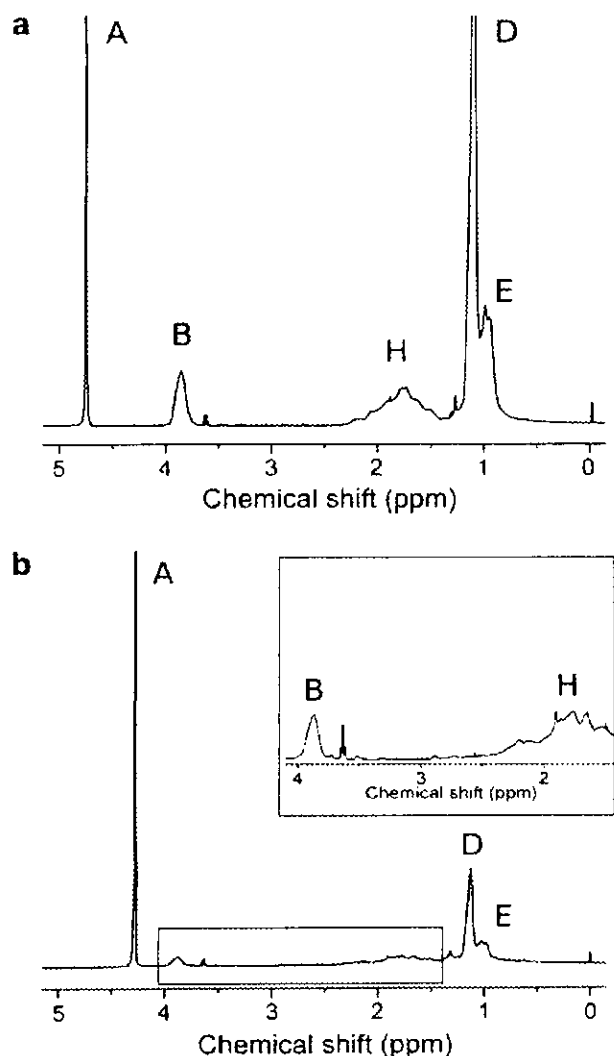


Fig. 1. ^1H NMR spectra of 75/25 (IPMAM/AAM monomer ratio in the reaction mixture) P(IPMAM/AAM) copolymer in D_2O solution ($c = 5$ wt%) measured at 298 K (a) and 340 K (b) under the same instrumental conditions. The inset in (b) shows the spectrum with higher amplification. Peak assignments are explained in the text.

determine the composition of the copolymers. The molar ratio of IPMAM and AAM units was calculated using the relation:

$$\frac{[\text{IPMAM}]}{[\text{AAM}]} = \frac{3I_B}{(I_H - 2I_B)} \quad (1)$$

where I_B and I_H are integrated intensities of the signals B and H, respectively. From Table 1 it follows that in all the cases the content of AAM units in the copolymer is somewhat lower than the content of AAM in the respective reaction mixture, thus indicating that AAM is less reactive than IPMAM. Nevertheless, taking into account the inherent error of this analysis, especially for the 95/5 polymer where the content of AAM units is small (cf. Table 1), hereafter we use for designation of P(IPMAM/AAM) copolymers the composition of the reaction mixture. Solutions of copoly-

mers with polymer concentration $c = 5$ wt% in D_2O (Sigma, 99.9% of deuterium) and in $\text{D}_2\text{O}/\text{EtOH}$ and $\text{D}_2\text{O}/\text{acetone}$ mixtures were prepared and filled into 5-mm NMR tubes, which were then degassed and sealed under nitrogen.

2.2. NMR measurements

All ^1H NMR measurements were made with a Bruker Avance 500 spectrometer operating at 500.1 MHz. Typical conditions were as follows: $\pi/2$ pulse width 14 μs , relaxation delay 10 s, spectral width 5 kHz, acquisition time 1.64 s, 8 scans. The integrated intensities were determined with a spectrometer integration software with an accuracy of $\pm 1\%$. The ^1H spin–spin relaxation times T_2 of HDO were measured using the CPMG [32] pulse sequence $90^\circ_x (t_d \ 180^\circ_y \ t_d)_n$ – acquisition with $t_d = 5$ ms. Every experiment was made with 4 scans, the relaxation delay between scans was 180 s, and spectral width 3.5 kHz. The ^1H spin–lattice relaxation times T_1 of HDO were measured using the inversion recovery pulse sequence $180^\circ - \tau - 90^\circ$ with 4 scans separated by a relaxation delay of 180 s, the spectral width being 3.5 kHz; the 10–18 τ values in the range 0.1–250 s were used. All the obtained T_1 and T_2 relaxation curves were monoexponential and the fitting process always made it possible to determine a single value of the relaxation time. In all measurements temperature was maintained constant within ± 0.2 K in the range 298–348 K using a BVT 3000 temperature unit. The samples were kept at the experimental temperature always for 15 min before the measurement.

3. Results and discussion

3.1. ^1H NMR spectra of D_2O solutions and phase-separated fraction

Fig. 1 shows high-resolution ^1H NMR spectra of a D_2O solution ($c = 5$ wt%) of P(IPMAM/AAM) copolymer with the IPMAM/AAM molar ratio in the polymerization mixture 75/25, measured under the same instrumental conditions at two temperatures (298 and 340 K). The assignment of resonances to various proton types is shown directly in the spectrum measured at 298 K, i.e., below the LCST transition. The strong peak A corresponds to HDO. Peaks B, D and E correspond to CH, $(\text{CH}_3)_2$ and $\alpha\text{-CH}_3$ groups of IPMAM units, respectively. In the band H the signal of CH_2 protons of the IPMAM units overlaps with CH and CH_2 signals of AAM units. Weak peaks at 1.29 and 3.63 ppm are due to residual ethanol. The most important effect observed in the spectrum measured at a higher temperature (340 K) is a marked decrease in the integrated intensity of all polymer lines. With the exception of the CH_3 protons of isopropyl groups of IPMAM units, other signals of the IPMAM component almost disappeared. While at 298 K the copolymer dissolves, at 340 K the solution is phase-separated and shows a milk-white turbidity. Evidently, at temperatures above the LCST the mobility of most IPMAM copolymer units in polymer chains in rather compact mesoglobules is reduced to such an extent that the corresponding lines become too broad to be detected in

high-resolution NMR spectra measured using a liquid-state spectrometer. For segments forming globular-like structures in D₂O solutions of PIPAAm or poly(*N,N*-diethylacrylamide) (PDEAAm), line widths of 3–4 kHz were detected [10,33,34], which are similar to the spectral width 5 kHz used in our measurements.

To quantitatively characterize the phase separation of IPMAm units in copolymers, we have used the values of fraction *p* of phase-separated IPMAm units (of significantly reduced mobility) determined from the relation:

$$p_{\text{IPMAm}} = 1 - \left(\frac{I}{I_0} \right) \quad (2)$$

where *I* is the integrated intensity of the given line of IPMAm units in a partly phase-separated system and *I*₀ is the integrated intensity of this line if no phase separation occurs [10,19–24,26]. For *I*₀, we took values based on integrated intensities as obtained for the respective D₂O solution at 298 K and using the fact that the integrated intensities decrease with absolute temperature as 1/*T*. Temperature dependences of the phase-separated fraction (*p*_{IPMAm}) as determined from the signal of CH protons of IPMAm units are shown for investigated polymers (including PIPMAm homopolymer) in Fig. 2. Temperature dependences of *p*_{IPMAm} determined from the signals of other proton types in IPMAm units are similar. The results obtained from the signal of CH protons in PAAm homopolymer are also shown in Fig. 2 for comparison. Both for PAAm homopolymer and P(IPMAm/AAm) copolymer with the IPMAm/AAm molar ratio in the polymerization mixture 60/40, virtually no phase separation was detected by ¹H NMR spectra, in accord with the fact that these solutions do not show any turbidity. For the remaining systems it follows from Fig. 2 that an increasing fraction of hydrophilic AAm units in the copolymer significantly

shifts the transition towards higher temperatures, broadens the transition interval and reduces the maximum value of the phase-separated fraction *p*_{max}. While for PIPMAm homopolymer the transition temperature (in the middle of the transition interval) is 316 K, the transition is relatively sharp (transition width 3 K) and *p*_{max} = 0.96, for P(IPMAm/AAm) copolymer with the IPMAm/AAm molar ratio in the polymerization mixture 75/25, the transition temperature is 330 K, the transition is ca. 14 K broad and *p*_{max} = 0.68. We assume that with increasing content of hydrophilic AAm units in P(IPMAm/AAm) copolymers the mesoglobules are more porous and disordered. Hence a relatively large portion of IPMAm segments is highly mobile even above the transition temperatures. Similar behaviour was previously observed also with aqueous solutions of negatively charged P(IPMAm-co-sodium methacrylate (MNA)) copolymers and gels of crosslinked P(DEAAm-co-MNA) copolymers [19,24,34].

Though the analysis of the phase-separation of AAm units in P(IPMAm/AAm) copolymers is complicated by the fact that CH and CH₂ signals of AAm units overlap with the CH₂ signal of IPMAm units (cf. Fig. 1), the phase-separated fraction *p* of AAm units can be obtained via the composition of the part which is directly detected in ¹H NMR spectra at temperatures above the phase transition. We made this analysis for copolymers with IPMAm/AAm molar ratios in the polymerization mixture 75/25 and 85/15 where the content of AAm units is not too small (cf. Table 1) and the phase-separated fractions of IPMAm units are still relatively high (cf. Fig. 2). By using Eq. (1) we have found that for the 75/25 copolymer the IPMAm/AAm molar ratio in the mobile part directly detected in ¹H NMR spectrum at 345 K, i.e. at temperature above the phase transition, is (IPMAm/AAm)_{above} = 63/37, while the real composition of this copolymer as determined at 310 K (below the phase transition) is (IPMAm/AAm)_{real} = 84/16 (cf. Table 1). Similarly we have found for the 85/15 copolymer that the IPMAm/AAm molar ratio in the part that is directly detected in ¹H NMR spectrum at 334 K (IPMAm/AAm)_{above} = 75/25 while the real composition of this copolymer as determined at 310 K (IPMAm/AAm)_{real} = 94/6. For both the copolymers, the higher AAm fraction in the part directly detected in NMR spectra at temperatures above the phase transition in comparison with their real composition indicates that the phase-separated fraction of AAm units is significantly smaller than the fraction of phase-separated IPMAm units as depicted in Fig. 2. The molar fraction of AAm units in the part directly detected in NMR spectra above the phase transition is given by the relation:

$$(AAm)_{\text{above}} = (1 - p_{\text{IPMAm}})(IPMAm)_{\text{real}} \left(\frac{AAm}{IPMAm} \right)_{\text{above}} \quad (3)$$

where (IPMAm)_{real} is the real molar fraction of IPMAm units in the copolymer determined at temperature below the phase transition. For the 75/25 copolymer we obtained (AAm)_{above} = (1 - 0.68) × 0.84 × (37/63) = 0.16. This value is the same as the real mole fraction of AAm units in the copolymer; hence, all AAm units are visible in the ¹H NMR spectrum measured at the temperature above the phase transition. Therefore, the fraction of phase-separated

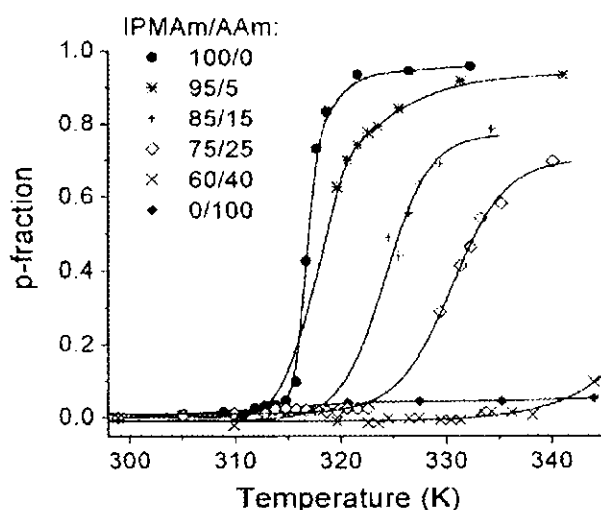


Fig. 2. Temperature dependences of the phase-separated fraction *p*_{IPMAm} as determined in D₂O solutions (*c* = 5 wt%) from integrated intensities of the CH line of IPMAm units in PIPMAm and P(IPMAm/AAm) copolymers with various IPMAm/AAm monomer ratios in the polymerization mixture. The plot obtained from integrated intensities of the CH signal of PAAm in D₂O solution is also shown.

AAM units $p_{\text{AAM}} = 0$. Also for the 85/15 copolymer, the molar fraction $(\text{AAM})_{\text{above}} = (1 - 0.77) \times 0.94 \times (25/75) = 0.07$ is virtually the same as the real molar fraction of AAM units in the copolymer (0.06). These results show that virtually all hydrophilic AAM units show high mobility even at temperatures above the phase transition. Because these AAM units are incorporated in polymer chains forming phase-separated globular structures, their rather high mobility implies that they are hydrated. The finding that AAM units in the copolymer retain a high mobility at temperatures above the phase transition throws light upon a part of IPMAM units, which retain high mobility above the phase transition (fraction $(1 - p_{\text{IPMAM}})_{\text{max}}$), which increases with increasing content of AAM units in the copolymer (cf. Fig. 2). We assume that these units are in short hydrated IPMAM sequences surrounding the AAM sequences while only sufficiently long IPMAM sequences show cooperative phase transition. These long IPMAM sequences are dehydrated and their mobility is strongly reduced. The overall picture is consistent with mesoglobules that are rather porous and disordered, as mentioned above.

3.2. Phase separation in mixed solvents $\text{D}_2\text{O}/\text{EtOH}$ and $\text{D}_2\text{O}/\text{acetone}$

Temperature dependences of the phase-separated fraction p_{IPMAM} , as determined from the signal of CH protons of IPMAM units, are shown for PIPMAM homopolymer and P(IPMAM/AAM) copolymers in mixed solvents $\text{D}_2\text{O}/\text{EtOH}$ and $\text{D}_2\text{O}/\text{acetone}$ in Figs. 3 and 4, respectively. Also in these cases the temperature dependences of p_{IPMAM} , as determined from signals of other IPMAM proton types, were virtually the same as those shown in Figs. 3 and 4. For PIPMAM homopolymer it follows from Fig. 3 that the phase transition in $\text{D}_2\text{O}/\text{EtOH}$ mixed solvent containing 20 vol% of EtOH appears at 307 K, i.e., at the temperature which is by 9 K lower in comparison with the D_2O solution. At the same time, the maximum value of the phase-separated fraction $p_{\text{max}} \approx 1$ in both cases. Similar behaviour was observed also for the 95/5 (molar IPMAM/AAM ratio in the polymerization mixture)

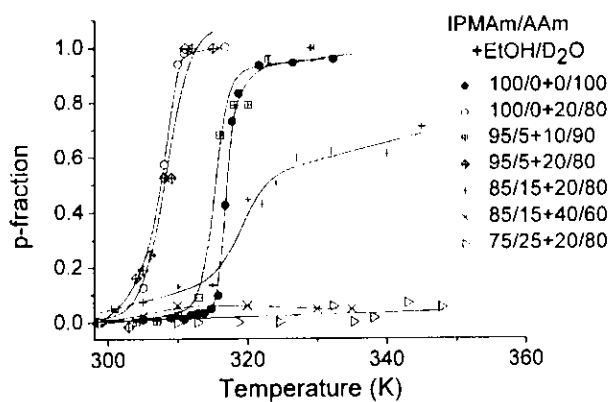


Fig. 3. Temperature dependences of the phase-separated fraction p_{IPMAM} as determined from integrated intensities of the CH line of IPMAM units in PIPMAM and P(IPMAM/AAM) copolymers with various IPMAM/AAM monomer ratios in the polymerization mixture in $\text{D}_2\text{O}/\text{EtOH}$ solutions ($c = 5 \text{ wt}\%$) of various EtOH/ D_2O volume composition.

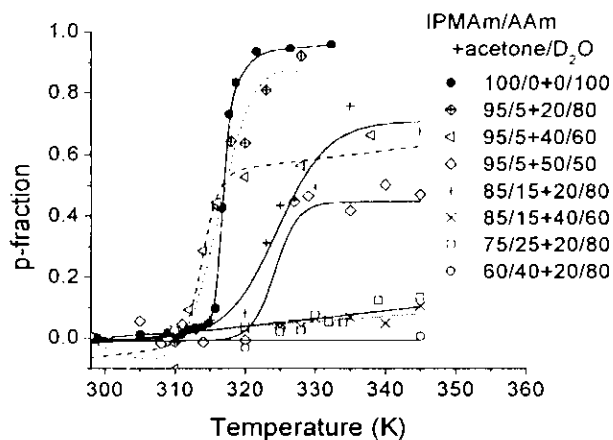


Fig. 4. Temperature dependences of the phase-separated fraction p_{IPMAM} as determined from integrated intensities of the CH line of IPMAM units in PIPMAM and P(IPMAM/AAM) copolymers with various IPMAM/AAM monomer ratios in the polymerization mixture in $\text{D}_2\text{O}/\text{acetone}$ solutions ($c = 5 \text{ wt}\%$) of various acetone/ D_2O volume composition.

P(IPMAM/AAM) copolymer where the transition temperature in $\text{D}_2\text{O}/\text{EtOH}$ containing 20 vol% of EtOH is ca. 7 K lower than in the $\text{D}_2\text{O}/\text{EtOH}$ containing 10 vol% of EtOH while $p_{\text{max}} \approx 1$ for both systems (cf. Fig. 3). For the 85/15 P(IPMAM/AAM) copolymer in $\text{D}_2\text{O}/\text{EtOH} = 80/20$ mixed solvent the transition shifts towards higher temperatures, it is broader and the maximum phase-separated fraction p_{max} is significantly reduced. No phase separation was detected for this copolymer in $\text{D}_2\text{O}/\text{EtOH}$ containing 40 vol% of EtOH, for 75/25 P(IPMAM/AAM) copolymer in $\text{D}_2\text{O}/\text{EtOH} = 80/20$ mixed solvent (cf. Fig. 3) as well as for copolymers with higher AAM fractions and/or higher EtOH contents in the mixed solvent. The shift of the LCST towards lower temperatures was previously reported for PIPAAm solutions in water/methanol mixtures with the methanol content below 50 vol% and explained by the cononsolvent character of these mixtures [35]. On the other hand, the described behaviour is in contrast to what was observed with PVME solutions in $\text{D}_2\text{O}/\text{ethanol}$ mixtures where the transition region continuously shifts towards higher temperatures and the maximum values of the phase-separated fraction p_{max} decrease with increasing EtOH content in the $\text{D}_2\text{O}/\text{EtOH}$ mixture [36].

For the 95/5 P(IPMAM/AAM) copolymer in $\text{D}_2\text{O}/\text{acetone}$ mixed solvent the transition temperature was virtually the same for the solvents containing 20 and 40 vol% of acetone, but the maximum phase-separated fraction p_{max} was significantly lower in the latter case. A further increase in the acetone fraction in $\text{D}_2\text{O}/\text{acetone}$ reduced the p_{max} and shifted the transition of this copolymer towards higher temperatures (Fig. 4). From this figure it also follows that virtually no phase separation was found for the 85/15 P(IPMAM/AAM) copolymer in $\text{D}_2\text{O}/\text{acetone}$ mixture containing 40 vol% of acetone, and for 75/25 and 60/40 P(IPMAM/AAM) copolymers in $\text{D}_2\text{O}/\text{acetone} = 80/20$ mixed solvent.

We assume that the described complex behaviour of PIPMAM and P(IPMAM/AAM) copolymers in $\text{D}_2\text{O}/\text{EtOH}$ solutions is mainly in connection with cononsolvency effects where water–EtOH interactions (hydrogen bonds)

are preferred to polymer–water hydrogen bonds. However, it is clear that the presence of AAm units in the copolymer chains weakens the resulting consolvency effect. Among others, this manifests itself by the fact that while for PIPMAm homopolymer the largest consolvency effect was found at 298 K in D₂O/EtOH mixture containing 40 vol% of EtOH ($p \approx 1$) [25,26], for the 85/15 P(IPMAm/AAm) copolymer in the same mixed solvent $p \approx 0$ not only at 298 K, but in the whole temperature range. The different behaviour of PIPMAm homopolymer and P(IPMAm/AAm) copolymers on the one hand and PVME on the other can be associated with the fact that while polymer–polymer hydrogen bonds might be formed between IPMAm sequences in the globular state, as shown by infrared spectroscopy for aqueous solutions of PIPMAm and P(IPMAm-co-MNA) copolymers [14,24,37], similar polymer–polymer hydrogen bonding cannot exist in PVME solutions. Moreover, the thermodynamic quality of the components of the mixed solvent relative to polymer is probably also important.

Similarly to D₂O solutions discussed in Section 3.1, also in D₂O/EtOH and D₂O/acetone mixed solvents we analyzed the phase-separation behaviour of AAm units in P(IPMAm/AAm) copolymers. For this purpose we used the 85/15 P(IPMAm/AAm) copolymer where the phase-separated fraction of IPMAm component at 345 K $p_{IPMAm} = 0.69$ and 0.67 in D₂O/EtOH and D₂O/acetone mixed solvents containing 80 vol% of D₂O, respectively. Using Eq. (1) we have found that the IPMAm/AAm molar ratio in the mobile part which can be directly detected in ¹H NMR spectrum at 345 K is (IPMAm/AAm)_{above} = 83/17 and 86/14 for D₂O/EtOH and D₂O/acetone, respectively, whereas the real composition of this copolymer is (IPMAm/AAm)_{real} = 94/6 (cf. Table 1). Using Eq. (3) it follows for the D₂O/EtOH mixed solvent that the mole fraction of AAm units in the part directly detected in NMR spectra at 345 K, (AAm)_{above} = 0.06; for D₂O/acetone mixed solvent, (AAm)_{above} = 0.05. The first value is exactly the same and the other value is virtually the same as the real mole fraction of AAm units in the copolymer. Thus, also in both mixed solvents virtually all hydrophilic AAm units are rather mobile and, therefore, directly detected in high-resolution NMR spectra even at temperatures above the phase transition. Similarly, as we discussed in Section 3.1., for D₂O solutions this implies that AAm sequences as well as short surrounding IPMAm sequences in polymer chains forming mesoglobules extensively interact with solvent molecules.

3.3. ¹H spin-lattice and spin-spin relaxation times of HDO

Figs. 5 and 6 show the temperature dependences of spin-lattice relaxation time T_1 and spin-spin relaxation time T_2 , respectively, of HDO molecules in D₂O solutions of PIPMAm homopolymer and 95/5 P(IPMAm/AAm) copolymer. For these two polymers, the temperature dependences of the phase-separated fraction p_{IPMAm} are rather similar (cf. Fig. 2). Similar T_1 and T_2 values as shown in these figures for the 95/5 P(IPMAm/AAm) copolymer were obtained at selected temperatures also for HDO in a D₂O solution of 85/15 P(IPMAm/AAm) copolymer where at 301 K and 332 K, $T_1 = 26.7$ and 6.0 s, and $T_2 = 3.8$ and 0.8 s, respectively. Both

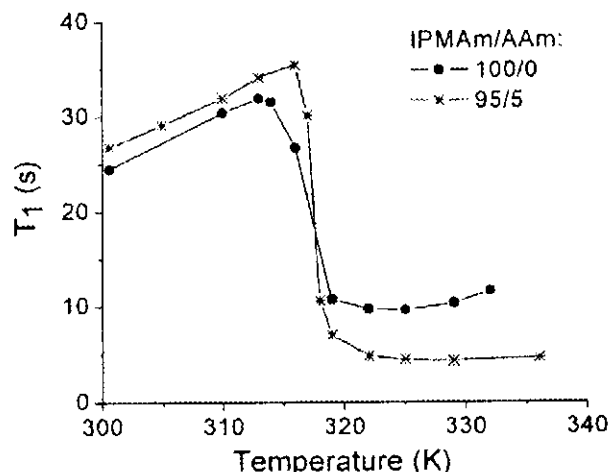


Fig. 5. Temperature dependences of ¹H spin–lattice relaxation time T_1 of HDO in D₂O solutions ($c = 5$ wt%) of PIPMAm (●) and 95/5 P(IPMAm/AAm) copolymer (*).

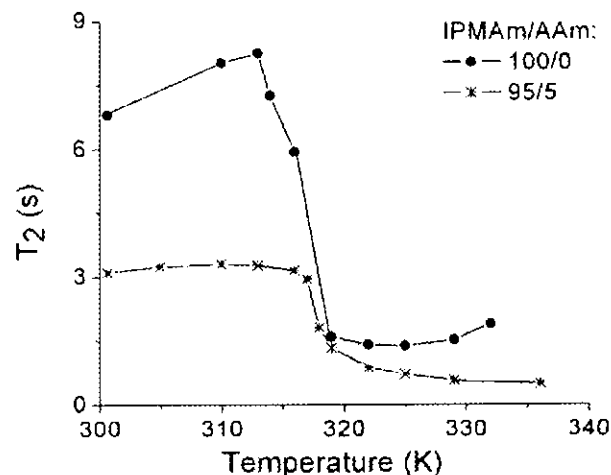


Fig. 6. Temperature dependences of ¹H spin–spin relaxation time T_2 of HDO in D₂O solutions ($c = 5$ wt%) of PIPMAm (●) and 95/5 P(IPMAm/AAm) copolymer (*).

T_1 and T_2 dependences show a decrease in the LCST transition; the transition region (Figs. 5 and 6) is in accord with that obtained from the phase-separated fraction p_{IPMAm} (cf. Fig. 2). Similar dependences of spin–lattice and spin–spin relaxation time of water (HDO) were previously reported for aqueous solutions of PIPAAm, P(IPAAm-co-ethylene glycol) and P(IPMAm-co-MNA) copolymers, and PIPMAm/PVME mixtures [20,24,38,39]. The reduced T_1 and T_2 values of HDO at temperatures above the phase transition show the existence of a portion of HDO molecules that exhibit a lower, spatially restricted mobility. Evidently, this portion corresponds to HDO bound in mesoglobules [10]. The single-exponential character of relaxation curves indicates a fast exchange between bound and free water molecules. In such case, the observed relaxation rates at temperatures above the LCST transition are given as weighted average of the relaxation rates of bound and free HDO molecules

[20,22–24,27]. While T_1 dependences for D_2O solutions of PIPMAm and the 95/5 P(IPMAm/AAm) copolymer are rather similar, at least at temperatures below the phase transition (Fig. 5), more pronounced differences between these two systems can be seen in T_2 dependences (Fig. 6). From Figs. 5 and 6 it follows that at temperatures below the phase transition the T_2 values of HDO in solution of 95/5 P(IPMAm/AAm) copolymer are significantly shorter than in PIPMAm solution and this tendency is preserved also at temperatures above the phase transition both for T_2 and T_1 values. This is probably associated with increasing polymer–water interactions in the system where polymer chains contain hydrophilic AAm units. At temperatures above the phase transition we assume that the shorter T_2 and T_1 of HDO in solutions of 95/5 and 85/15 P(IPMAm/AAm) copolymers, compared with PIPMAm solution, reflect the existence of hydrated AAm and IPMAm sequences in copolymer mesoglobules, which can result in larger amounts of bound HDO.

The LCST phase transition is also observed in temperature dependences of the line width $\Delta\nu$ of the HDO signal as shown in Fig. 7 where temperature dependences of $\Delta\nu$ are plotted for D_2O solutions of PIPMAm and P(IPMAm/AAm) copolymers. An increase in $\Delta\nu$ in the temperature region of the phase transition was observed both in solutions of PIPMAm homopolymer and 95/5 and 85/15 P(IPMAm/AAm) copolymers. With increasing content of AAm units in the copolymers the $\Delta\nu$ -transition shifts to higher temperatures, in accord with temperature dependences of the phase-separated fraction p_{IPMAm} shown in Fig. 2. From $\Delta\nu$ values of the HDO line (Fig. 7) it also follows that the transition is less pronounced in P(IPMAm/AAm) copolymers in comparison with PIPMAm homopolymer. Virtually no increase in the HDO line width was found for 75/25 and 60/40 P(IPMAm/AAm) solutions. The behaviour of $\Delta\nu$ of HDO in the phase transition is not surprising because, assuming a Lorentzian line shape, a simple relation exists between the line width $\Delta\nu$ and spin–spin relaxation time

T_2 , $\Delta\nu = (\pi T_2)^{-1}$. In contrast to this fact, the values of $\Delta\nu$ in Fig. 7 are at least one order of magnitude larger than those calculated from T_2 values. This implies that the $\Delta\nu$ values in Fig. 7 (both below and above the phase transition) are predominantly affected by inhomogeneous line broadening.

4. Conclusion

In this work we applied 1H NMR spectroscopy to investigate temperature-induced phase separation in D_2O , D_2O /ethanol and D_2O /acetone solutions of P(IPMAm/AAm) random copolymers. The effects of P(IPMAm/AAm) composition and the ethanol or acetone contents in mixed solvents on the temperature, width and extent (the phase-separated fraction) of the phase transition were quantitatively characterized. For copolymers prepared with the AAm content in the polymerization mixture not exceeding 25 mol%, we have found that at temperatures above the LCST transition the mobility of most IPMAm units is reduced to such an extent that corresponding NMR lines become too broad to be detected in high-resolution spectra, evidently due to phase separation and formation of mesoglobules. The increasing fraction of hydrophilic AAm units in the copolymer significantly shifts the transition towards higher temperatures, broadens the transition interval and reduces the maximum value of the phase-separated fraction p_{max} of IPMAm units with reduced mobility. Apart from the cononsolvency effect in D_2O /EtOH mixtures, the increasing content of acetone or EtOH in mixed solvents with D_2O affects the phase transition similarly to an increasing fraction of AAm units in the copolymer. In contrast to IPMAm units, virtually all hydrophilic AAm units are directly detected in high-resolution NMR spectra of these systems even at temperatures above the phase transition; therefore, the phase-separated fraction of AAm units $p_{AAm} \approx 0$. This applies to both D_2O solutions and solutions in D_2O /EtOH and D_2O /acetone mixtures. Because the AAm units are incorporated in polymer chains forming phase-separated globular structures, their rather high mobility suggests that they extensively interact with solvent molecules, i.e., they are hydrated in D_2O . This fact results in dynamic heterogeneity of copolymer chains in mesoglobules where AAm sequences and surrounding short IPMAm sequences are hydrated and mobile, while the sufficiently long IPMAm sequences are dehydrated and their mobility is strongly reduced. From temperature dependences of the spin–lattice relaxation time T_1 and spin–spin relaxation time T_2 of HDO molecules in D_2O solutions of PIPMAm homopolymer and 95/5 and 85/15 P(IPMAm/AAm) copolymers, it follows that a portion of HDO molecules is bound in mesoglobules. At the same time the amounts of bound HDO are probably larger in the mesoglobules formed by copolymer chains than with PIPMAm homopolymer. All these results are consistent with the assumption that P(IPMAm/AAm) copolymer mesoglobules are rather porous and disordered.

Thermoreversible copolymers in general enable convenient tuning of the LCST [8]. However, on the basis of the obtained results with P(IPMAm/AAm) copolymers, one can speculate that the changes in the character of the

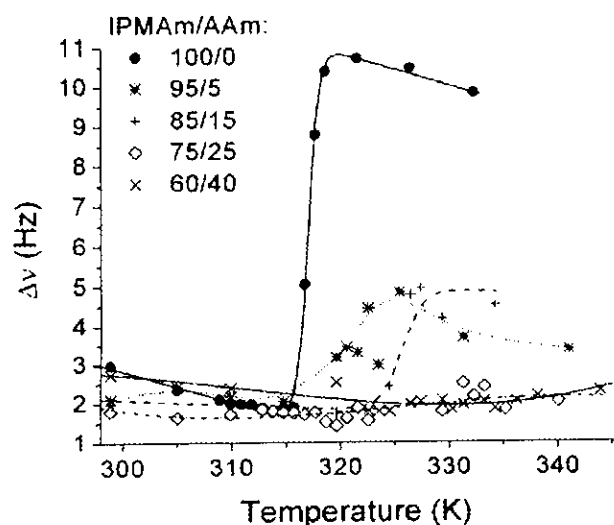


Fig. 7. Temperature dependences of HDO NMR line width $\Delta\nu$ for D_2O solutions ($c = 5$ wt%) of PIPMAm and P(IPMAm/AAm) copolymers with various IPMAm/AAm monomer ratios in the polymerization mixture.

transition (larger transition width) and in the structure of mesoglobules, i.e., the existence of domains with hydrated AAm and IPMAm sequences, might adversely affect the efficiency of random copolymers containing hydrophilic units in some applications, such as drug delivery.

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