# Polymer-Solvent Interactions in Solutions of Thermoresponsive Polymers Studied by NMR and IR Spectroscopy

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**Summary:** NMR relaxation and diffusion coefficient measurements revealed that a portion of water molecules is bound in mesoglobules formed in poly(*N*-isopropylmethacrylamide) (PIPMAm) and poly(vinyl methyl ether) (PVME) aqueous solutions above the LCST, with fast exchange between bound and free states (residence time ~1 ms). Two types of bound water molecules were assigned to water bound inside mesoglobules and on their surface. For highly concentrated PVME/$D_2O$ solutions ($c \geq 20$ wt%) a slow exchange was detected by NMR for bound water (residence time = 2.1 s). For PIPMAm aqueous solution IR spectra indicate a two-steps character of the phase transition. For PIPMAm in $D_2O$/ethanol (EtOH) mixtures the globular structures were observed by NMR at 298 K for certain compositions of the mixed solvent (cononsolvency effect). Virtually no EtOH is bound in these globular structures, in contrast to the temperature-induced globular structures.

**Keywords:** FT-IR; NMR; phase separation; poly(*N*-isopropylmethacrylamide); poly(vinyl methyl ether)

## Introduction

It is well known that some acrylamide-based polymers and other polymers with amphiphilic character like poly(vinyl methyl ether) (PVME) exhibit in aqueous solutions a lower critical solution temperature (LCST). They are soluble at low temperatures, but heating above the LCST results in phase separation which is especially at polymer concentrations $c \geq 1$ wt% macroscopically manifested by milk-white turbidity of the solution.[1,2] On the molecular level both phase separation in solutions and similar volume phase transition (collapse) in crosslinked hydrogels are assumed to be a macroscopic manifestation of a coil-globule transition, as was shown for poly(*N*-isopropylacrylamide) (PIPAAm) in water, e.g., by light scattering,[2,3] followed by further aggregation and formation of rather compact globular-like structures, also called mesoglobules,[2] which are colloidally stable in solution. The phase transition is probably associated with the changed balance between various types of interactions, mainly hydrogen bonds and hydrophobic interactions. Their thermosensitivity makes these systems interesting for possible biomedical and technological applications, e.g., as drug release systems. A similarity to the LCST behaviour of elastin-like polypeptides[4] and to thermal denaturation of proteins in aqueous solution also makes them interesting from an academic point of view. Among various methods used in the investigations of phase separation behaviour, both NMR[5] and vibrational (infrared (IR) and Raman)[6] spectroscopies provide information on phase-separated

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globular structures and interactions in these systems.

In this work we deal with phase transitions in solutions of poly(*N*-isopropylmethacrylamide) (PIPMAm) and PVME. PIPMAm in aqueous solution represents here systems where the LCST is well below the temperature of the glass transition $T_g$ of the respective polymer in bulk and therefore where one can expect that polymer segments in globular-like structures are rather rigid. For PIPMAm ($T_g = 449$ K)[7] aqueous solutions the LCST is around 315 K. On the other hand PVME in aqueous solution represents systems where the LCST is well above the $T_g$ of the respective polymer in bulk and consequently the polymer segments in globular-like structures are highly flexible. For PVME aqueous solutions the LCST is around 308 K, while $T_g$ values in the range $T_g = 191$–$251$ K are reported for PVME in bulk.[8] We present here some new results on hydration and other polymer-solvent interactions during the temperature-induced and solvent-composition-induced phase separation as obtained on solutions of PIPMAm and PVME using NMR methods, in some cases in combination with IR spectroscopy.

## Experimental Part

### Samples

PVME (purchased from Aldrich, supplied as 50 wt% solution in water; molecular weight determined by GPC in THF: $M_w =$ 60 500, $M_w/M_n \cong 3$, tacticity by $^1$H NMR: 59% of isotactic diads[9]) was used after drying to prepare PVME/$D_2O$ (99.9% of deuterium) solutions with polymer concentrations in the range c = 5–60 wt%. PIPMAm was prepared by polymerization of IPMAm initiated by 4,4′-azobis(4-cyanopentanoic acid) and carried out in ethanol/water mixture (94/6 by volume); the volume fraction of the monomer in the mixture was 0.2. After polymerization and subsequent drying the solutions of PIPMAm in $D_2O$ [$D_2O$/ethanol (EtOH) mixtures in some cases] of desired polymer concentration (c = 5–20 wt%) were prepared. All solutions for NMR measurements were degassed and kept in 5 mm NMR tubes sealed under argon.

### NMR Measurements

$^1$H and $^{13}$C NMR spectra were mostly recorded with a Bruker Avance 500 spectrometer operating at 500.1 and 125.7 MHz, respectively. Some $^1$H NMR relaxation and diffusion measurements were done on Bruker Avance 300 NMR spectrometer at 300.1 MHz; in the pulsed field-gradient diffusion experiments a water cooled gradient probe and a special gradient unit BGU2 were used. In all measurements the temperature was maintained constant within ±0.2 K using a BVT 3000 temperature unit. The $^1$H spin-spin relaxation times $T_2$ of HDO were measured using the CPMG[10] pulse sequence $90^\circ_x$-($t_d$-$180^\circ_y$-$t_d$)$_n$-acquisition with $t_D = 5$ ms. The $^{13}$C $T_2$ relaxation times of ethanol carbons were measured using CPMG sequence with $^1$H 180° pulse added to remove cross-correlation between chemical shift anisotropy and dipole-dipole interactions.[11] All obtained $T_2$ relaxation curves had the monoexponential character. The BPPSTE pulse sequence[12] was used in measurements of the diffusion coefficients while the PGSE pulse sequence[13] was used in $^1$H $T_2$ measurements with diffusion filter. A modified DPFGSE pulse sequence[14] was used in one-dimensional exchange $^1$H NMR experiment to detect a slow exchange in highly concentrated PVME/$D_2O$ solution. This sequence was combined with a spin-echo $T_2$ filter to suppress the broad signal from PVME protons;[15] the Gaussian-shaped pulses[16] were used for selective excitation of the main HDO signal.

## Results and Discussion

### PIPMAm and PVME in $D_2O$ – Hydration in Globular Structures

Figure 1 shows the results of the measurements of spin-spin relaxation times $T_2$ of HDO in $D_2O$ solutions ($c = 5$ wt%) of

 

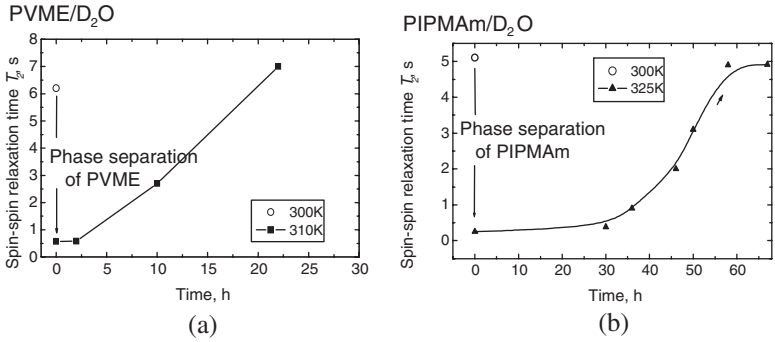


***Figure 1.***
Time dependences of spin-spin relaxation times $T_2$ of HDO in $D_2O$ solutions ($c = 5$ wt%) of PVME (a) and PIPMAm (b) measured at 500.1 MHz and 310 K (a) and 325 K (b).[21]

PVME and PIPMAm. In both cases the $T_2$ values at temperatures above the phase transition (310 and 325 K for PVME and PIPMAm solutions, respectively) were 1 order of magnitude shorter than those at 300 K, i.e., at temperature below the transition. This shows that at temperatures above the transition there is a portion of HDO molecules that exhibit a lower, spatially restricted mobility. Evidently, this portion corresponds to HDO bound in globular-like structures.[5,15,17–21] Interestingly enough, from Figure 1 it follows that at temperatures above the phase transition $T_2$ values increase with time showing that originally bound water is very slowly released from globular-like structures, in contrast to the fact that phase

separation itself is probably rather fast (faster than 1 s in PIPAAm aqueous solutions).[22,23] The main reason for the large difference both in the induction period (it characterizes the ''plateau'' in the time dependence of $T_2$) and in the time characterizing the process of expelling water from mesoglobules (during this time $T_2$ values increase) as found for PVME and PIPMAm is probably much higher mobility of PVME segments in mesoglobules in comparison with PIPMAm segments which are in glassy state in mesoglobules.[21] In both cases there was a single line of HDO in $^1$H NMR spectrum and the $T_2$ relaxation curves were exponential, indicating a fast exchange between bound and free sites. A contribution from the chemical exchange to the spin-spin relaxation rate $(T_2)^{-1}$ is also important as documented in Figure 2. This figure shows the dependence of spin-spin relaxation rate on the time interval $t_D$ in CPMG pulse sequence for HDO protons in PIPMAm/$D_2O$ solution ($c = 20$ wt%) at 320 K, i.e., above the LCST. Solid curve in this figure shows the best fit as obtained using the equation[24]

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

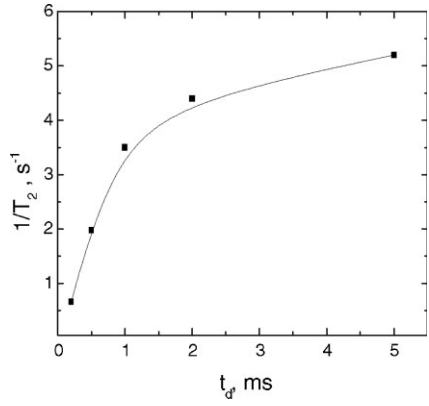


***Figure 2.***
Dependence of spin-spin relaxation rate $(T_2)^{-1}$ on the interval $t_d$ in CPMG sequence as obtained at 300.1 MHz for HDO protons in PIPMAm/$D_2O$ solution ($c = 20$ wt%) kept at 320 K.

 

with $k_{ex} = 2660\ s^{-1}$. Here $k_{ex}$ is the rate constant for exchange process, $(R_2)^0$ is the average spin-spin relaxation rate in the absence of the exchange, $p_A$ and $p_B$ are populations of the states, $\Delta$ is the chemical shift difference between the states and $\omega$ is the resonance frequency. For PIPMAm$D_2O$ solution at 320 K therefore the exchange time (or the residence time of the bound HDO) $\tau_{ex} = 1/k_{ex}$ is $\tau_{ex} = 0.4$ ms. For PVME/$D_2O$ ($c = 6$ wt%) at 309.5 K we found that $\tau_{ex} = 1.2$ ms.[25] We assume that slightly faster exchange as found for PIPMAm/$D_2O$ solution in comparison with PVME/$D_2O$ solution is mainly due to higher temperature.

The evidence that a certain portion of water is bound in mesoglobules formed above the LCST follows also from NMR diffusion measurements. Figure 3 shows the diffusion curve, i.e., dependence of signal intensity on the gradient strength for HDO in PIPMAm/$D_2O$ solution ($c = 5$ wt%) at 320 K. While the diffusion curve of HDO in the neat $D_2O$ measured at the same temperature is monoexponential with diffusion coefficient $D = 3.1 \times 10^{-9}$ m$^2$/s, in PIPMAm/$D_2O$ solution the diffusion curve is biexponential with diffusion coefficients $D_1 = 5.7 \times 10^{-9}$ m$^2$/s and $D_2 = 4.8 \times 10^{-11}$ m$^2$/s. While the diffusion coefficient $D_1$ can be assigned to the free HDO, the two orders of magnitude smaller diffusion coefficient $D_2$ apparently corresponds to HDO molecules bound in mesoglobules.

The fact that diffusion of HDO molecules bound in mesoglobules is significantly slower in comparison with free HDO can be used to separate the $T_2$ values from various types of HDO existing in PIPMAm/$D_2O$ solution above the LCST which all undergo a fast exchange and contribute to the same NMR signal. First, by using so-called spin-echo $T_2$ relaxation filter the signal from the rapidly relaxing bound HDO molecules can be suppressed and only the signal of free HDO can be detected. For free HDO in PIPMAm/$D_2O$ solution ($c = 20$ wt%) at 320 K we found at 300.1 MHz in this way the value $T_2 = 7.2$ s. Using a diffusion filter with the sufficient diffusion filter time the signal from the rapidly diffusing free HDO is suppressed and for $T_2$ we obtained the value $T_2 = 0.2$ s. We assigned this $T_2$ to the HDO bound inside the mesoglobules. Finally, a combination of relaxation and diffusion filters results in intermediate $T_2 = 2.9$ s; we assumed that this value corresponds to HDO bound on the surface of mesoglobules. These results therefore show that three types of HDO molecules are simultaneously present in PIPMAm/$D_2O$ solution at temperature above the LCST: (i) free HDO; (ii) HDO interacting with the surface of mesoglobules; (iii) HDO bound inside the mesoglobules. Figure 4 shows the time dependences of $T_2$ values corresponding to the HDO interacting with the mesoglobules surface and to the HDO bound inside the mesoglobules. The investigated PIPMAm/$D_2O$ sample was kept for all the time at 320 K, i.e, above the LCST. While in the first case the $T_2$ values are virtually time independent, in the latter case the $T_2$ values after some induction period slowly increase with time in similar way as shown in Figure 1. From this result it follows that the releasing process of HDO molecules as documented already in Figure 1 is connected with HDO originally bound inside the mesoglobules.

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 shows a slow exchange process.[15] At

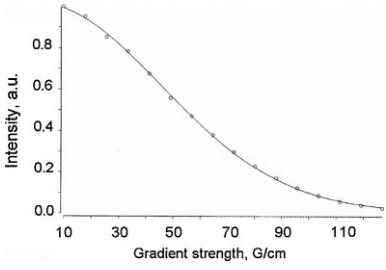


***Figure 3.***
Diffusion curve for HDO in PIPMAm/$D_2O$ solution ($c = 5$ wt%) at 300.1 MHz and 320 K.

 

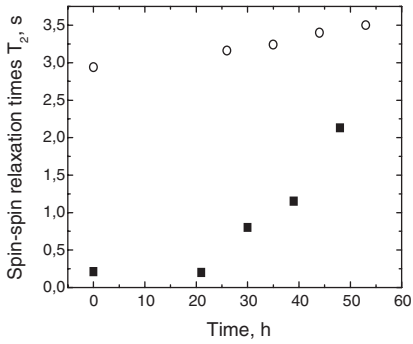


*Figure 4.*
Time dependences of spin-spin relaxation times $T_2$ of two components of "bound" HDO in PIPMAm/$D_2O$ solution ($c = 20$ wt%) as obtained at 300.1 MHz and 320 K using a diffusion filter (■) and a combination of relaxation and diffusion filters (○).

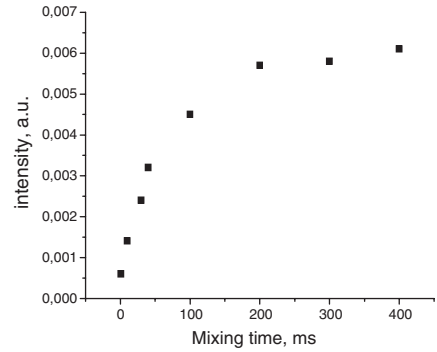


*Figure 5.*
Dependence of the difference intensity of the signal of the bound HDO as a function of the mixing time in 1D DPFGSE NMR exchange experiment for concentrated PVME/$D_2O$ solution ($c = 50$ wt%) at 500.1 MHz and 309.5 K.

the same time the fractions of bound water were unchanged even for 70 h; from their values it follows that the polymer concentration in mesoglobules is 89 wt%, in accord with the phase diagram.[25,26] To detect a slow exchange in highly concentrated PVME/$D_2O$ solution ($c = 50$ wt%) we applied an one-dimensional exchange $^1H$ NMR experiment with selective excitation of the main HDO signal. The dependence of the difference intensity of the signal of the bound HDO as a function of the mixing time is shown in Figure 5. From the initial linear portion of this curve we obtained for the rate constant of the exchange process the value $k_{F\rightarrow B} = 0.063\ s^{-1}$; here subscripts F and B correspond to the free and bound HDO, respectively. Taking into account that the following equation holds

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

where $b$ is the fraction of the bound HDO (for PVME/$D_2O$ with $c = 50$ wt%, $b = 0.12$[15]), we obtain for the residence time of the bound HDO the value $\tau_B = 1/k_{B\rightarrow F} = 2.1$ s. This value is three orders of magnitude larger than the residence time of bound HDO for PVME/$D_2O$ solution with $c = 6$ wt%.

From IR studies of PIPMAm/$H_2O$ solutions (Golden Gate™ Heated Diamond ATR Top-Plate was used in these measurements) it follows that heating above the LCST results in the shift of amide I (1610–1625 $cm^{-1}$) and amide II (1525–1540 $cm^{-1}$) bands towards higher and lower wavenumbers, respectively.[6,17,27,28] For amide I band, which is mainly due to the C=O stretching vibrations, these changes are in connection with the fact that while at temperatures below the LCST this band contains a single component that reflects C=O...H–O polymer-water hydrogen bonds, the second component due to C=O...H–N polymer-polymer hydrogen bonds exists at temperatures above the LCST.[6] Also the $CH_3$ antisymmetric stretching band (2975–2982 $cm^{-1}$) exhibits a shift towards lower wavenumbers at temperatures above the LCST which indicates dehydration of the alkyl groups.[6,28] In Figure 6 the wavenumbers of amide I and $CH_3$ antisymmetric stretching bands are plotted as function of temperature during gradual heating and cooling of PIPMAm/$H_2O$ solution ($c = 10$ wt%). From comparison of the temperature dependences (during heating) of the band positions it follows that the transition temperature of hydrophilic C=O groups (as reflected in amide I band) is ~2 K higher than the transition temperature of hydrophobic $CH_3$ groups (as reflected

 

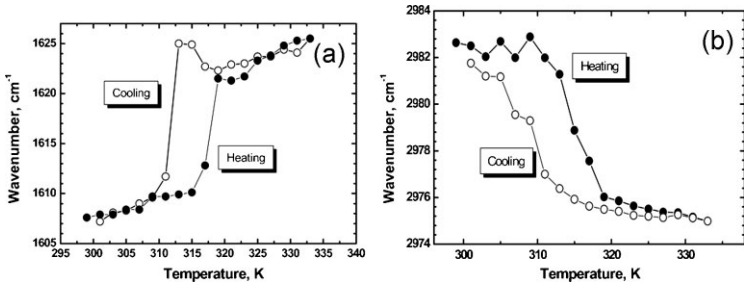


***Figure 6.***
Temperature dependences of wavenumbers of amide I (a) and $CH_3$ antisymmetric stretching (b) bands during gradual heating and cooling in ATR IR spectra of PIPMAm/$H_2O$ solution ($c = 10$ wt%).[17]

in $CH_3$ stretching band).[17] This result suggests a two-steps character of the phase transition. Similar result was recently reported from 2D IR spectra for PVME aqueous solutions.[29] Figure 6 also shows a hysteresis for gradual heating and cooling; the transition temperature during cooling is ~6 K lower than that during gradual heating. In accord with other authors we assume that this hysteresis is associated with polymer-polymer hydrogen bonding in the globular state.[6,17]

### PIPMAm in $D_2O$/Ethanol Mixtures – Phase Separation Due to Cononsolvency

Figure 7 shows three $^1$H NMR spectra of PIPMAm solutions ($c = 5$ wt%), all measured at room temperature. The spectrum on the top is from PIPMAm/$D_2O$ solution while other two spectra were obtained in $D_2O$/ethanol (EtOH) mixtures containing 30 or 40 vol% of EtOH. From the figure it follows that intensities of all polymer signals are strongly reduced in the spectrum measured in the $D_2O$/EtOH mixture containing 30 vol% of EtOH and in the mixture containing 40 vol% of EtOH all polymer signals completely disappeared in the spectrum due to the marked line broadening. Together with the fact that both samples in $D_2O$/EtOH mixtures are cloudy therefore $^1$H NMR spectra also show a phase separation and formation of globular-like structures in these systems.[5,9,17,19,21] In contrast to previous part where phase separation was induced by temperature, in $D_2O$/EtOH mixtures the phase separation has to be ascribed to the cononsolvency. While both $D_2O$ and EtOH are good solvents of PIPMAm, their mixture with 40 vol% of EtOH is evidently a nonsolvent of this polymer. Similarly as established for PIPAAm in water/alcohol mixtures[30,31] we assume that also for PIPMAm in $D_2O$/EtOH mixtures the cononsolvency results from the fact that water-EtOH interactions are preferred to PIPMAm-water hydrogen bonds. The values of the phase-separated fraction $p$ (units in globular-like structures) were determined from the comparison of absolute integrated intensities of $^1$H NMR lines;

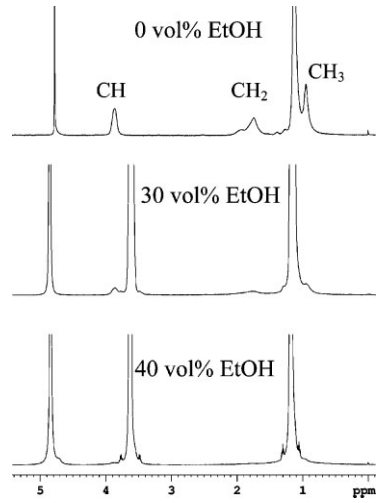


***Figure 7.***
$^1$H NMR spectra of PIPMAm in $D_2O$ and $D_2O$/EtOH mixtures measured at 500.1 MHz and 298 K.

 

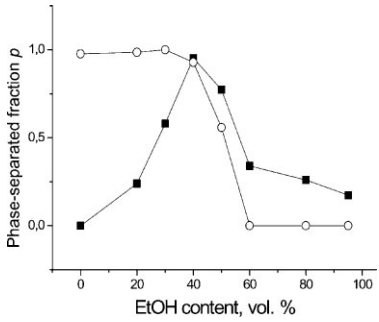


***Figure 8.***
Phase-separated fraction $p$ for PIPMAm solutions in $D_2O$/EtOH mixtures ($c = 5$ wt%) as determined for $CH_2$ protons of PIPMAm at 500.1 MHz and 298 K (■) and 328 K (○).

figure it follows that at 298 K all $T_2$ values are almost the same showing that virtually no EtOH is bound in globular-like structures. On the other hand at 328 K, i.e., at temperature above the LCST, $T_2$ value observed for PIPMAm solution in $D_2O$/EtOH mixture containing 20 vol% EtOH (where phase-separated fraction $p = 1$, cf. Figure 8) is one order of magnitude shorter in comparison with other cases. This result shows that a certain portion of EtOH molecules is bound in globular structures induced by temperature in PIPMAm solution in $D_2O$/EtOH mixture containing 20 vol% EtOH, similarly as we previously found for PVME solutions in $D_2O$/EtOH

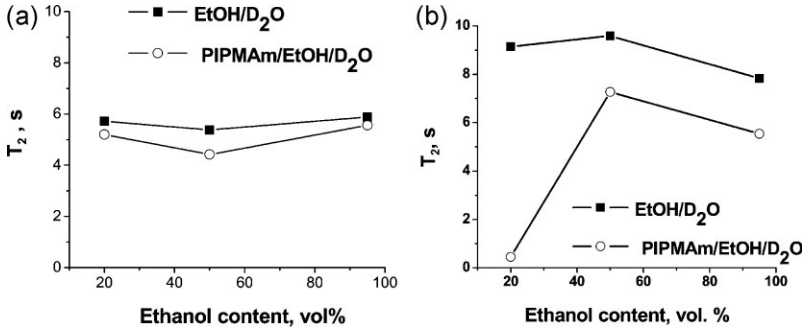


***Figure 9.***
$^{13}C$ spin-spin relaxation times of EtOH $CH_3$ carbons for PIPMAm solutions in $D_2O$/EtOH mixtures ($c = 5$ wt%) and $D_2O$/EtOH mixtures (without PIPMAm) measured at 125.7 MHz and 298 K (a) and 328 K (b).

they are plotted for two temperatures as function of EtOH content in $D_2O$/EtOH mixtures in Figure 8. While at 298 K the fraction $p$ shows a relatively sharp maximum for EtOH content 40 vol%, at 328 K, i.e., at temperature above the LCST there is a flat maximum for EtOH content 0–40 vol%. At room temperature a similar cononsolvency efffect was found for PIPMAm networks in water/EtOH mixtures; for EtOH content ~40 vol%, a pronounced swelling minimum was observed.[32]

Figure 9 shows $^{13}C$ spin-spin relaxation times $T_2$ of EtOH $CH_3$ carbons; in addition to PIPMAm solutions in $D_2O$/EtOH mixtures ($c = 5$ wt%) the values obtained for $D_2O$/EtOH mixtures (without polymer) are also shown for comparison. From this mixtures.[33] The finding that no EtOH is bound in globular structures formed in $D_2O$/EtOH mixtures due to the cononsolvency, while a portion of EtOH is bound in globular structures induced by temperature indicates a different character of globular structures in both cases.

## Conclusion

By using $^1H$ NMR methods, combined with IR spectroscopy in some cases, we investigated hydration and other polymer-solvent interactions in aqueous solutions of PVME and PIPMAm. The main results can be summarized as follows: (*i*) NMR relaxation and diffusion coefficient measurements

 

revealed that a portion of water molecules is bound in mesoglobules formed in PIPMAm and PVME aqueous solutions ($c \approx 5$ wt%) above the LCST, with fast exchange between bound and free states (residence time $\sim$1 ms). Two types of bound water molecules were assigned to water bound inside mesoglobules and on their surface. (*ii*) For highly concentrated PVME/$D_2O$ solutions ($c \geq 20$ wt%) a slow exchange process was detected by NMR for bound water (residence time = 2.1 s). (*iii*) For PIPMAm aqueous solution IR spectra indicate a two-steps character of the phase transition; transition temperatures for the hydrophilic C=O groups are $\sim$2 K higher in comparison with hydrophobic $CH_3$ groups. (*iv*) For PIPMAm in $D_2O$/EtOH mixtures the globular structures were observed by NMR even at room temperature for certain compositions of the mixed solvent (cononsolvency effect). Virtually no EtOH is bound in these globular structures, in contrast to the temperature-induced globular structures.

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