Stimuli-Responsive Polymers in Solution Investigated by NMR and Infrared Spectroscopy

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Summary: Temperature-induced and solvent composition-induced phase separation in solutions of poly(*N*-isopropylmethacrylamide) (PIPMAm) and other thermoresponsive polymers as studied by NMR and infrared (IR) spectroscopy is discussed. The fraction *p* of phase-separated units (units with significantly reduced mobility) and subsequently, e.g., thermodynamic parameters characterizing the coil-globule phase transition induced by temperature, were determined from reduced integrated intensities in high-resolution ^{1}H NMR spectra. This approach can be especially useful in investigations of phase separation in solutions of binary polymer systems. Information on behaviour of water during temperature-induced phase transition was obtained from measurements of ^{1}H NMR relaxation times of HDO molecules. NMR and IR spectroscopy were used to investigate PIPMAm solutions in water/ethanol (D_2O/EtOH) mixtures where the phase separation can be induced by solvent composition (cononsolvency). Some differences in globular-like structures induced by temperature and solvent composition were revealed by these methods.

Keywords: aqueous solutions; infrared spectroscopy; NMR; phase separation; stimuli-responsive polymers

Introduction

It is well known that some acrylamide-based polymers, including poly(*N*-isopropylacrylamide) (PIPAAm), poly(*N*-isopropylmethacrylamide) (PIPMAm) and other polymers with amphiphilic character, 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.[1–3] 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 followed by further aggregation and formation of so-called mesoglobules,[3] which are colloidally stable in solution. Their thermosensitivity makes these systems interesting for various biomedical and technological applications, e.g., as drug release systems.[4–6] In addition to temperature, which represents an external stimulus which is most often used, phase separation can be induced also by change of other factors such as solvent composition, pH, electric field, etc. Of various methods used in investigations of phase-separation behaviour, NMR spectroscopy can also provide important information on phase-separated globular structures and interactions in these systems.[7]

The present paper is divided into two parts. In the first part we present an overview of our recent ^{1}H NMR studies dealing with phase transitions in D_2O solutions of several thermoresponsive polymers. We concentrated mainly on behaviour of PIPMAm aqueous solutions which in comparison with PIPAAm solutions were not studied so often; nevertheless, selected results obtained on solutions of several other homopolymers (PIPAAm,

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poly(*N*,*N*-diethylacrylamide) (PDEAAm), poly(vinyl methyl ether) (PVME), poly-(*N*-vinylcaprolactam (PVCL)) and thermoresponsive P(IPMAm/AAm) random copolymers are also mentioned for comparison. In the second part we used NMR and infrared (IR) spectroscopy to investigate PIPMAm solutions in water/ethanol (EtOH) mixtures where the phase separation can be induced by solvent composition. At the same time some new results are presented in both parts.

^{1}H NMR Studies of PIPMAm and other Thermoresponsive Polymers in Aqueous Solutions

Formation of globular-like structures during phase separation in aqueous polymer solutions affects mobility of polymer segments and subsequently shape and intensities in high-resolution NMR spectra, as well as NMR relaxation times of polymer and solvent. The most significant effect of coil-globule transition on NMR spectra is illustrated in Figure 1 where ^{1}H NMR spectra (measured with a Bruker Avance 500 spectrometer operating at 500.1 MHz) of PIPMAm/D_2O solution ($c = 10$ wt%) at two slightly different temperatures 314 K and 317 K (LCST = 315 K) are shown. The assignment of resonances to various types of protons of PIPMAm (molecular weight $M_w = 37\ 900$, $M_w/M_n = 1.54$) is shown directly in a spectrum measured at 314 K, i.e., below the LCST transition. The strong line on the left is a signal of HDO. The most important effect observed in the spectrum measured at higher temperature (317 K) is a marked reduction in the integrated intensity of all PIPMAm lines. This is due to the fact that at temperatures above the LCST the mobility of most PIPMAm units (included in mesoglobules) is reduced to such an extent that corresponding lines become too broad to be detected in high-resolution NMR spectra.

When we plot the dependence of integrated intensity of given polymer line as function of the temperature, the integrated intensity transitionally decreases with increasing temperature in the LCST region.[7] Such dependence can be easily transformed in temperature dependence of the fraction p of phase-separated units (units in globular-like structures with significantly reduced mobility) by using the relation $p = 1 - (I/I_0)$, where I is the integrated intensity of the given polymer line in the LCST transition region (or above this region) and I_0 is the integrated intensity of this line if no phase separation occurs. For I_0 we usually took values based on integrated intensities obtained at 298 K and using the fact that integrated intensities should decrease with absolute temperature as $1/T$. Figure 2 shows temperature dependences of the phase-separated fraction p as determined from ^{1}H NMR integrated intensities for D_2O solutions of PIPMAm and several other thermoresponsive polymers of various concentration.[9–12] From this Figure it follows that for PIPMAm/D_2O solutions the transition interval is ~ 6 K broad and independent of polymer concentration in the range $c = 0.1 - 10$ wt%.

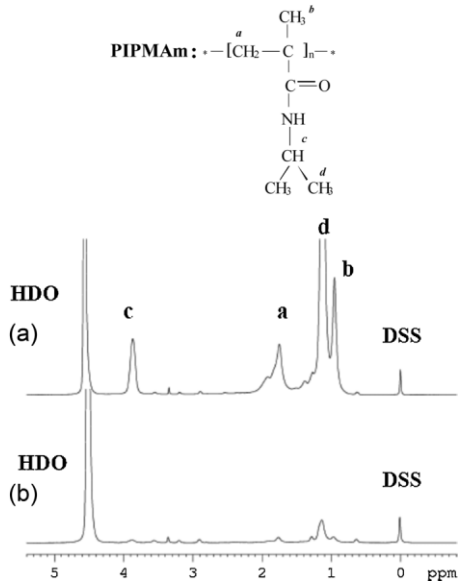

Figure 1.
500 MHz ^{1}H NMR spectra of PIPMAm/D_2O solution ($c = 10$ wt%) measured at 314 K (a) and 317 K (b) under the same instrumental conditions.[8]

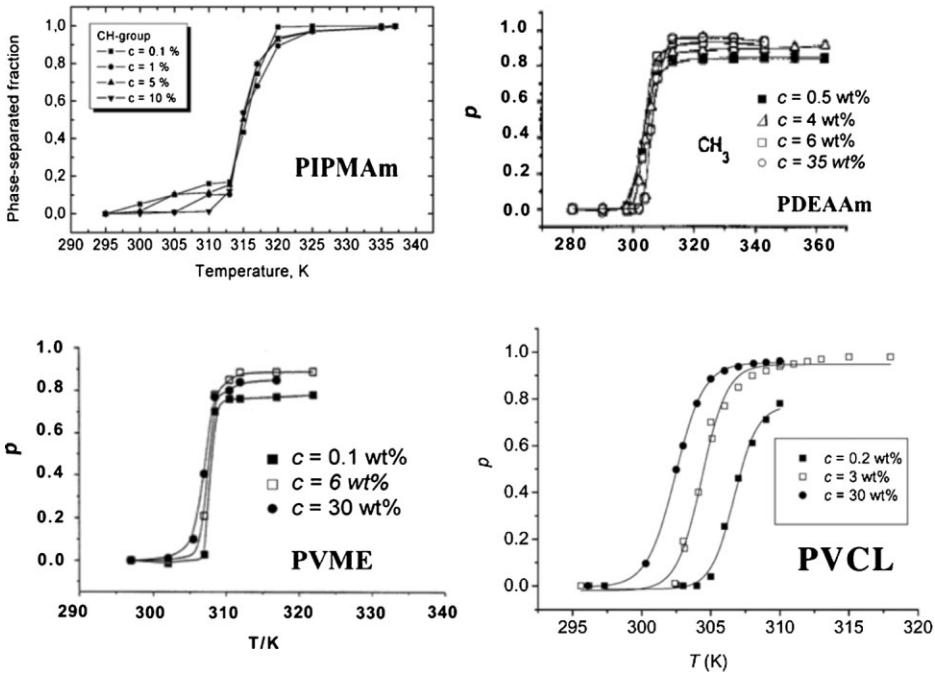

Figure 2.
Temperature dependences of phase-separated fraction p for D_2O solutions of PIPMAm and several other thermoresponsive polymers with various concentrations.

Similar behaviour was found also for PDEAAm solutions while for concentrated PVME ($M_w = 60\ 500$, $M_w/M_n = 3$)/D_2O solution ($c = 30$ wt%) the transition is somewhat shifted towards lower temperatures and is ~3 K broad. A strong concentration dependence of the transition temperatures was found for PVCL ($M_w = 40\,000$, $M_w/M_n = 3.33$)/D_2O solutions; transition temperatures for $c = 30$ wt% are here 5 K lower in comparison with $c = 0.2$ wt% (cf. Figure 2).

From time dependences of ^1H NMR integrated intensities in the transition region it follows that the respective change in the integrated intensity is rather fast, mostly in first ~3 min (this time is necessary to reach the desired temperature in the sample) and then the integrated intensities do not change with time;[9–14] this means that p-values as shown in Figure 2 represent equilibrium values. It is then possible to determine the thermodynamic parameters (ΔH, ΔS) of the coil-globule transition by using the approach originally suggested for selfaggregation in solutions of syndiotactic poly(methyl methacrylate);[15] a similar approach was more recently applied by Rice to PIPAAm hydrogel.[16] In terms of p-values, it holds for the equilibrium constant $K(T)$ of the coil ⇔ globule transition, $K(T) = p/(1 - p)$. Values of the changes of the enthalpy ΔH and entropy ΔS then can be determined from van't Hoff plots

$$\ln K = \ln[p/(1-p)] = -(\Delta H/RT) + (\Delta S/R) \quad (1)$$

In accord with other authors[17] we assumed that cooperative unit is the whole macromolecule; then ΔH_m and ΔS_m values related to monomer unit were obtained by dividing the original ΔH and ΔS values as determined from Eq. (1) by the respective degree of polymerization. For PIPMAm/D_2O solutions of various concentration the values $\Delta H_m \cong 4.5$ kJ/mol and $\Delta S_m \cong 14.4$ J mol^{-1} K^{-1} were determined.

Similar ΔH_m and ΔS_m values were obtained from NMR data for D_2O solutions of PIPAAm ($M_w = 139\,000$, $M_w/M_n = 1.47$) and PVME, in accord with values reported for PIPMAm, PIPAAm and PVME aqueous solutions by other authors using DSC.[17–19] For PVCL/D_2O solutions somewhat higher values ($\Delta H_m \cong 7$ kJ/mol and $\Delta S_m \cong 23\,\mathrm{J mol^{-1} K^{-1}}$) were found by NMR analysis. Both breakdown of polymer-water hydrogen bonds and disruption of local structure of water molecules surrounding hydrophobic groups of polymer units (hydrophobic hydration) probably contribute to the positive ΔH_m and ΔS_m values.

NMR spectroscopy can be a suitable method in investigations of phase separation in solutions of binary or multicomponent polymer systems because it can provide quantitative information on the behaviour at LCST of both polymer components separately. Two phase transitions were detected in D_2O solutions of PIPMAm/PVME and PIPMAm/PIPAAm mixtures by ^{1}H NMR measurements.[9,20] While the phase transition temperatures of PVME or PIPAAm component (appear at lower temperatures in comparison with PIPMAm component) are not affected by the presence of PIPMAm in the mixtures, the temperatures of the phase transition of PIPMAm component are affected by the phase separation of PVME or PIPAAm component. A direct connection between the state of the mesoglobules (hydrated or dehydrated) formed by the component with lower LCST (PVME, PIPAAm) and the temperatures of the phase transition of the PIPMAm component was established by ^{1}H NMR spectroscopy.[14] In contrast to mixtures of two homopolymers, only single phase transition was found for P(IPMAm/IPAAm) random copolymers containing 25–75 mol% of IPMAm units. At the same time, the phase-transition temperatures strongly depend on the composition of the copolymer.[20] Recently we studied the phase transition in solutions of P(IPMAm/AAm) random copolymers containing 1-29 mol% of AAm units.[21] We found that the increasing content of hydrophilic AAm units in the copolymer significantly shifts the transition of IPMAm component towards higher temperatures, broadens the transition interval and reduces the maximum value of the fraction of phase-separated IPMAm units with reduced mobility. In contrast to IPMAm copolymer units, virtually all AAm units are directly detected in high-resolution ^{1}H NMR spectra of these systems even at temperatures above the phase transition. These results suggest a dynamic heterogeneity of copolymer chains in mesoglobules where AAm units (sequences) and surrounding IPMAm sequences are hydrated and therefore mobile, while most IPMAm sequences are dehydrated and their mobility is strongly reduced.

Information on behaviour of water and polymer-solvent interactions (hydration) during temperature-induced phase transition in aqueous solutions of thermoresponsive polymers can be obtained from measurements of NMR relaxation times of the solvent. Figure 3 shows the temperature dependences of spin-lattice relaxation time T_1 and spin-spin relaxation time T_2 of HDO molecules in D_2O solutions of PIPMAm. Both T_1 and T_2 dependences show a decrease in the LCST transition.[21] 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.[7] One order of magnitude shorter T_2 values of HDO at temperatures above the phase transition than those at temperatures below the phase transition were found also for D_2O solutions of PVME and PIPAAm, again showing that a certain portion of water molecules is bound in mesoglobules induced by temperature.[14,22] For D_2O solutions of PVCL the difference in T_2 values of HDO at temperatures above and below the phase transition was much smaller. The single-exponential character of relaxation curves indicates a fast exchange between bound and free water molecules; the residence time ~1 ms was

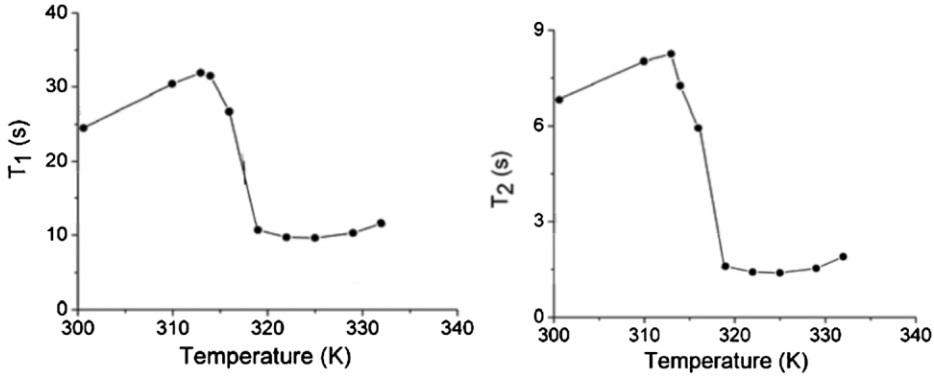

Figure 3.
Temperature dependences of ^{1}H spin-lattice relaxation time T_1 and spin-spin relaxation time T_2 of HDO in D_2O solution of PIPMAm ($c = 5$ wt%).

found for the bound HDO in PIPMAm, PVME and PVCL solutions (for PVME solutions this holds only for concentrations $c = 2$–10 wt%).[23,24]

When the sample was kept at the temperature above the phase transition, an increase of T_2 values of HDO with time shows that originally bound water is very slowly released from globular-like structures,[14,22] in contrast to the fact that phase transition itself is rather fast (faster than 1 s in PIPAAm aqueous solutions[25]). The main reason for the large difference in the induction period, as found for PVME on the one hand and PIPMAm and PIPAAm on the other hand, is probably much higher mobility of PVME segments in mesoglobules (for PVME aqueous solutions the LCST $\approx$ 308 K is well above the T_g of PVME in bulk where values in the range $T_g = 191$–251 K are reported) in comparison with PIPMAm or PIPAAm segments which are in glassy state in mesoglobules.[14,22]

NMR and IR Study of PIPMAm in Water/Ethanol (EtOH) Mixtures

Figure 4 shows dependences of the phase-separated fraction p in PIPMAm/D_2O/EtOH solutions as function of EtOH content in D_2O/EtOH mixtures, as obtained from integrated intensities of polymer CH and CH_2 protons in ^{1}H NMR spectra at two temperatures.[26] From this figure it follows that the dependence of the fraction p on EtOH content measured at 298 K exhibits a maximum for the solution with 40 vol% of EtOH where $p = 0.95$ and 0.8 for the CH_2 and CH protons, respectively. This result shows that in this case most of PIPMAm units are involved in globular-like structures formed by aggregated polymer chains with restricted mobility. This interpretation is corroborated by the fact that PIPMAm solutions in D_2O/EtOH mixtures with EtOH content 30 and 40 vol% are lightly turbid at room temperature. Phase separation induced at room temperature by solvent composition has to be ascribed to cononsolvency;[27–29] while both D_2O and EtOH are good solvents of PIPMAm, their mixture with $\sim$40 vol% of EtOH is a nonsolvent of this polymer. For higher temperatures the maximum in p values is extended towards lower EtOH content and at 328 K the maximum phase separation exists also in D_2O solution because this temperature is above the respective LCST. As EtOH content is increasing above 40 vol%, the fraction of PIPMAm units with restricted mobility is decreasing and for the solution with the highest EtOH content (95 vol%) the p-fraction is negligible. From Figure 4a it also follows that the values of p-fraction as determined at 298 K

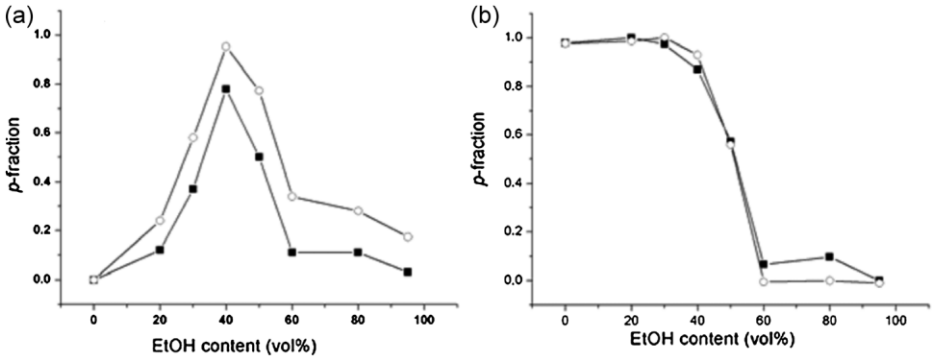

Figure 4.
Fraction p of CH_2 (○) and CH (■) PIPMAm protons with reduced mobility for PIPMAm solutions in D_2O/EtOH mixtures ($c = 5$ wt%) as a function of EtOH content as determined at 298 K (a) and 328 K (b).[26]

from integrated intensities of the main-chain CH_2 protons are systematically higher in comparison with values of p corresponding to the side-chain CH protons. This indicates that some side-chains in phase-separated PIPMAm units are still in interaction with solvent molecules when phase separation is due to the cononsolvency. This behaviour is in contrast to the phase-separation induced by temperature (328 K, cf. Figure 4b) where the dependences of the p-fraction on the EtOH content are virtually the same for the main-chain CH_2 and side-chain CH polymer protons. The same behaviour of main chain and side chain protons during the temperature-induced phase separation was found also for D_2O solutions of PDEAAm and PVME.[10–12]

Figure 5 shows ^{13}C spin-spin relaxation times T_2 of EtOH CH_3 carbons as obtained for PIPMAm solutions in D_2O/EtOH mixtures at 125.7 MHz and temperatures 298 K and 328 K.[26] 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 $p \approx 1$, cf. Figure 4) is one order of magnitude shorter in comparison with other cases, showing that a certain portion of EtOH molecules is bound in globular structures induced by temperature. In contrast, at 298 K all T_2 values are almost the same and nothing indicates that some portion of EtOH is bound in globular-like structures formed in D_2O/EtOH mixture containing 50 vol% of EtOH (cf. Figure 4).

Phase separation in PIPMAm aqueous solutions was studied in the past also by IR spectroscopy[30–32] and therefore we applied this method also to PIPMAm solution in water/EtOH mixture. Figure 6 shows FTIR spectra of the solid powder of PIPMAm and the spectra of the PIPMAm solutions ($c = 5$ wt%) in EtOH, water and in the mixture EtOH/water (40/60 by volume; for this composition $p \approx 1$ at room

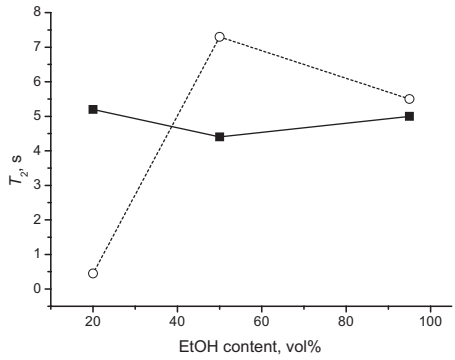

Figure 5.
^{13}C spin-spin relaxation times T_2 of EtOH CH_3 carbons for PIPMAm solutions in D_2O/EtOH mixtures ($c = 5$ wt%) as a function of EtOH content as determined at 298 K (■) and 328 K (○).

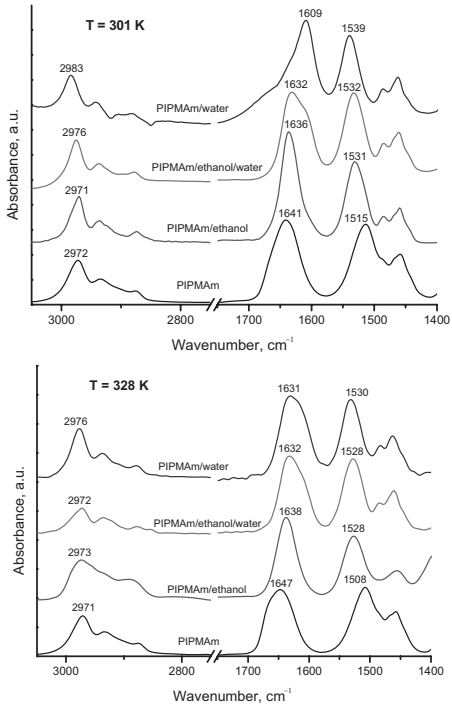

Figure 6.
ATR FTIR spectra of solid PIPMAm and solutions of PIPMAm (c = 5 wt%) in EtOH, water and mixture EtOH/water (40/60 by volume) measured at 301 and 328 K.

temperature, cf. Figure 4) at 301 K and 328 K. A Nicolet Nexus 870 FTIR spectrometer purged with dry air and equipped with a cooled mercury-cadmium-telluride (MCT) detector was used for the acquisition of these ATR FTIR spectra. Samples were measured on a horizontal micro-ATR Golden Gate unit (SPECAC), having a control-heated top plate with a diamond prism. Spectral resolution was 4 cm^{-1} and all the spectra were processed by baseline correction and advanced ATR correction using OMNIC software, version 8. In Figure 6 spectra of the solvents were subtracted; C–H stretching region (3000–2800 cm^{-1}) in the spectra of PIPMAm in EtOH may be affected by artifacts due to strong overlap of the bands of solute and solvent.

In the following, two regions of the IR spectra will be discussed: region of the asymmetric stretching vibration of methyl groups at 2980 cm^{-1} and the regions of Amide I (1600–1700 cm^{-1}) and Amide II (1500–1600 cm^{-1}). It was found that while CH stretching vibrations enable us to detect the hydrophobic interactions of methyl groups with surrounding envelope of water molecules, Amide I and Amide II vibrations are sensitive to the hydrophilic interactions of water molecules with the C=O and N–H groups by means of strong hydrogen bonds.[32,33] In the spectrum of the water solution of PIPMAm measured at 301 K, the asymmetric CH stretching band of methyls is shifted to higher frequencies (2983 cm^{-1}) in comparison with the neat solid PIPMAm (2972 cm^{-1}). This blue shift is caused by relatively strong interactions of methyl groups with clusters of water molecules.[32] On heating from 301 to 328 K the CH stretching band is shifted from 2983 to 2976 cm^{-1}. This corresponds to the partial disintegration of the hydration shells of the methyl groups. It is seen in Figure 6 that practically no solute-solvent interaction is detected in the CH stretching region for the EtOH solution of PIPMAm. In the spectra of the solutions in EtOH/water mixture the CH stretching band is shifted from 2976 to 2972 cm^{-1} so indicating only partial hydration of methyl groups at 301 K; it is fully removed at 328 K.

Observed red shifts of the Amide I band in the spectra of solutions with respect to dry solid PIPMAm (Figure 6) indicate that inter-chain bonds C=O...H–N in polymer are replaced in solutions by stronger solute-solvent hydrogen bonds C=O...H–O. By analogy, the shifts of the Amide II band to the higher frequencies prove an analogous role of the strong N–H...O–H solute-solvent hydrogen bonds in the PIPMAm solutions. From comparison of wavenumbers of Amide I band for PIPMAm/water and PIPMAm/EtOH solutions it follows that hydrogen bonds PIPMAm-water are stronger than PIPMAm-EtOH hydrogen bonds. The number and strength of the solute-solvent hydrogen bonds increases in the direction: EtOH → EtOH/water → water. In contrast with water solution, just a

small portion of strong hydrophilic interactions of PIPMAm with water is present at 301 K in the mixed solvent ethanol/water (40/60) (Figure 6) and it is changed only slightly with increasing temperature. This result is in accord with idea suggested for PIPAAm in water/alcohol mixtures[28,29] that cononsolvency results from the fact that water-EtOH interactions are preferred to polymer-water hydrogen bonds.

Conclusion

In the paper we dealt with temperature-induced and solvent composition-induced phase separation in solutions of PIPMAm and other thermoresponsive polymers as studied by NMR and IR spectroscopy. In the first part we present an overview of our recent ^{1}H NMR studies dealing with solutions of thermoresponsive polymers. The use of the NMR in our studies is based: (*i*) On the reduction of integrated intensities of polymer NMR lines in high-resolution NMR spectra in the system undergoing the coil-globule phase transition; (*ii*) On changes in NMR relaxation times of the solvent. In the first case the fraction p of phase-separated units (units with significantly reduced mobility) and subsequently, e.g., thermodynamic parameters characterizing the coil-globule phase transition can be determined. This approach was useful in investigations of phase separation in solutions of binary polymer systems such as PIPMAm/PVME and PIPMAm/PIPAAm mixtures or P(IPMAm/AAm) random copolymers. Measurements of NMR relaxation times of the solvent (water) provide information on behaviour of water during phase transition.

In the second part we used combination of NMR and IR spectroscopy to investigate PIPMAm solutions in water/EtOH (D_2O/EtOH) mixtures where the phase separation due to cononsolvency can be induced by solvent composition. Some differences in globular-like structures induced by temperature and solvent composition were revealed from NMR measurements (including ^{13}C spin-spin relaxation times T_2 of EtOH CH_3 carbons) and from IR spectra.

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