Cononsolvency of Poly(*N*-isopropylacrylamide): A Look at Spin-Labeled Polymers in Mixtures of Water and Tetrahydrofuran

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ABSTRACT: The electron paramagnetic resonance (EPR) spectra of solutions of a nitroxide-labeled poly(*N*-isopropylacrylamide) (PNIPAM-T/880, M_v 2.9 × 10^6; label, 4-amino-2,2,6,6-tetramethylpiperidine 1-oxide (TEMPO); label content, 1 × 10^{-5} mol g^{-1}) were recorded in mixtures of water and tetrahydrofuran (THF) in order to investigate the phenomenon of cononsolvency exhibited by the PNIPAM/water/THF ternary system. A detailed line-shape analysis of the EPR signals was carried out for spectra recorded at constant temperature as a function of solvent composition and as a function of temperature (−10 to +35 °C) for several water/THF mixtures. Changes in the rotational correlation time and in the isotropic hyperfine coupling constant of the label were correlated to the macroscopic polymer phase-separation process. The data support a model involving preferential water adsorption along the polymer chains in mixed water/THF solutions. This situation was compared to that offered by the PNIPAM/water/methanol system, another ternary mixture which exhibits the phenomenon of cononsolvency. Differences between the two systems are described. They are interpreted in terms of the relative hydrogen-bonding abilities of the two organic cononsolvents.

Introduction

Among all liquids, water has certainly been studied for the longest time and in most detail.[1] Yet the structure of water remains a very controversial issue. New models are put forward to account for the "unusual" properties of this familiar liquid.[2] The dissolution in water of polar substances, such as NH_3, is an exothermic process with energy attributed to H-bond formation. When nonpolar solutes, such as hydrocarbons or carbon tetrachloride, are dissolved in water, appreciable heat is released and large entropies of solvation are involved. This exothermic dissolution of nonpolar solutes in water has been ascribed to the formation of a solvation shell, a "clathrate", of H-bonded water molecules around the solute. The magnitude of these effects is related in subtle ways to the hydrophobic–hydrophilic balance in the solute molecule. Studies of aqueous solutions of alcohols have indicated that, in general, the larger the hydrophobic group, the larger the effects on water. Ethers form nonideal solutions in water. In this case, the origin of the deviation from ideality has been associated with H-bond formation between water molecules and the ether oxygen, a H-bond acceptor. Curiously, these perturbations are larger in the case of tetrahydrofuran (THF) than in the case of dioxane,[3] despite the fact that a THF molecule has only a single oxygen. Studies of the THF/water solution by dynamic light scattering[4] and ultrasonic absorption[5] have highlighted the importance of fluctuations in this system. The THF/water system presents a liquid–liquid lower critical solution point (near 71.85 °C and 22.5 mol % THF),[6] but concentration fluctuation effects are sensed even at 25 °C, far from the critical point.

The dissolution of polymers in water is also accompanied by unusual effects. Neutral polymers are often soluble in cold water over a large concentration range, but they separate from solution above a critical temperature (lower critical solution temperature, LCST).[7] Temperatures and concentrations of phase separation are related to the hydrophobic–hydrophilic balance of the substituents attached to the polymer backbone.[8] Examples of polymers which possess a lower critical solution temperature in water include cellulose ethers, such as (hydroxypropyl)cellulose (HPC), and poly(*N*-alkylacrylamides), such as poly(*N*-isopropylacrylamide) (PNIPAM).[9] The addition of a water-miscible solvent to an aqueous polymeric solution may have unexpected results. For example, PNIPAM is soluble in cold water and in methanol, but it is not soluble in certain mixtures of the two solvents.[10] Note that HPC also is soluble in pure methanol and in cold water, but unlike PNIPAM it is soluble in methanol/water mixtures of all proportions. A more comprehensive survey of PNIPAM solvents reported by Schild et al.[11] revealed that the cononsolvency of this polymer is not limited to aqueous alcoholic solutions. Other water-miscible solvents, including dioxane and tetrahydrofuran (THF), induce phenomena analogous to those observed in MeOH/water mixtures. They attributed the PNIPAM cononsolvency in mixed aqueous solutions to changes in local contacts between the polymer and the solvents.

The work described here originates from our previous investigations on the use of electron paramagnetic resonance (EPR) and ^{13}C nuclear magnetic resonance (NMR) spectroscopy to explore the mechanism of the heat-induced

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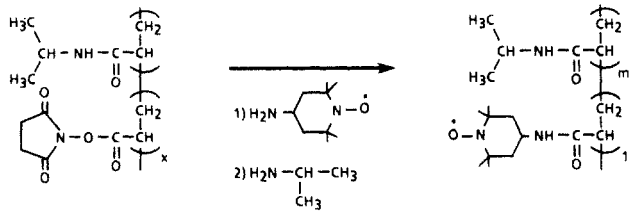


Figure 1. Structures of the polymers used in this study.

Table I. Physical Properties of the Polymers

polymer	$[\eta]^a$ ($cm^3\ g^{-1}$)	$M_v{}^b$	$M_n{}^c$	$M_w{}^c$	LCST (°C at 1 g L^{-1})
PNIPAM	72 ± 1	0.92×10^6	55 000	120 000	32.5
PNIPAM-T/880	151 ± 3	2.9×10^6	90 000	340 000	32.2

[a] From THF solutions. [b] From $[\eta] = 9.59 \times 10^{-3} M_v$ (Fujishige, S. *Polym. J.* **1987**, *19*, 297). [c] GPC in THF, calibrated against polystyrene standards.

phase separation of aqueous solutions of PNIPAM in pure water and in water/methanol mixtures.[12] EPR spectroscopy provides information on the dynamics of a labeled polymer and on the polarity of different environments.[13] It proved to be an excellent tool to monitor local solvent concentration fluctuations near nitroxide-labeled poly(*N*-isopropylacrylamides) as their solutions passed through their LCST. The overall results supported a model involving preferential adsorption of methanol to the polymer chains in mixed methanol/water solutions, as the main contributor to the cononsolvency phenomenon. We report here the results of an investigation of the phase-transition phenomenon in water/THF mixtures. The nitroxide-labeled polymer employed as a probe, poly(*N*-isopropylacrylamide)/TEMPO (PNIPAM-T/880, see Figure 1), was the same as the polymer used in the water/MeOH system. Hence, we will be able to compare directly the effects induced on the EPR spectra of the labeled polymer in the two cononsolvent systems below and above the LCST. Reported as well are control experiments performed with the EPR probe 2,2,6,6-tetramethylpiperidine 1-oxide (TEMPO) dissolved in several water/THF mixtures and in mixed solutions of PNIPAM.

Experimental Section

Materials. All commercial chemicals were purchased from Aldrich Chemical Co., unless otherwise noted. *N*-Isopropylacrylamide was purchased from Eastman Kodak Chemicals. The EPR probes 2,2,6,6-tetramethylpiperidine 1-oxide (TEMPO) and 4-amino-2,2,6,6-tetramethylpiperidine 1-oxide (TEMPamine) were used without purification. The TEMPO-labeled polymer (PNIPAM-T/880) was prepared by reaction of 4-amino-2,2,6,6-tetramethylpiperidine 1-oxide with a copolymer of *N*-isopropylacrylamide and *N*-(acryloxy)succinimide, as described previously.[12] Physical data of the polymers used are listed in Table I. Water was deionized with a Millipore Milli-Q water purification system. HPLC-grade solvents were used for all spectroscopic measurements.

Instrumentation. Cloud points were determined by spectrophotometric detection of changes in turbidity of solutions (1 g L^{-1}) heated at a constant rate (0.2 °C min^{-1}) in a magnetically stirred fluorescence cell placed in the thermally-controlled sample compartment of a SPEX DMB1 fluorescence spectrometer. The solutions were irradiated at 500 nm. The scattered light intensity was collected at a right angle to the incident beam. Its intensity was measured as the counting rate of the single-photon-counting detector. EPR spectra were recorded with a Bruker ESP 300 spectrometer with ESP 1600 data analysis software. Temperature control ($T \pm 1$ °C) was achieved with a Bruker ER 4111T variable-temperature accessory.

Analysis of the EPR Spectra. In the case of labeled polymers the analysis of the signals was performed by using the procedure reported by Schneider and Freed.[14] This procedure is particularly well-suited for the analysis of signals originating from nitroxides in slow motion conditions, i.e., correlation times for rotational diffusion between 5×10^{-7} and $(1\text{–}2) \times 10^{-9}$ s. Best fits were obtained by setting parameters such as the following: (1) the input magnetic parameters for the **g** and **A** tensor components (for the Zeeman and hyperfine interactions, respectively) evaluated by computing the spectra in the slowest motion, i.e., at liquid-nitrogen temperature. At 77 K the aqueous polymer solution formed a glass for which magnetic parameters could be obtained with good resolution. The spectral parameters were evaluated by simulating the signal from polymer powder using a SIM 15Fl program; (2) the model for the rotational diffusional motion (Brownian motion, free motion, or jump motion); (3) the principal components of the diffusion tensor. In the computation of the EPR spectra two different approaches were followed. In the first approach, the simulations included the tilt angle of the rotational diffusional axes with respect to the magnetic molecular axes. In this case the simulations yielded an exact N value ($N = D_\parallel/D_\perp$, where $D_\parallel$ and $D_\perp$ are the parallel and perpendicular components of the rotational diffusional tensor, respectively). For these calculations, the following parameters were employed. THF: g components, $g_{xx} = 2.0087$, $g_{yy} = 2.0064$, $g_{zz} = 2.0038$; A components, $A_{xx} = 6.0$ G, $A_{yy} = 7.5$ G, $A_{zz} = 34.5$ G; $N = 50$; tilt angle = 90°, $z' = x$ axis corresponding to the N–O direction. Water: g components, $g_{xx} = 2.0087$, $g_{yy} = 2.0064$, $g_{zz} = 2.0038$; A components, $A_{xx} = 6.5$ G, $A_{yy} = 7.5$ G, $A_{zz} = 36.5$ G; $N = 4$ ($N = D_\parallel/D_\perp$). Tilt angles: component A, 90°; component B, 30°; component C, 0°; $z' = z$ axis, corresponding to the direction of the p_z orbital containing the unpaired electron. A second simulation protocol was employed to obtain reliable values of the rotational correlation times for motion of the TEMPO label. The parameters used for these computations were as follows. THF: $A_{xx} = 6.0$ G, $A_{yy} = 7.0$ G, $A_{zz} = 33.5$ G. THF/H_2O mixtures larger than 30% THF (v/v): $A_{xx} = 6.0$ G, $A_{yy} = 7.5$ G, $A_{zz} = 35.5$ G. H_2O and THF/H_2O mixtures where the THF concentration was lower than 30% (v/v): $A_{xx} = 6.5$ G, $A_{yy} = 7.5$ G, $A_{zz} = 36.5$ G. An N value ranging from 4 to 6 was used in order to obtain the best fits. In cases where more than one signal contributed to the EPR spectra, each signal was computed separately and added in a ratio appropriate to simulate the experimental spectrum. A subtraction–addition procedure was performed in order to identify the spectrum line shapes. The accuracies in the determination of rotational correlation times were ±10% and ±0.05 G in the determination of hyperfine coupling constants and ±1% in the determination of the relative intensity of the signal components.

Preparation of Samples for Analysis. 1. From Polymer Solutions in Water and THF. Solutions of the labeled polymer were prepared by dissolving the polymer (4 g L^{-1}) in either water or THF. Adequate time (ca. 24 h at 25 °C) was allowed to ensure complete dissolution of the polymer in water. Solutions employed in the mixed-solvent experiments were prepared by mixing aliquots of each solution in the appropriate volume ratio, except in the case of the 70% v/v and 75% v/v solutions which were prepared by weighing the two solutions prior to mixing. The total sample volume was 0.2 mL. Unless otherwise specified the mixed solutions were equilibrated for 12 h at 25 °C. They were then cooled to 0 or −15 °C for complete dissolution. Spectra were recorded over a period of time for some solutions. We observed a slow decrease in signal intensity with time, until after approximately 1 month no signal could be detected. The heat-induced phenomena, monitored by light scattering, were not affected in these aged solutions. The loss of the EPR signal may be attributed to autoxidation of THF by oxygen and subsequent spin trapping of stable nitroxides.[15] Solutions of TEMPO (probe) and PNIPAM in water, THF, or methanol were prepared by dissolving the polymer (4 g L^{-1}) in a solution of TEMPO (5×10^{-5} mol L^{-1}) in water, THF, or methanol. Solutions employed in the mixed-solvent experiments were prepared as described in the case of the labeled polymer solutions. Solutions in tetrahydrofuran and in water/THF mixtures were degassed by a 20-min purge with argon.

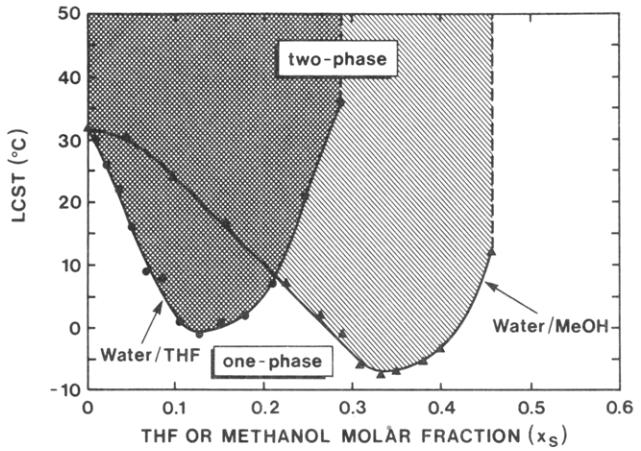


Figure 2. Phase diagram of the PNIPAM-T/880/THF/water ternary system determined from turbidity measurements. Temperature: 20 °C. Polymer concentration: 4 g L^{-1}.

2. From Dissolution of the Labeled Polymer in Premixed Solvent. The labeled polymer was dissolved at 0 °C in a water/THF mixture (20% THF v/v) and degassed by a 20-min purge with argon. This solution was transferred in an EPR sample tube and warmed in the spectrometer sample compartment, first to 15 °C and then to 17 °C. It was kept at this temperature for 24 h. Spectra were recorded at several temperatures during the warm-up period and after various time periods at 17 °C.

Results

Phase Diagram of the TEMPO-Labeled Polymer in Water/Tetrahydrofuran. From turbidity measurements we determined the phase diagrams of PNIPAM and PNIPAM-T/880 in water/THF mixtures. We detected no difference in the phase diagrams of the labeled and unlabeled polymers. The results for PNIPAM agreed well with those of Schild, Muthukumar, and Tirrell for a PNIPAM sample of different molecular weight.[11] The phase diagram of PNIPAM-T/880 (Figure 2) consists of three solvent composition ranges: (i) for THF molar fractions x_T below 0.13 (THF volume fraction: 40%) the LCST decreases sharply from 32 °C in pure water to reach a minimum of −1 °C; (ii) for $0.13 < x_T < 0.30$ the LCST increases sharply; (iii) for $x_T > 0.30$ it is not possible to detect an LCST: solutions in this solvent composition range can be heated to 100 °C without showing any measurable turbidity. Figure 2 also presents the phase diagram of PNIPAM-T/880 in mixed water/methanol solutions. The phase diagrams in the two aqueous mixtures differ in several features. The coordinates of the minimum in the LCST curves are different: the minimum LCST value is higher in the case of THF (−1 °C versus −7.5 °C in H_2O/MeOH), and it occurs in a solvent mixture of higher water molar content in THF (0.87 versus 0.65 in H_2O/MeOH). The cononsolvency effect of MeOH is noticeable only for $x_M > 0.05$, whereas in the case of THF it takes place in H_2O/THF mixtures of lower cononsolvent concentration ($x_T > 0.012$). Such differences on the macroscopic scale are diagnostic of differences of the molecular interactions between the polymer chain and the two solvent systems. These molecular phenomena were monitored by the EPR experiments described next.

EPR Spectra of the Labeled Polymers in Solution. The EPR spectrum of PNIPAM-T/880 exhibits the typical three-line pattern due to coupling of the electronic spin of the nitroxide function with the nuclear spin ($I = 1$) of the ^{14}N nucleus. The spectral line shape presents different features in the three solvents tested here (Figure 3). EPR line shapes are usually analyzed by taking into account

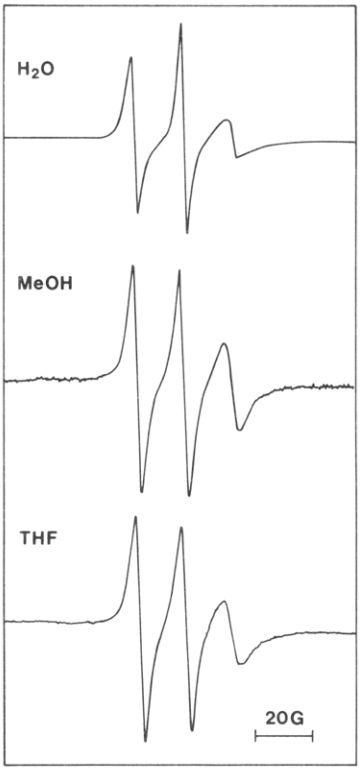


Figure 3. EPR spectra of TEMPO-labeled poly(N-isopropylacrylamide), PNIPAM-T/880, in water, methanol, and tetrahydrofuran. Temperature: 20 °C. Polymer concentration: 4 g L^{-1} (from ref 12).

Table II. Spectral Characteristics[a] of PNIPAM-T/880[b] in Water and in THF

	water		THF	
	25 °C	35 °C	20 °C	35 °C
τ_c(A) (s)	2×10^{-10}	2×10^{-10}		
%	12	12		
$\langle A_N \rangle$ (G)	17.0	17.0		
τ_c(B) (s)	1×10^{-9}		7×10^{-10}	5×10^{-10}
%	88		100	100
$\langle A_N \rangle$ (G)	16.0		15.4	15.4
τ_c(C) (s)		8.4×10^{-9}		
%		88		

[a] τ_c: rotational correlation time. $\langle A_N \rangle$: hyperfine coupling constant. [b] Polymer concentration: 4 g L^{-1}.

mobility and structural parameters to solve the spin Hamiltonian and the relaxation matrix. Among the various parameters which can be extracted from a spectral analysis the most useful in characterizing a system are the components of the hyperfine coupling tensor, the line widths, and the correlation times for rotational motion. The correlation times for motion provide information on the local mobility of the probe. Under conditions of highly anisotropic motion, as, for example, in the case of the nitroxide group of PNIPAM-T/880 in THF solution ($N = 50$), it is possible to determine the correlation times for the motion of the probe in different directions. Thus, the analysis of this spectrum which takes into account the tilt of the main rotational axis toward the N–O direction ($z' = x$) indicates a rather fast mobility of the probe along the N–O direction ($\tau_c = 9.9 \times 10^{-11}$ s) and a much slower motion ($\tau_c = 5 \times 10^{-9}$ s) in the perpendicular direction. This is an indication of the restriction of motion experienced by a nitroxide group attached to the PNIPAM chain. The value of τ_c (7×10^{-10} s) reported in Table II represents an average over all directions. This value is much slower than that of the free probe 4-amino-TEMPO ($\tau_c \approx$ (1–6)

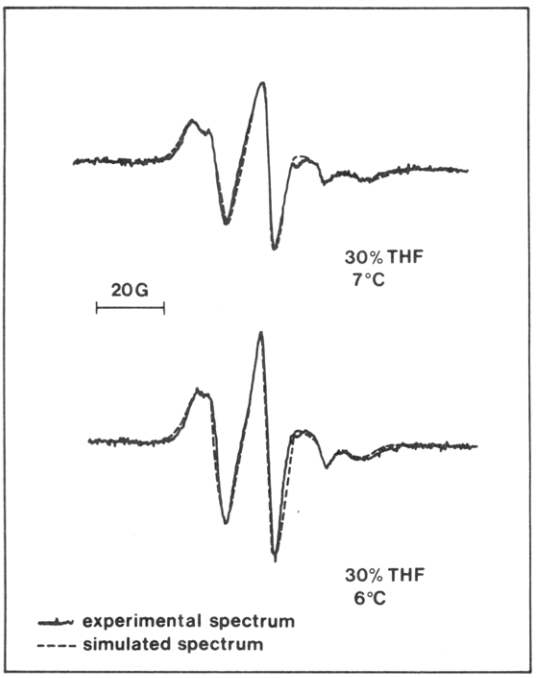


Figure 4. Experimental and simulated EPR spectra of TEMPO-labeled poly(*N*-isopropylacrylamide), PNIPAM-T/880, in a water/tetrahydrofuran mixture (water/THF (v/v) 70/30). The spectra were recorded at 6 and 7 °C.

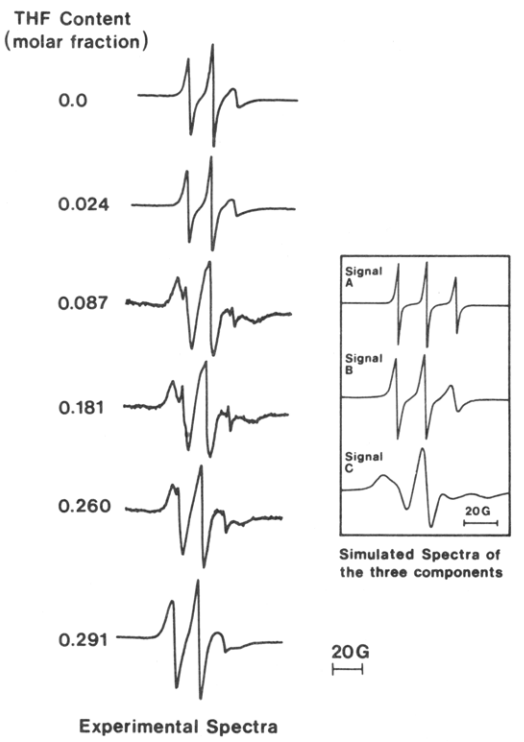


Figure 5. EPR spectra of TEMPO-labeled poly(*N*-isopropylacrylamide), PNIPAM-T/880, in water/tetrahydrofuran mixtures (x_T: molar fraction of THF). Temperature: 20 °C. Polymer concentration: 4 g L^{-1}. Inset: simulated EPR signals of the three components A–C employed in the analysis of the EPR spectra of TEMPO-labeled poly(*N*-isopropylacrylamide).

$\times 10^{-11}$ s, in water),[16] confirming that the nitroxide group is indeed attached to the polymer chain. In conditions of fast motion ($\tau_c < (1–2) \times 10^{-9}$ s) the anisotropies are averaged to zero and the isotropic hyperfine coupling constant $\langle A_N \rangle$ can be used. It becomes an extremely valuable parameter which reveals changes in the polarity of the microenvironment experienced by the nitroxide.

The spectra of the labeled polymer in water and in mixed water/THF solutions consisted of more than one component; therefore, the spectra were computed as a sum of several signals and the different percent contribution of each spectral component was evaluated. The following procedure was adopted. First, a substraction–addition routine was used to identify each component. Then, magnetic and mobility parameters were calculated by the spectral simulation method developed by Schneider and Freed,[14] assuming Lorentzian line shape of the signal and isotropic motion of the label and choosing as magnetic parameters those reported for 4-amino-TEMPO in water. The simulated spectra thus generated reproduced closely the experimental spectra, as illustrated in Figure 4. With these assumptions the EPR signals of PNIPAM-T/880 in water (25 °C) were analyzed as a sum of two components: a major component (B; $\tau_c \approx 1 \times 10^{-9}$ s) and a minor component associated with a faster motion of the label (A; $\tau_c \approx 2 \times 10^{-10}$ s) (Table II).

Solutions of PNIPAM-T/880 were prepared in a series of water/THF mixtures, and their EPR spectra were recorded at 20 °C (Figure 5). Several features of the EPR signals showed a marked dependence on solvent composition. Most significant was the sudden growing-in of a new signal (component C) at the expense of component B, as the solution underwent macroscopic phase separation. The new component is characterized by a longer correlation time ($\tau_c = 6 \times 10^{-9}$ s). Component A detected in pure water, but not in pure THF, underwent only minor changes in terms of correlation time and relative contribution to either the signals B or C. However, a significant decrease in its hyperfine coupling constant $\langle A_N \rangle$ took place with increasing THF content (Figure 6), from 17.2 G in pure

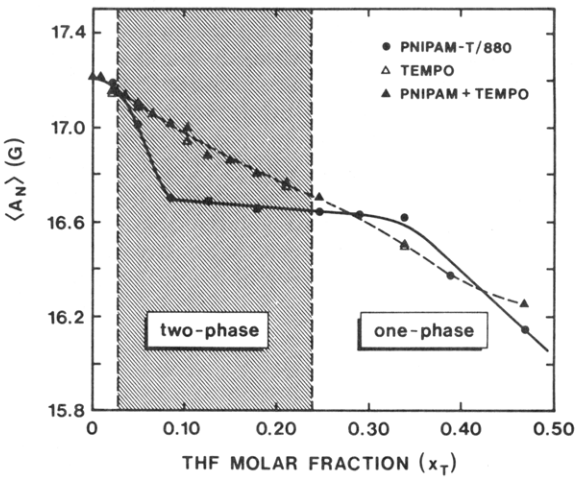


Figure 6. Hyperfine coupling constants, $\langle A_N \rangle$, of component A, as a function of a tetrahydrofuran molar fraction in water/tetrahydrofuran mixtures for solutions of PNIPAM-T/880 (open circles), TEMPO (full triangles), and TEMPO and PNIPAM (full circles). Polymer concentration: 4 g L^{-1}. Temperature: 20 °C.

water to 16.7 G at the solvent composition corresponding to macroscopic phase separation ($x_T = 0.038$). The hyperfine coupling constant of A remained constant and then decreased further as the THF concentration was increased to take a value of 15.4 G in the spectrum of the THF solution of the polymer. These changes in $\langle A_N \rangle$ are not linear, the sharpest drop in $\langle A_N \rangle$ corresponding to macroscopic phase transition (Figure 6), signaling a sudden change in the micropolarity of the probe environment.

To determine the microscopic interpretation of this observation, we performed a series of control measurements with solutions containing unlabeled polymer (4 g L^{-1}) and a free EPR probe, 2,2,6,6-tetramethylpiperidine

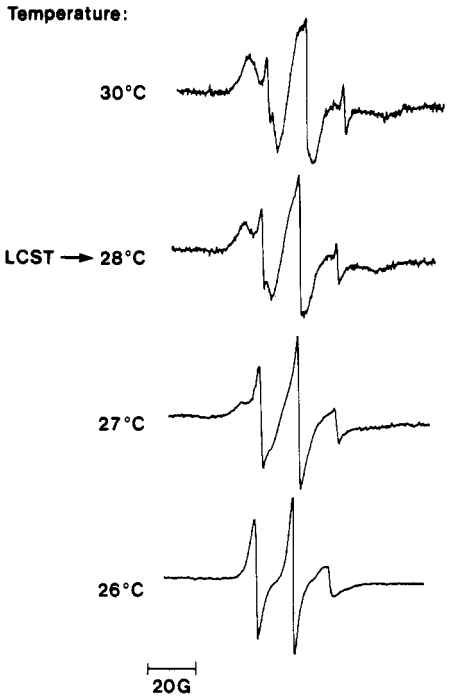


Figure 7. EPR spectra of TEMPO-labeled poly(*N*-isopropylacrylamide), PNIPAM-T/880, in a water/tetrahydrofuran mixed solution (10% THF by volume) at several temperatures below and above the LCST (28 °C). Polymer concentration: 4 g L^{-1}.

1-oxide (TEMPO, 5×10^{-5} mol L^{-1}). Spectra of the probe (5×10^{-5} mol L^{-1}) in the absence of polymer were recorded as well. The hyperfine coupling constant of the probe signal underwent a monotonic decrease with increasing THF concentration in the mixtures, whether or not PNIPAM was present in solution (Figure 6).

Temperature Effects. We monitored the effects of changes in solution temperature on the spectra of the labeled polymer in water, in THF, and in mixed water/THF solutions. The EPR signal of PNIPAM-T/880 in THF was hardly affected by changes in temperatures from 0 °C to the boiling point of THF. Significant changes were observed only in the spectra of solutions which underwent temperature-induced phase separation. In the case of an aqueous solution of PNIPAM-T/880, the effects were detected as the temperature reached 32 °C. Most significant was the sudden growing-in of a new signal (component C) at the expense of component B. The new component is characterized by a longer correlation time ($\tau_c \approx 7.5 \times 10^{-9}$ s at 33 °C). The minor contributor, component A, did not respond to temperature changes as dramatically: its correlation time decreased slightly, but is contribution to the intensity of the total signal remained constant.

The changes observed in the EPR spectra of PNIPAM-T/880 in mixed water/THF solutions are exemplified here for a water/THF solution with 10% by volume of THF ($x_T = 0.024$) (Figure 7). Solutions of the polymer in a solvent of this composition have an LCST of 28 °C (see Figure 2). Again, as in pure water, the most significant effect observed is the growing-in of component C at the expense of component B. The computed EPR parameters of the three components of the EPR signal are listed in Table III for several mixed-solvent compositions and at temperatures below and above the corresponding phase-transition temperatures. A small, but consistent, difference in the spectral parameters merits mention. The issue of concern is the correlation time of motion for component C detected above the LCST. In the case of water the correlation time takes the value of 7.5×10^{-9} s at all temperatures above 32 °C, while in mixed solutions it takes an intermediate value (ca. 5.5×10^{-9} s) at the temperature of phase transition, before reaching a constant value ca. 7.5×10^{-9} s) at all higher temperatures. The same trend was observed also in water/methanol mixed solutions.[12] The origin of the effect is not known. An artifact related to the data-fitting procedure cannot be ruled out at this point.

Sample Preparation and Aging Effects in Mixed Water/THF Systems. The data reported to this point were obtained from measurements performed on pre-equilibrated mixed solutions prepared from solutions of the polymer in each solvent. Reproducible results could be collected only from solutions kept below their LCST for at least 12 h. When such a precaution was not taken, we noticed small variations (ca. ± 1.5 °C) in the temperature at which the phase-transition-induced changes in EPR parameters took place. One set of measurements was performed on a polymer solution prepared by dissolving PNIPAM-T/880 in a premixed water/THF (20% v/v) solution. This solution was warmed up to 15 °C, the corresponding LCST (see Figure 2), and then to 17 °C. EPR spectra were recorded during the heating process and at 17 °C after several time periods up to 24 h (Figure 8). During the warm-up period, ca. 1 h overall, we observed that the EPR signal corresponds closely to that of the labeled polymer in pure THF, indicating preferential solvation of the polymer by THF under these circumstances (Figure 8a). The spectra recorded at 17 °C exhibited a marked time dependence (Figure 8b). Gradually with time, over a period of 12 h, component C corresponding to the slow motion of the nitroxide label, grew-in at the expense of the component related to the faster motion of the label. Only after 24 h was the signal absolutely stable with time at 17 °C. Note that the EPR parameters themselves ($\langle A_N \rangle$, τ_c) of the signal are constant with time; only its relative contribution to the total signal is time-dependent.

Discussion

Three Components of the EPR Signal of PNIPAM-T/880. The EPR spectra of PNIPAM-T/880 recorded under a variety of experimental conditions can be simulated in terms of a superposition of up to three components, termed A, B, and C, distinguishable primarily by their different rotational correlation times. These components are assumed to correspond to the motions of the label in structurally different environments. In THF, only one component, analogous to the signal of component B, is present. Two components, A and B, are required to simulate the spectra of solutions of the polymer in water and in mixed water/THF solutions in temperature and concentration domains that do not include the LCST. The local structures of the two components A and B are closely related. Component A (minor contributor) is characterized by rotational motions with a correlation time of ca. 0.2×10^{-9} s, while the motion of component B has a correlation time of ca. 1×10^{-9} s. The two components exist in equilibrium, which has to be slower than the time scales probed by the rotation of the nitroxide label. We suggested previously[12] that this equilibrium may originate from a syn–anti isomerism of the primary amide bond. The isomeric state of the amide may affect the local conformation of the polymer chain and place the label in regions of different solvent composition and of different mobility.

At the phase transition, the contribution of component B decreases dramatically and a new component, C, characterized by a slower motion becomes predominant. However, at the phase transition component A is hardly

Table III. EPR Parameters of the TEMPO Label in Water Tetrahydrofuran Solutions of PNIPAM-T/880

		component A[a]			component B[a]			component C[a]		
THF content	T (°C)	%	$\tau_\perp$[b]	$\langle a \rangle$ (G)[c]	%	$\tau_\perp$[b]	$\langle a \rangle A'_\parallel$ (G)[d]	%	$\tau_\perp$[b]	$A'_\parallel$ (G)[d]
0	30	12	0.2	17.0	88	1.0	16.9			
	31	12	0.2	17.0	35	1.0	16.9	53	7.5	61.5(1)[e]
	33	12	0.2	17.0	2	*e*	*e*	86	7.3	61.0(1)
	35	12	0.2	17.0				88	8.4	65.3(1)
10% v/v (x_T = 0.024)	26	12	0.2	16.9(1)[e]	88	1.8	16.5(1)			
	27	12	0.2	16.9(1)	88	1.8	16.5(1)			
	28	12	0.2	16.9(1)	48	1.8	16.5(1)	40	5.5	58.0(2)
	29	12	0.2	16.9(1)	20	1.5	16.5(1)	68	7.5	61.0(2)
	30	12	0.2	16.9(1)				88	7.7	61.5(2)
20% v/v (x_T = 0.052)	13	12	0.8	16.2(1)	88	3.5	47.0(1)			
	14	12	0.8	16.2(1)	88	3.5	47.0(1)			
	15	12	0.8	16.2(1)	53	3.2	46.9(1)	35	5.5	51.0(3)
	16	12	0.8	16.2(1)	18	3.2	46.9(1)	70	7.5	57.0(3)
	17	12	0.8	16.2(1)				88	7.2	56.0(3)
30% v/v (x_T = 0.087)	4	12	1.5	16.4(1)	88	5.0	51.5(2)			
	5	12	1.5	16.4(1)	88	4.9	51.3(2)			
	6	12	1.5	16.4(1)	70	4.8	51.0(2)	18	6.0	52.0(3)
	7	12	1.5	16.4(1)	18	4.7	50.9(2)	70	6.8	54.5(3)
	8	12	1.5	16.4(1)				88	7.0	55.0(3)
40% v/v (x_T = 0.128)	0	12	1.5	16.2(2)	88	4.5	48.5[d](3)			
	1	12	1.5	16.2(2)	88	4.5	48.5[d](3)			
	2	12	1.5	16.2(2)	58	4.3	48.5(3)	30	5.0	49.5(3)
	3	12	1.5	16.2(2)	18	4.3	48.5(3)	70	6.3	52.5(3)
	4	12	1.5	16.2(2)				88	6.8	54.5(3)

[a] See text for the definitions of components A, B, and C. [b] $\tau_\perp$ (10^9 s): perpendicular component of the rotational correlation time for motion ($\tau_\perp = 1/D_\perp$, where $D_\perp$ is the perpendicular component of the diffusion tensor). [c] $\langle A_N \rangle$: isotropic hyperfine coupling constant evaluated as the peak-to-peak distance between the three hyperfine absorptions of the computed A and B components in fast motion conditions ($\tau_\perp < 2 \times 10^{-9}$ s). [d] $A'_\parallel$: fictitious parallel component of the hyperfine coupling tensor evaluated from the distance between the lowest and highest field peaks in the computed B and C components in slow motion conditions. [e] Different choices of hyperfine coupling tensor components in the computation (see text, Experimental Section): (1) A_{ii} = 6.5, 7.5, 36.5 G. (2) A_{ii} = 6.0, 7.5, 35.5 G. (3) A_{ii} = 6.0, 7.0, 33.6 G.

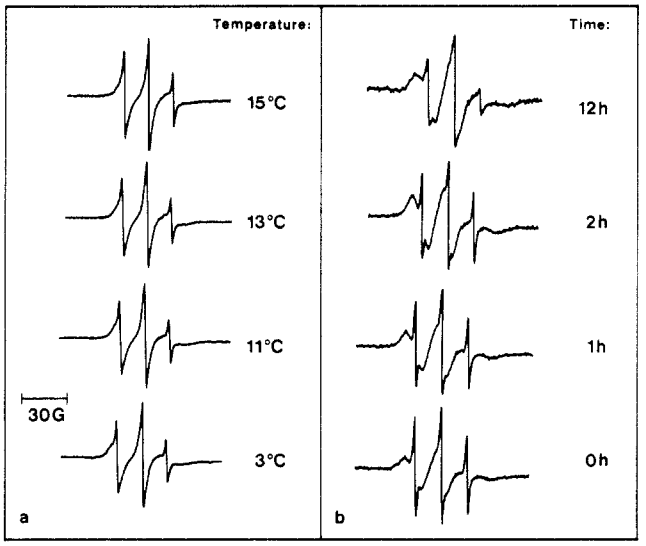
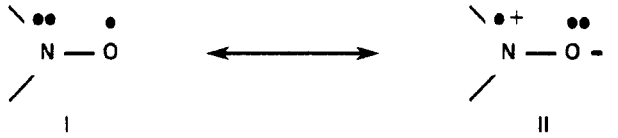


Figure 8. Temperature and time-dependent EPR spectra of TEMPO-labeled poly(*N*-isopropylacrylamide), PNIPAM-T/880, in a water/tetrahydrofuran mixed solution (20% THF by volume) as it is heated from 0 to 17 °C and then kept at this temperature for 12 h. Polymer concentration: 4 g L^{-1}.

affected, in terms of either its relative contribution to the total signal or the correlation time of its motion. Component A exists now in equilibrium with component C, presumably via a mechanism analogous to the equilibrium between A and B, below the phase transition. Note that the relative contribution of signal A (*ca.* 10%) to the total EPR spectrum is of the same magnitude for the polymer in water and in mixed water/THF or water/methanol systems. That the contribution of signal A is not severely affected may indicate conservation of the *syn*-amide isomer to *anti*-amide isomer equilibrium in these three media.

Significance of the Changes in Hyperfine Coupling Constants, $\langle A_N \rangle$ or $A_\parallel$. The hyperfine coupling constant reports on the polarity of the environment sensed by the label. To appreciate the implications of the changes in $\langle A_N \rangle$ values under different conditions, it is useful to remember the molecular origin of this effect. The nitroxide three-electron bond exists in two canonical forms, I and II, which make about equal contribution to the overall



electronic distribution in the absence of specific interactions of the radical with its environment. Polar, polarizable, and hydrogen-bonding solvents stabilize the dipolar form II, thereby increasing the spin density on the nitrogen atom. The hyperfine coupling constant $\langle A_N \rangle$ measures directly perturbations of the ground-state wave function of the radicals in solution. Water and hydroxylic solvents can form specific hydrogen bonds with the nitroxide: an alcohol or water molecule would presumably be bonded to the relatively exposed lone pair of the nitroxide oxygen atom. Thus this specific H-bond should stabilize the canonical structure II, thus enhancing $\langle A_N \rangle$ to a greater extent than a polar but not hydroxylic solvent such as THF. If the H-bond is broken, the canonical form II loses its predominant contribution, thus resulting in a decrease of $\langle A_N \rangle$.

A nearly linear dependence on the THF molar fraction of the hyperfine coupling constant $\langle A_N \rangle$ for the signal A is exhibited by TEMPO in water/THF solutions in the absence of polymer and in the presence of unlabeled PNIPAM (see Figure 6). In contrast, when the nitroxide is attached to the polymer chain, the $\langle A_N \rangle$ values drop sharply at the solvent composition corresponding to phase

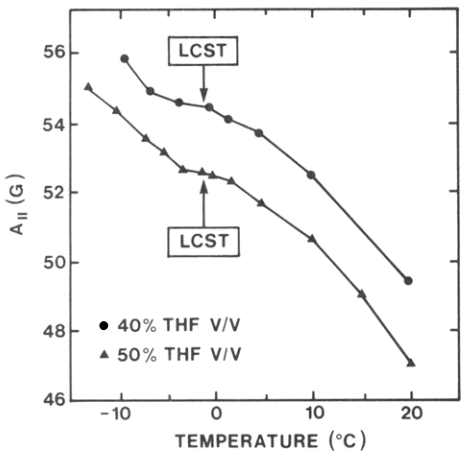


Figure 9. Changes in the anisotropic hyperfine **A** tensor, $A_{\parallel}$, as a function of temperature for solutions of PNIPAM-T/880 in water/THF mixtures: (●) water/tetrahydrofuran (v/v) 60/40; (▲) water/tetrahydrofuran (v/v) 50/50.

separation and remains constant in all phase-separated systems. The polarity sensed by the probe decreases, signaling some disruption of the hydrogen bonds between the polymer and its closest solvation layer. However, the $\langle A_N \rangle$ values are closer to those reported by the probe in water than in THF, therefore allowing one to conclude that the separated polymer-rich phase retains water adsorbed onto the polymer chain. In the solvent mixtures which solubilize the polymer, the variations of $\langle A_N \rangle$ of the label with water/THF compositions closely match those of free TEMPO.

Trends in the changes with temperature of the hyperfine coupling constant $A_{\parallel}$ also provide evidence that the polarity sensed by the probe in the precipitated phase does not change significantly at the LCST, even for mixtures with high THF content. This feature is illustrated in Figure 9 where are presented the temperature-induced changes of $A_{\parallel}$ of TEMPO in labeled polymer systems containing 40 and 50% THF by volume. In each ternary system the values $A_{\parallel}$ decrease with increasing temperature, but a plateau value characteristic of each water/THF composition is maintained in a short temperature range around the corresponding LCST. Thus the solvent composition in the immediate vicinity of the polymer chain is preserved during the coil/globule collapse. Upon further heating of the phase-separated systems, the values of $A_{\parallel}$ decrease almost linearly. This decrease in $A_{\parallel}$ may be related to an increase in the mobility of the probe or to the occurrence of an exchange process between bulk solvent and solvent molecules adsorbed on the polymer chain, resulting in a net increase in THF concentration in close proximity to the chain.

Further circumstantial evidence corroborates the conclusion that the polymer remains hydrated throughout the phase-separation process in the water/THF ternary system. The ratio N of the principal values $D_{\parallel}$ and $D_{\perp}$ of the diffusion tensor was determined to lie between 4 and 6 from simulations of the PNIPAM-T/880 spectra in mixed water/THF solutions containing up to 80% THF by volume. This N value is much closer to that used to simulate the spectra of the labeled polymer in water (N = 4) than in pure THF (N = 50).

Cononsolvency of PNIPAM in Mixed Solvents. A Comparison of the Water/THF and the Water/MeOH Ternary Systems. 1. Review of Key EPR Parameters. Significant differences exist among corresponding EPR parameters obtained for PNIPAM-T/880 in water, water/THF, and water/methanol. They are emphasized by the data presented in Table IV and in Figures 10 and 11. Table IV reports the values of correlation times of motion, hyperfine coupling constants, and N values derived from EPR spectra recorded for phase-separated PNIPAM-T/880 in the three systems. The variations with solvent composition of the hyperfine coupling constant $\langle A_N \rangle$ of

Table IV. Spectral Characteristics of Phase-Separated PNIPAM-T/880[a] in Water, Water/THF, and Water/MeOH

solvent	water (35 °C)	water/THF	water/MeOH
τ_c(C) (s)	8.4×10^{-9}	$(7.2–7.5) \times 10^{-9}$	8.1×10^{-9}
%	88	88	88
$\langle A_N \rangle$ (G)	17.0	16.6–16.7	16.1–16.5
τ_c(A) (s)	2×10^{-10}	$(8–15) \times 10^{-10}$	$(2–4) \times 10^{-10}$
%	12	12	12
$A'_{\parallel}$ (G)	65.3	55–61	58.5–62
N	4	4–6	15

[a] Polymer concentration: 4 g L^{-1}.

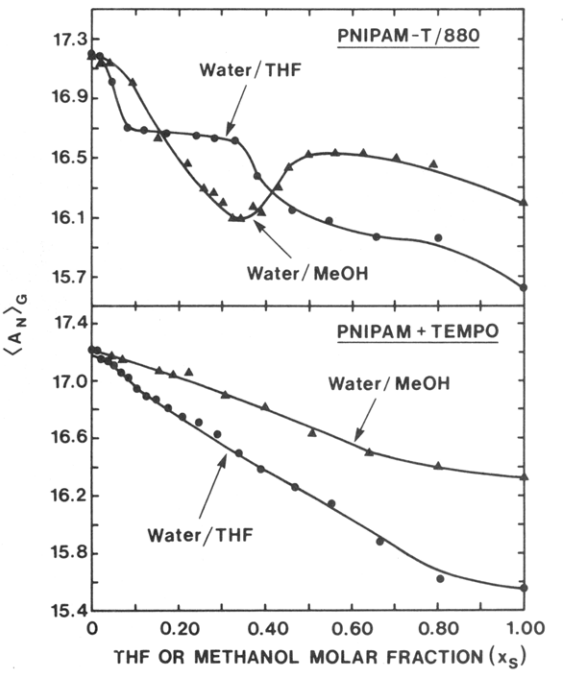


Figure 10. Hyperfine coupling constant, $\langle A_N \rangle$, of component A of the EPR signal, as a function of THF or MeOH molar fraction (x_s) for mixed solutions of PNIPAM-T/880 in water/tetrahydrofuran and water/methanol mixtures. Polymer concentration: 4 g L^{-1}. Temperature: 20 °C.

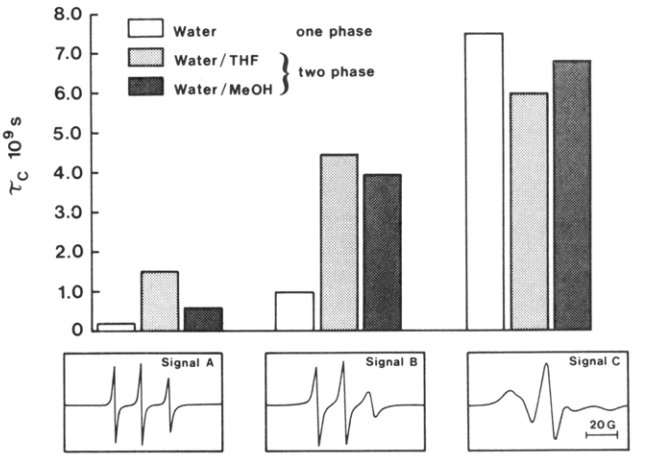


Figure 11. Correlation times for motion (τ_c) of components A–C computed from experimental EPR spectra of PNIPAM-T/880 in water, water/tetrahydrofuran, and water/methanol. Temperature: 20 °C. Polymer concentration: 4 g L^{-1}. Methanol or tetrahydrofuran molar fracton in the mixed solution: x_s = 0.125.

the signal A are shown in Figure 10, and correlation times of motion for the three spectral components are compared in Figure 11.

One major difference between the two ternary systems concerns the hyperfine coupling constant, $\langle A_N \rangle$, of the component A in the phase-separated systems (Figure 10). Unlike the situation in mixed water/THF, in the mixed water/methanol system the quantity $\langle A_N \rangle$ reaches a minimum value (*ca.* 16.0 G). In the water/THF system this quantity reaches a plateau value of 16.7 G closer to that of the TEMPO label in water.

Correlation times for motion also exhibit distinct trends in the two ternary systems (Figure 11). First, the difference in τ_c values for components B and C are smaller in the case of the water/THF system than in water/methanol. Second, the value τ_c(C) in water/THF is larger than τ_c(C) in water/methanol. Thus, the mobility experienced by the label in the polymer-rich phase separated from a water/THF system is higher.

2. Implications Concerning the Composition of the Separated Polymer Phase. The solvent effects on nitroxide radicals have been examined by a large number of researchers over the last 30 years.[14] It is now accepted that the $\langle A_N \rangle$ value increases with solvent polarity as a result of the stabilization of the resonance structure II of the nitroxide group (see above). For hydroxylic solvents specific hydrogen bonding with the nitroxide oxygen provides further stabilization of the canonical form II. Thus the comparatively large value of $\langle A_N \rangle$ for nitroxide probes in water is well understood. The situation in mixed alcohol/water solutions remains somewhat controversial. In agreement with the data reported by Symons and Pena-Nuñez,[17] we observed a steady fall with increasing methanol content in the $\langle A_N \rangle$ values of TEMPO whether the solutions contained PNIPAM or not.[12] Similarly, the $\langle A_N \rangle$ values of TEMPO decrease also, upon addition to water of the nonhydroxylic solvent THF. In this case the absolute value of the decrease is larger (0.80 G vs 0.45 G), since THF cannot provide stabilization of the dipolar resonance form II via the hydrogen-bonding mechanism. This interpretation accounts well for the behavior of the EPR probe in the ternary solutions. In the following discussion we will assume that the same molecular interactions exist between the solvents and the EPR label attached to the polymer chain.

The solubility of PNIPAM in water is usually explained by evoking the formation of hydrogen bonds between water molecules and the amide groups of the polymer.[18] Hydrogen bonding lowers the free energy of mixing. It triggers the creation of a layer of highly organized water along the polymer, resulting in a decrease in the entropic term of the mixture. Addition of THF to the polymeric solution in water disrupts the structure of water, both in the bulk and in the vicinity of the polymer chain, as evidence by the changes in $\langle A_N \rangle$ values of the label, in particular as the solvent composition reaches the value which promotes macroscopic phase separation. At these conditions of temperature and solvent composition the balance between the entropy and enthalpy of the system is such that the free energy of mixing becomes unfavorable and the polymer precipitates. The important information that our results bring to the understanding of the ternary system concerns the composition of the separated polymer-rich phase. Evidence from trends in the $\langle A_N \rangle$ and correlation time values of the various components of the EPR signals concur to suggest that the polymer remains hydrated as it undergoes phase separation. One or several water layers remain bound to the collapsed polymer chain.

Water confined to small volumes often differs in properties from that of bulk water. This phenomenon has been the focus of much research, especially in the case of water compartmentalized in the pores of inorganic solids such as activated charcoal or zeolites. Cross-linked hydrogels provide an example of an organic system in which water molecules are confined in small volumes. In this context it is instructive to compare our results to those reported recently by Windle and Scherrer in their study by EPR spin probe techniques of the water trapped within microbeads of covalently cross-linked dextran-based hydrogels.[19] They determined the partition coefficient of a variety of probes, including TEMPO and TEMPamine. In each case they observed that for the probe located within the gel network the correlation times of motion increased and the hyperfine coupling constants decreased with increasing cross-linking and decreasing water content, indicating increased microviscosity and decreasing dielectric constant and hydrophilicity for gel water. In addition, the $\langle A_N \rangle$ values indicated that two hydrogen bonds were formed to the nitroxide group of the probes in water[20] and that, in the hydrogels, the hydrogen-bonding contribution to $\langle A_N \rangle$ progressively increased as the water content decreased. Our results show the same qualitative trend but do not allow us to comment on the details of the interactions between the label nitroxide group and the water molecules. It is clear, however, that the THF content within the phase-separated layer is very low and that the polymer chains are involved primarily in complexes with water only. Whether these complexes involve one or two molecules of water per nitroxide group cannot be determined from our data analysis.

Conclusion

The phenomenon of cononsolvency is readily observed: a milky suspension forms immediately when two clear solutions of PNIPAM are mixed, one in water and the other either in THF or in methanol. Techniques probing macroscopic quantities are generally unable to detect differences in the composition of the precipitated polymer phases. The data reported demonstrate the power of EPR spectroscopy in detecting small differences in local solvent composition. Experimental parameters, such as the label hyperfine coupling values $\langle A_N \rangle$ and $A_\parallel$, the correlation times for motion of the label, and the values of the N parameters required in spectral simulations indicated that in the water/THF/PNIPAM ternary mixture water molecules bind preferentially to the polymer chains. Our results do not allow us to exclude the occurrence of some local THF–polymer interactions, but they indicate clearly that the water concentration in the immediate vicinity of the polymer chains is much higher in THF/water mixtures than in methanol/water mixtures of identical molar compositions.

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