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NMR Methods to Study Effects of Additives on Phase Separation of Thermoresponsive Polymer

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Summary: NMR methods including ^{1}H NMR spectroscopy, 2D NOESY spectroscopy, pulsed-field gradient (PFG) NMR as well as spin-spin relaxation times T_2 measurement were employed to study the effects of one surfactant (sodium *n*-dodecyl sulfate, SDS) and three inorganic salts (NaCl, Na_2SO_4, Na_3PO_4) on the phase transition of poly(*N*-isopropylacrylamide) (PNIPAM) in aqueous solution. The molecular interactions between SDS and PNIPAM during the coil-to-globule transition of PNIPAM have been accurately observed through 2D NOESY spectra and PFG NMR. Through the combination of ^{1}H NMR spectra and T_2 measurements, the salts effects have been found not only shift the phase transition of PNIPAM but also lead to difference in the final conformational state of PNIPAM in aqueous solution after phase transition. Diffusion coefficients of Na^+ cations support the idea that the major effect of salts on phase transition of PNIPAM in aqueous solution is produced by anions.

Keywords: NMR; phase separation; poly(*N*-isopropylacrylamide); salt; surfactant

Introduction

Poly(*N*-isopropylacrylamide) (PNIPAM)[1–3] is a well-known thermosensitive polymer that exhibits a lower critical solution temperature (LCST) at around 32 °C in aqueous solutions. The coil-to-globule transition of this polymer can be induced by a small temperature variation (1–2 K) accompanied by abrupt conformational changes. The LCST behavior of PNIPAM has been attracting research interests for several decades because of its implication in a number of living phenomena, especially on protein folding and DNA packing.[4,5] However, some additives in solutions,[6] such as various cosolvents,[7] electrolytes,[8] and surfactants[9] may influence its LCST.

In the presence of anionic surfactant sodium *n*-dodecyl sulfate (SDS) at concentration of SDS above critical aggregation concentration (CAC), the LCST of PNIPAM increases as a result of their peculiar interactions. The surfactant effects on the conformational change of PNIPAM in water have been extensively studied using various techniques such as laser light scattering (LLS),[7] conductometric measurements,[10] nuclear magnetic resonance,[11] fluorescence spectroscopy,[12] and small angle neutron scattering.[13] However, the possible morphologies of PNIPAM and SDS in the compact globule state have remained ambiguous for decades. Walter et al.[12] suggested that the surfactants form a surface layer with the polymer globule to prevent phase separation when the temperature increases up to the LCST. Meanwhile, Wu and Zhou[14] proposed a PNIPAM/SDS complex structure above the LCST using LLS. When the temperature was increased, the polymer-bound SDS micelles gradually disintegrated into individual molecules and expelled from PNIPAM, followed by the collapse of the surfactant-free polymer network.

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Salts have been found to produce evident effects on the LCST of PNIPAM in aqueous solution. Unlike surfactants, most salts advance the coil-to-globule transition process and depress the LCST transition. The ability of depressing the LCST follows the Hoffmeister sequence.[15] However, there is no widely accepted interpretation for the mechanism of salts influence on the phase transition of PNIPAM in solution. On one hand, some researchers thought it is the anions that interrupt the solvation of PNIPAM and lower the LCST.[16] Cremer et al. proposed that there exist direct interactions between some anions and amide groups of PNIPAM.[17] On the other hand, some results about cations influence on LCST though direct binding to PNIPAM have been published.[18,19]

High-resolution NMR is a powerful technique of studying the structures of various materials as well as the molecular interactions among the components of a mixture under various conditions. NMR spectra, NMR relaxation times, and diffusion coefficients were used to study the coil-to-globule transition in a number of thermosensitive polymers, copolymers, and chemically cross-linked hydrogels.[11,20] In addition, two-dimensional nuclear Overhauser effect spectroscopy (2D NOESY)[21] can provide detailed information on the proximity in space of two different components. In current study, the effects of SDS and three inorganic salts on the phase transition of PNIPAM in aqueous solution were systematically investigated by various NMR methods including ^{1}H NMR spectroscopy, 2D NOESY spectroscopy, pulsed-field gradient (PFG) NMR as well as spin-spin relaxation time T_2 measurements.

Experimental Part

Materials

N-Isopropylacrylamide (NIPAM) was purchased from Aldrich. The monomer was recrystallized three times in a benzene/hexane mixture before use. PNIPAM was prepared[22] in the laboratory via free radical polymerization in benzene, initiated by recrystallized azobis(isobutyronitrile) (AIBN). The resultant PNIPAM sample was carefully fractionated by successive dissolution-precipitation cycles in a mixture of extremely dried acetone and n-hexane at ambient temperature. The average molecular weight of the sample was 1.2×10^5 g/mol, and the ratio of the weight-average to the number-average molar mass was 1.77, as determined by size exclusion chromatography. SDS was recrystallized three times from methanol before use. NaCl, Na_2SO_4, Na_3PO_4 were purchased in analytical reagent grade. Deuterium oxide (D_2O) was purchased from Cambridge Isotopes Laboratories.

NMR Measurements

SDS Effects on Phase Transition of PNIPAM

The NMR experiments were performed using a Varian 700 MHz spectrometer equipped with a 5 mm standard probe and a 70 G/cm maximum available gradient pulse field. NOESY 2D experiments were conducted at 298, 323, and 338 K with 400 ms mixing time. PFG NMR was used to measure the self-diffusion coefficient. The PSTE pulse sequence[23] included a 2 ms offset-independent adiabatic inversion pulse along with a 7.26 G/cm gradient pulse for the selective excitation of an 0.65 cm central sample region. Using these parameters, the molecular diffusion coefficient was quantitatively determined. The recycle time was set at 10 s, and the gradient pulse duration was 2 ms. The diffusion time was 200 ms. The gradient pulses were calibrated on a water sample (10% D_2O and 90% H_2O) under the experimental conditions used in the PNIPAM/SDS/D_2O system.

Salt Effects on Phase Transition of PNIPAM

Temperature dependences of ^{1}H NMR spectra were acquired with Bruker Avance DPX 300 spectrometer operating at 300.1 MHz. The width of 90° pulse was 12 μs with relaxation delay 10 s. The acquisition time was 1.73 s with 16 scans.

Before measurement the sample was kept 10 min at each target temperature.

The ^{1}H spin-spin relaxation times T_2 of HDO were measured with Bruker Avance III 600 spectrometer at 600.2 MHz using the with CPMG pulse sequence.[11] The relaxation delay between scans was 80–100 s, acquisition time was 0.82 s with 8 scans. All PNIPAM solutions in 5 mm NMR tubes were degassed and sealed under nitrogen in the same way.

The diffusion experiments were recorded with a Bruker Avance 500 spectrometer operating at 500.1 MHz and 132.3 MHz for ^{1}H and ^{23}Na nuclei, respectively. The self-diffusion coefficients of HDO molecules and Na^{+} cations were measured using double stimulated echo sequence with bipolar gradients pulses and spoil gradients. Sine-shaped gradient pulses were used with a duration of 1 ms together with a diffusion time of 100 and 70 ms for ^{1}H and ^{23}Na experiments, respectively. Data were accumulated by linearly varying the diffusion encoding gradients over a range from 2% to 95% of maximum for 32 gradient increment values. The gradient pulses were calibrated on a water sample (1% H_2O in D_2O) under the experimental conditions used in PNIPAM/D_2O systems.

Results and Discussion

2D NOESY and PFG NMR to Study Effects of SDS on Phase Transition of PNIPAM

With the aim to study SDS effects on phase transition quantitatively and qualitatively, a method composed of 2D NOESY and PFG NMR was employed as shown in Figure 1. NOESY can provide detailed information on the spatial correlation between different spins, which can be used for modeling the interactions of SDS and PNIPAM. The molecular self-diffusion coefficients measured by PFG diffusion NMR were used to calculate the proportion of SDS binding to PNIPAM. Figure 2 and 3 show ^{1}H NMR spectrum of the PNIPAM/SDS/D_2O solution at 298 K and the 2D NOESY spectra at 298, 323, and 338 K measured with a mixing time of 0.4 s, respectively, At 298 K, strong positive cross-peaks between PNIPAM and SDS appear between the Pa-S4, Pa-S2, Pa-S3, and Pb-S2 proton pairs. This result indicates that the SDS alkyl protons are in close proximity (<0.5 nm) to the polymer side chains. PNIPAM and SDS complex structure formed through the hydrophobic interactions between the SDS alkyl chain and PNIPAM isopropyl groups can thus be obtained. This complex model is consistent with the previously proposed pearl-

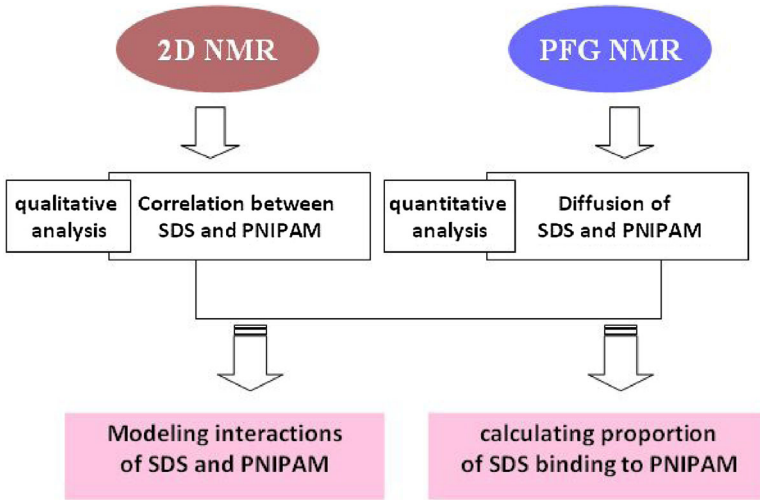

Figure 1.
NMR methods to study effect of SDS on phase transition of PNIPAM.

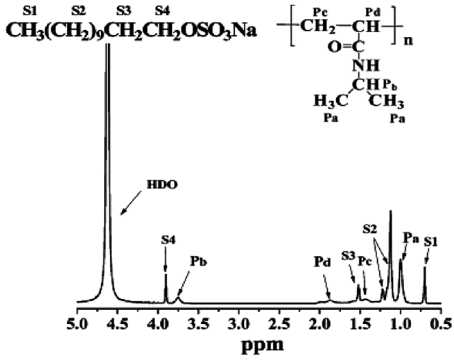

Figure 2.
^{1}H NMR spectrum of the PNIPAM/SDS/D_2O solution at 298 K.

necklace-like polymer-surfactant complex structure.[24] The anionic SDS is firmly associated with the polymer chain; thus, the PNIPAM/ SDS complexes bear the characteristics of a polyelectrolyte.

The polyelectrolyte-like complexes of PNIPAM and SDS greatly affect the coil-to-globule transition of PNIPAM in an aqueous solution. The electrostatic repulsions of the charged chains resist the hydrophobic interactions between the polymer chain segments when heating induces the solvent to deteriorate, which results in the retardation of the PNIPAM chain collapse and an increase in the LCST. On the other hand, the interchain electrostatic repulsions inhibit the interchain aggregation so that the PNIPAM single-chain compact globule can be obtained. However, the noticeable change in the 2D NOESY spectra in Figure 3 shows the marked decrease in the signal intensity of the crosspeaks between PNIPAM and SDS with increasing temperature, although about half of the PNIPAM units are detected in the NMR spectra above the LCST.[25] Slice 1D spectra were extracted from the 2D NOESY spectra and are shown in the right part of Figure 3. Compared with the extracted slice spectra, the signal intensities of the cross-peaks at the Pa-S4, Pa-S2, and Pa-S3 proton pairs are clearly reduced with increasing temperature, and almost no cross-peak is observed between PNIPAM and SDS above the LCST. This temperature dependence of the cross-peak indicates a disassociation between the polymers and SDS molecules. At high temperatures, most of the original polymer-bound SDS are assumed to be expelled from PNIPAM. This expulsion is ascribed to the electrostatic repulsions and steric hindrance effects accompanying the continuous collapse of the polymer chain.

PFG NMR analyses of the PNIPAM/ SDS/D_2O solutions were also performed. Two types of SDS, namely, polymer-bound and free SDS, exist in the PNIPAM and SDS aqueous mixture below the LCST. Therefore, biexponent fitting strategies for attenuation curves were employed to analyze the diffusion coefficients of SDS. Consequently, based on the Stejskal–Tanner equation[26] the SDS diffusion data were calculated using the equation:

$$\frac{I}{I_0} = f\exp(-D_{free}^{SDS}(\Delta - \delta/3)(\gamma\Delta g)^2 + (1-f)\exp(-D_{bound}^{SDS}) \times (\Delta - \delta/2)(\gamma\Delta g)^2) + A \quad (1)$$

where the polymer-bound and free SDS diffusion coefficients are expressed as D_{bound}^{SDS} and D_{free}^{SDS}, f is the molar fraction of the polymer-free surfactant in solution, $\frac{I}{I_0}$ is the ratio of the signal intensity to the outset value, $\gamma = \gamma_H = 2.6571 \times 10^8$ T^{-1} s^{-1} is the gyromagnetic ratio of proton, δ is the duration of the field gradient pulse, Δ is the time period between the two field gradient pulses, and g is the gradient pulse field, and A is a constant.

The diffusion coefficients of SDS and PNIPAM at various temperatures are summarized in Table 1. At 298 K, which is below the LCST, SDS clearly exists as two components, most of which is in the individual state but roughly 16% is bound to the PNIPAM chain and forms polymer-bound SDS micelles. The diffusion coefficient of the polymer-bound SDS is lower than that of individual ones by one order of magnitude but approaches that of PNIPAM. When the temperature is increased to a value above LCST, only one

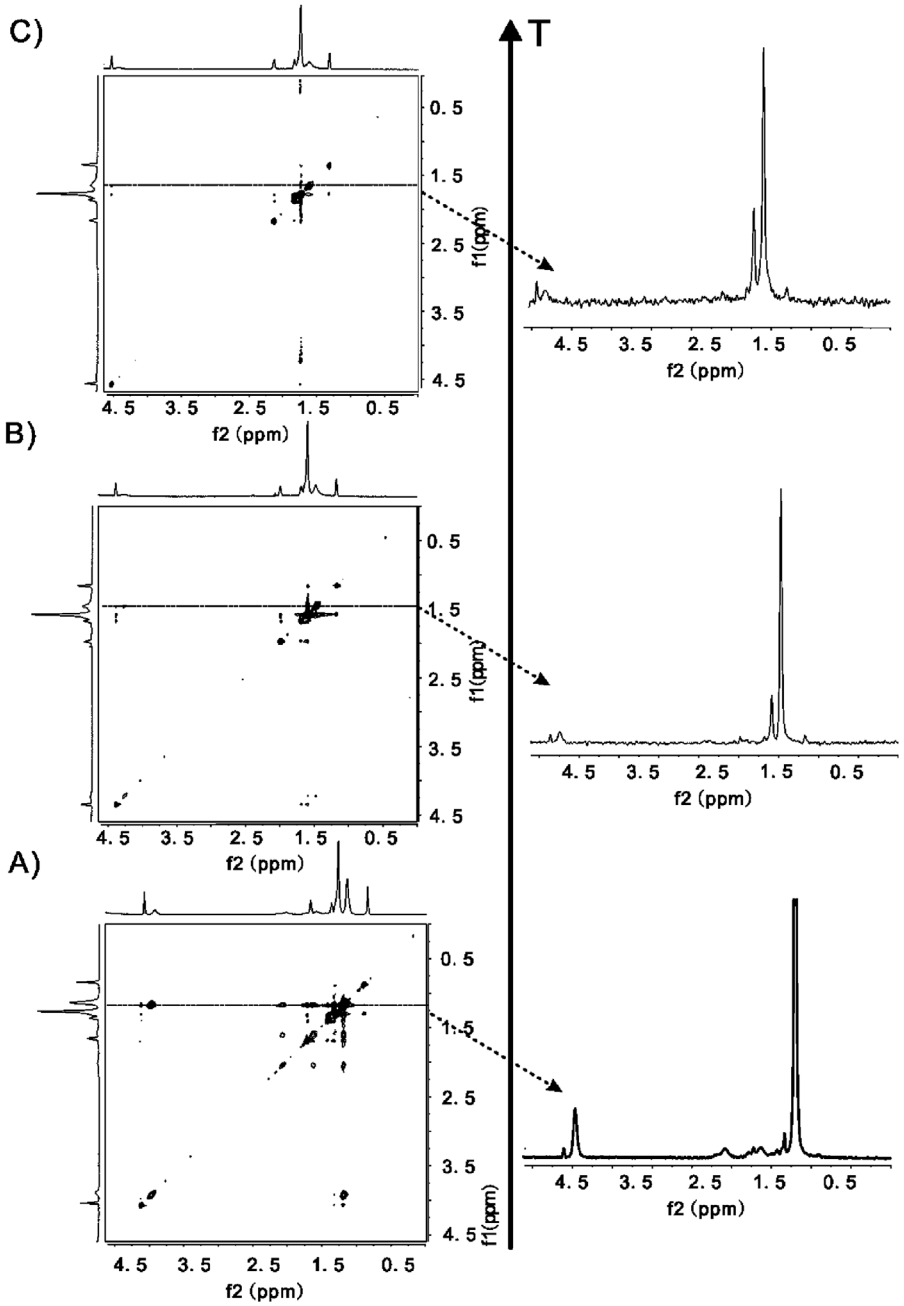

Figure 3.
Left: 2D NOESY spectra at various temperatures: 298 K (A), 323 K (B), and 338 K (C). Right: 1D spectra extracted from the underlined slices in the left spectra.[25]

component of SDS is observed at 323 and 338 K. In addition, the normalized number of SDS is almost constant around 1.0 (Table 1), and all SDS are detected in the NMR spectra during both the heating and cooling processes. Thus, all SDS at the elevated temperatures are uniform and exist as one component. The diffusion

Table 1.
Diffusion coefficients (in m^2/s) of SDS and PNIPAM in the PNIPAM/SDS/D_2O solution at various temperatures.[25]

Temperature (*K*)	SDS							PNIPAM	
	individual SDS				polymer-bound SDS				
	f^{SDS}_{free}	$f^{SDS}_{free-error}$	D^{SDS}_{free}	$D^{SDS}_{free-error}$	f^{SDS}_{bound}	D^{SDS}_{bound}	$D^{SDS}_{free-error}$	D^{PNIPAM}	D^{PNIPAM}_{error}
298	0.84	±0.04	6.90E-10	±0.70E-10	0.16	0.58E-10	±0.38E-10	1.13E-10	±0.01E-10
323	1.0	±0.1	8.23E-09	±0.71E-09	0			1.01E-09	±0.58E-09
338	1.0	±0.2	9.43E-08	±2.20E-08	0			3.71E-08	±0.71E-08

coefficients of SDS are much higher than those of PNIPAM, which is due to the conversion of all SDS moieties into the free individuals ones above the LCST.[25]

The 2D NOESY spectra shown in Figure 3 confirm the dissociation and expulsion of a portion of the original polymer-bound SDS from PNIPAM above the LCST, possibly because of electrostatic repulsions and steric hindrance effects during the continuous collapse of the polymer chain.

NMR Spectra, Relaxation Times T_2 and Diffusion Coefficients to Study Effects of Salts on Phase Transition of PNIPAM

PNIPAM aqueous solutions after addition of the salt (NaCl, Na_2SO_4, Na_3PO_4) at two salt concentrations have been investigated by ^{1}H NMR spectroscopy. Figure 4 shows the ^{1}H NMR spectrum of PNIPAM in Na_2SO_4 aqueous solution ($C_{Salt} = 0.046$ M) at various temperatures.

From integrated intensities in ^{1}H NMR spectra the phase-separated fraction p can be calculated[11] using the relation

$$p = 1 - (I_{(T)}/I_{0(T)}) \quad (2)$$

where

$$I_{0(T)} = I_{(T0)} \times (T_0/T)$$

Here $I_{(T)}$ is the integrated intensity of a given resonance of PNIPAM measured in phase-separated system and $I_{0(T)}$ is the integrated intensity of this resonance if no phase separation occurs. For $I_{0(T)}$, we took values based on integrated intensities below the transition, using the expected $1/T$ temperature dependence. Temperature dependences of the fraction p of phase-separated units of PNIPAM as obtained from integrated intensities of the signal of CH proton in isopropyl group after addition of three salts at two concentrations are

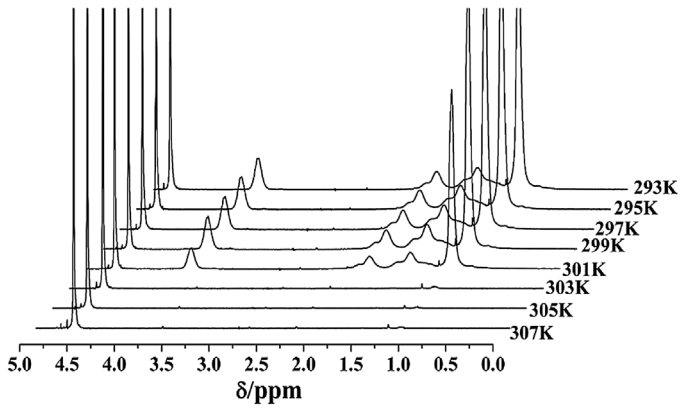

Figure 4.
^{1}H NMR spectrum of PNIPAM in Na_2SO_4 aqueous solution ($C_{Salt} = 0.046$ M) at various temperatures.

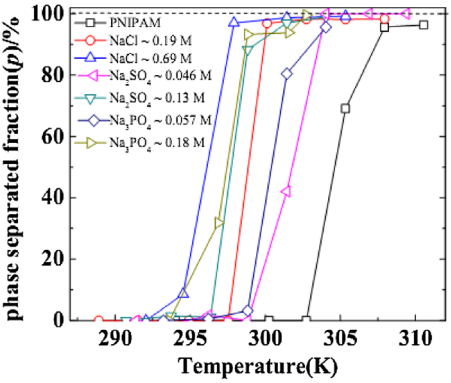

Figure 5.
Temperature dependences of phase-separated fraction p for isopropyl methine protons in D_2O solutions of PNIPAM containing different salts.

shown in Figure 5. In this figure the phase separated fraction p calculated from integrated intensities in ^1H NMR spectra shows the whole process of phase transition induced by temperature change. The shift of the LCST transition of PNIPAM in the presence of different salts at comparable concentration and those at different concentrations for the same salt are distinct. LCST transition shifts to lower temperature for all salt samples, and at low concentration of the salt the order of the ability to lower LCST for the three salts is $Na_3PO_4 \sim Na_2SO_4 > NaCl$, which is consistent with the order of Hoffmeister sequence. For the same salt, the shift of LCST transition depends strongly on the concentration of salt.

T_2 relaxation times of HDO at temperatures below (293 K) and above (310 K) the phase transition are shown in Table 2. At 293 K, the T_2 of water molecules exists as single component in all salt samples as well as in neat PNIPAM solution. This shows that the mobility of water molecules is effectively uniform below the phase transition of PNIPAM indicating a fast exchange between bound and free sites for all the sample solutions.[11] When temperature increased to 310 K and phase transition took place, the T_2 of HDO in the two NaCl solutions and Na_3PO_4, Na_2SO_4 solutions at low salt concentrations exhibited bi-component behavior with fast and slow T_2 relaxation. On the other hand the T_2 of HDO in the neat PNIPAM solution and solutions containing Na_3PO_4 or Na_2SO_4 at high concentration of the salt remained as single component. For the bi-component of T_2 for HDO in the four solution samples, the fraction for the portion of HDO molecules that exhibited a lower (spatially restricted) mobility does not exceed 10%. The results can indicate that this small portion of spatially restricted water corresponds to HDO molecules bound in globular-like structures when the phase transition took place. For the single component behaviour of T_2 for HDO in the rest three sample solutions the situation is somewhat complicated. In the case of the neat PNIPAM solution, the collapsed PNIPAM forms loose globules and the fast exchange of water molecules between bound and free sites still exists. However, in the solutions with Na_3PO_4 and Na_2SO_4 at high concentration, the collapsed PNIPAM is more compact compared to the sample with the same salt at lower salt concentration. Therefore, almost no water in the compact

Table 2.
Spin-spin relaxation time T_2 of HDO in PNIPAM/salt/D_2O solutions at 293 K and 310 K.

Salt	Salt/M	PNIPAM/mg/L	293 K		310 K		
			components	T_2/s	components	T_2/s	
–	–	10	1	1.57	1	2.12	
NaCl	0.19	10	1	1.52	2	0.12(≈10%)	2.98
NaCl	0.69	10	1	0.90	2	0.13(≈5%)	1.24
Na_2SO_4	0.046	10	1	1.29	2	0.31(≈5%)	2.33
Na_2SO_4	0.13	10	1	0.89	1	0.72	
Na_3PO_4	0.057	10	1	0.90	2	0.088(≈5%)	2.11
Na_3PO_4	0.18	10	1	0.86	1	0.75	

globule can be detected in the T_2 relaxation measurement and the single component of T_2 corresponds to HDO in the bulk solution which is largely affected by the addition of salts at higher concentration in comparison with the long T_2 component detected for the solution with the same salt at lower salt concentration.

Table 3 shows diffusion coefficients of HDO molecules and Na^+ cations as obtained on PNIPAM/salt/D_2O solutions by using ^1H and ^{23}Na NMR spectroscopy. The values D_{HDO} of the diffusion coefficient of HDO are at given temperature very similar for all investigated systems. At the same time D_{HDO} values at 310 K are significantly larger than those at 293 K, as expected for any polymer solution at higher temperature. Evidently D_{HDO} values do not reflect subtle changes as detected above the LCST transition in T_2 measurements (cf. Table 2). Similar behavior was previously found also for D_{HDO} in PNIPAM/D_2O gels during the volume phase transition.[27]

Concerning the diffusion coefficients of Na^+ cations, it follows from Table 3 that at 293 K the value D_{Na+} is significantly smaller for PNIPAM solution containing high concentration of Na_3PO_4 salt in comparison with other investigated systems. At 310 K, i.e., above the phase transition, D_{Na+} values are larger than those at 293 K and similar for all samples ($D_{Na+} = 1.3–1.5 \times 10^{-9}$ m^2/s). No change in D_{Na+} values, e.g., such as existence of the component with smaller D_{Na+}, was detected during LCST transition. In accord with other authors[17] this result indicates that the major effects on phase transition of PNIPAM in aqueous solution are produced by anions.

Conclusion

The effects of surfactant (SDS) and three salts (Na_3PO_4, Na_2SO_4 and NaCl) on the coil-to-globule transition of PNIPAM were studied by various methods of NMR spectroscopy. At SDS concentrations above the CAC and temperatures below the LCST SDS exists in two different forms, namely, as the polymer-bound and free surfactant. Approximately 16% of SDS bound to polymer chain greatly affects the conformational structure of the polymer, which exhibits the characteristics of a polyelectrolyte. The disappearance of the cross-peaks between PNIPAM and SDS in the 2D NOESY spectra above the LCST shows that most of the polymer-bound surfactants were dissociated and expelled from the polymer chain due to electrostatic repulsions and steric hindrance effects.

In the presence of inorganic salts, the values of phase-separated fraction p indicate the order of abilities of salts to lower the LCST at comparable concentration: $Na_3PO_4 \sim Na_2SO_4 > NaCl$. Moreover, the T_2 relaxation time of HDO indicates that the type of the salt and its concentration affects the conformation of the collapsed PNIPAM globules in the aqueous solution. Diffusion coefficients of Na^+ cations support the idea that the major effect on phase transition of PNIPAM in aqueous solution is produced by anions.

Table 3.
Diffusion coefficients D of HDO and Na^+ cations in PNIPAM/salt/D_2O solutions at 293 and 310 K.

Salt	Concentration Mol/L	PNIPAM Mg/mL	$D_{HDO}/([m^2/s]^*10^9)$		$D_{Na+}/([m^2/s]^*10^9)$	
			293 K	310 K	293 K	310 K
–	–	10	1.40	2.35	–	–
NaCl	0.19	10	1.33	2.20	0.896	1.42
NaCl	0.69	10	1.30	2.22	0.954	1.48
Na_2SO_4	0.046	10	1.37	2.23	0.904	1.50
Na_2SO_4	0.13	10	1.33	2.16	0.842	1.35
Na_3PO_4	0.057	10	1.28	2.21	1.02	1.35
Na_3PO_4	0.18	10	1.29	2.12	0.59	1.31

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