
ATR-FTIR Studies on the Effect of Strong Salting-out Salts on the Phase Separation Scenario in Aqueous Solutions of Poly(*N*-isopropylacrylamide) [PNIPA]†

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ABSTRACT

Attenuated total reflectance (ATR) FTIR spectroscopy was used to investigate the effect of two strong salting-out salts (Na₂SO₄ and K₂SO₄) on the temperature-induced phase separation process in aqueous solutions of poly(*N*-isopropylacrylamide) [PNIPA]. It was found that the addition of these salts not only depresses the phase transition temperature (as was pointed out by other groups in the past) but, in fact, changes the whole scenario below, at, and above the aggregation temperature. Deconvolution of the amide II peak into three subbands reflecting three types of populations that differ by their hydrogen bonding types revealed that the sulfate ions decreased the amount of amide–water hydrogen bonds, while increasing the amount of amide–amide intramolecular H-bond population. This led to a relatively compact structure even at low temperatures. Based on the behavior of the hydrophobic IR peaks it is suggested that addition of sulfate ions increases the importance of hydrophobic interactions below the phase separation temperature. This study demonstrates the potential of ATR-FTIR in providing information at a molecular level on phase separation phenomena in aqueous solutions of hydrophobic polymers and, in

particular, on the effects of co-solutes in such systems. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: FTIR; hydrogen bonding; poly(*N*-isopropylacrylamide); phase separation; sodium sulfate; potassium sulfate

INTRODUCTION

It is well known that many nonionic water-soluble polymers are temperature-dependent and exhibit entropy-driven phase separation phenomena. Understanding the scenario leading to the phase separation and explaining it in terms of pre-transition structural changes can be very useful in elucidating the mechanisms governing many biological systems such as the denaturation of large proteins [1]. A common model polymer for such studies is poly(*N*-isopropylacrylamide) [PNIPA], a polyvinyl polymer, containing both hydrophilic amide and hydrophobic isopropyl groups in its side chains (for a comprehensive review on PNIPA, see [2].) Owing to its side chains, the polymer is soluble in polar solvents (such as water) as well as in low-polarity solvents such as tetrahydrofuran. It has been proposed [3] that phase separation in PNIPA solutions is a two-step mechanism, initiated by the collapse of single chains and progressed by

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†This paper was presented at PAT 2001 – Eilat, Israel.

aggregation. According to this explanation, PNIPA chains in aqueous solution form expanded coils at room temperature that undergo a coil to globule transition when the temperature is increased to 34–35 °C (approximately the theta-temperature).

Although the gross scenario of the phase separation process in PNIPA solutions is well understood, the detailed scenario of the process is still under debate. More specifically, the relative role of PNIPA–water hydrogen bonding versus that of intramolecular hydrogen bonding and versus that of hydrophobic interactions is not yet fully understood [4–7]. To large extent, this is due to the fact that most of the experimental methods used for studying these phenomena (such as calorimetry [9–11], rheology [12] and fluorescence [13–15]) are silent with respect to providing detailed information on a molecular level. One of the few methods that may provide useful information on a molecular level is infrared spectroscopy. However, direct absorption measurements with aqueous solutions of polymers are problematic due to the high absorptivity of water. Implementation of the attenuated total reflectance (ATR) set up has given a way to overcome this obstacle [16–19]. In two pioneering works, ATR-FTIR was used in studies related to the temperature-dependent phase separation of PNIPA gels [20] or PNIPA/water solutions [21]. The same technique was used recently to clarify the role of water–polymer hydrogen bonding versus that of intramolecular hydrogen bonding in the phase separation process of aqueous solutions of PNIPA [22].

The temperature of the phase separation in PNIPA is known to be affected by the presence of co-solutes such as salts, sugars and surfactants. For salts, this effect follows, in most cases, the classical Hofmeister series [23], which ranks simple inorganic salts according to their relative ability to precipitate proteins (a salting-out effect) [24, 25]. According to the Hofmeister series the precipitating efficiency of anions is: $\text{SO}_4^{2-} > \text{Cl}^- > \text{Br}^- > \text{I}^- > \text{SCN}^-$. A similar series holds for cations; however, the effect of cations on the temperature-induced phase separation in aqueous solutions of PNIPA is generally smaller than that of anions [26]. In PNIPA hydrogels, an addition of a salting-out salt is manifested by a decrease in volume of the gel [26–28]. Here, the volume effects were attributed to distortion of the water molecules in the hydrophobic hydration shell around the PNIPA [29]. As was pointed out clearly in a recently published review [26] the effect of salts may act through one (or more) of the following mechanisms: (1) direct stabilization or destabilization of the nonpolar isopropyl groups, (2) change in the water structure or (3) direct interaction of salts with the amide groups. The first mechanism is in line with observations on the effect of salts on the activity coefficients of trimethyl amine [30], a nonionic solute having a structure similar to that of the pendent isopropyl groups in PNIPA. Evidence for the validity of the second mechanism is based on correlating between the viscosity B coefficient

(VBC) of salts solutions and the phase separation temperature [31]. According to this explanation, small and polyvalent ions are water-structure makers, who have positive VBC values, which are manifested by enhancing hydrophobic interactions, thus decreasing the phase separation temperature. These two mechanisms seem to fit the relative effect of anions on the phase separation temperature. Nevertheless, both mechanisms also imply a significant effect of the type of cations on the phase separation temperature, in contrast to observations. The possibility that the effect of salts is due to direct interactions between the salts and the amide groups (the third mechanism) is based on the observations that salts bind to the amide groups in poly(acrylamide) [32], and that vicinyl methyl groups (as exist in PNIPA) may enhance selective binding to the amide groups [33].

Specific binding between salts and segments in the polymer may affect the intramolecular bond strength. In that case, one may expect some shifts in the vibrational spectrum of the polymer, in particular in those vibrational modes which are strongly coupled with the relevant functional groups. The effect of salts on the LCST aqueous solutions of PNIPA was investigated, concluding that salts did not have any influence on the IR spectrum profile [34, 35].

In the work described herein, ATR-FTIR was used to study the effect of salts on the phase separation scenario in PNIPA. In particular we aimed to find out whether the presence of salt introduces any specific changes in the scenario, apart from the well-known change in the transition temperature. Within this context, the effect of the divalent anion SO_4^{2-} , ranked as a strong “salting-out” anion is presented. A parallel study on the effect of a strong “salting-in” anion, CNS^- , is currently in progress.

EXPERIMENTAL

PNIPA samples (7 ± 1 MDa) were prepared by employing a redox polymerization in aqueous solution at 4 °C with a similar protocol to that used by Otake *et al.* [36]. Details on the preparation scheme can be found in an already published paper, describing the phase separation scenario in a water–PNIPA system, i.e. without the co-solute [22]. Measurements with the ternary system water–PNIPA–salt were carried out with Na_2SO_4 and with K_2SO_4 at concentrations of 0.09 M and 0.18 M. The latter concentration is equivalent approximately to the number of NIPA monomer units in the polymer solution.

ATR-FTIR measurements of aqueous solutions of PNIPA (with and without co-solutes) were performed by a Bruker IFS55 machine equipped with a temperature-controlled circle cell accessory (CC-ATR). The accessory was made of a ZnSe cylindrical crystal (75 mm long, 6 mm in diameter, 45° edges), fixed by a leak-proof O-ring at the center of a temperature-controlled stainless steel

vessel (60 mm long, 15 mm inner diameter) containing the PNIPA solution. To increase signal to noise ratio, the IR beam was first de-collimated, then redirected onto the crystal edges in a circular manner that made it possible to probe the whole circumference of the embedded crystal, with a reflecting angle of 45°. The IR beam that impinged on the edges of the crystal, outside of the vessel, propagated through the crystal and exited through the other end where it was redirected by another set of mirrors towards the IR detector. The short distance from which the signal is collected by this method (a few micrometers) made it possible to overcome problems related to the high absorption coefficient of the solvent. The temperature of the sample solution was monitored by a thermocouple inserted into the vessel. The ATR-FTIR spectra were taken in the range of 10–48°C with a resolution of 1°C. The circle cell was equipped with a transparent PMMA cover, making it possible to follow the temperature-induced transition from a clear solution into a turbid one.

All measurements were taken using the empty circle cell as a background. Since the IR spectrum of water is known to change as a function of temperature, mainly due to breaking of hydrogen bonds [37], care was taken to record a set of temperature-dependent spectra of water, later to be subtracted from the temperature-dependent spectra of the polymer solutions.

RESULTS

Figure 1 presents the ATR spectra of aqueous solution of PNIPA at temperatures of 22, 34, 35, 36, 37, and 45°C. These spectra were obtained by subtracting the spectra of water at the corresponding temperatures from that of the raw data. To account for the effects of dilution, changes in penetration depth, and interaction between the solvent and the polymer, a multiplication coefficient was fitted to the water spectra. Fitting was based on obtaining a perfect elimination of the water wide combination band peaked at 2125 cm⁻¹, a peak that is known to be a good indication for water quantity [38]. Another reason for using this peak as a subtraction guide was the fact that this spectral range is clear of polymer-related peaks, unlike, for example, the HOH bending peak at 1635 cm⁻¹ that partially overlaps with the amide I peak of PNIPA. For a 2.5% solution the multiplication factor below the phase transition temperature ranged between 0.98 and 0.99, whereas above the phase separation point the value of this factor was no more than 0.94–0.95. The phase transition at *ca.* 35°C is clearly indicated by the intensity enhancement in the amide I (mostly C=O stretch) band and amide II (mostly N–H in-plane deformation) band at 1628 cm⁻¹ and 1556 cm⁻¹ respectively [39] as well as in the coupled band of C–H and CH₃ at 1460 cm⁻¹. In parallel, an increase in the signal of the isopropyl group [20] at 1355–1400 cm⁻¹ was observed, together with that of the

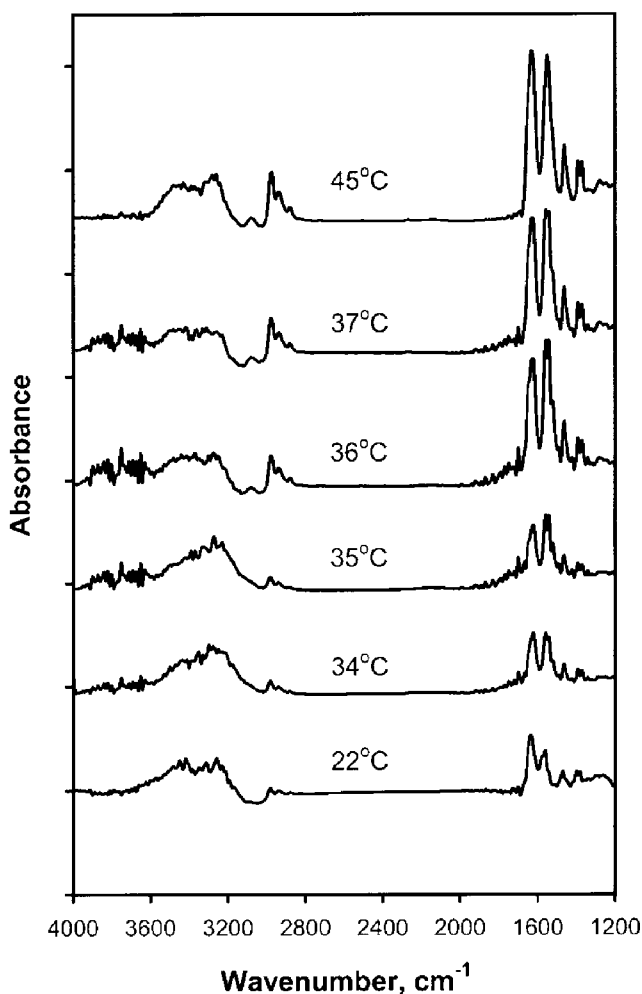


FIGURE 1. Subtraction spectra of a 2.5% PNIPA solution at six different temperatures. The origin of the difference between the 35°C spectrum and the 36°C spectrum is the phase transition occurring at that interval.

CH₂ antisymmetric mode and the CH₃ symmetric and antisymmetric stretching modes. Here, the phase separation was manifested by a sharp change in the location of the peaks towards lower frequencies: the CH₃(s) peak was shifted from 2882 to 2878 cm⁻¹, the CH₂(a) peak was shifted from 2939 to 2936 cm⁻¹ and the CH₃(a) peak was shifted from 2982 to 2975 cm⁻¹. Line-shape fitting of these peaks revealed a gradual decrease in their width below the phase transition point, following by an increase in their width at the phase separation temperature. Above the phase separation point another three weak peaks could be resolved. These were the amide II overtone at 3070 cm⁻¹, H-bonded N–H stretch at 3310 cm⁻¹ and another peak at 3550 cm⁻¹ (free N–H stretch?) with intensities of 5%, 10%, and 7% of that of the amide II, respectively. The abrupt increase in all the polymer-related peaks and the abrupt decrease in all the water-related peaks at the phase separation point were attributed to the formation of a polymer-enriched film on the surface of the ZnSe crystal [22]. Subtraction spectra of PNIPA solutions

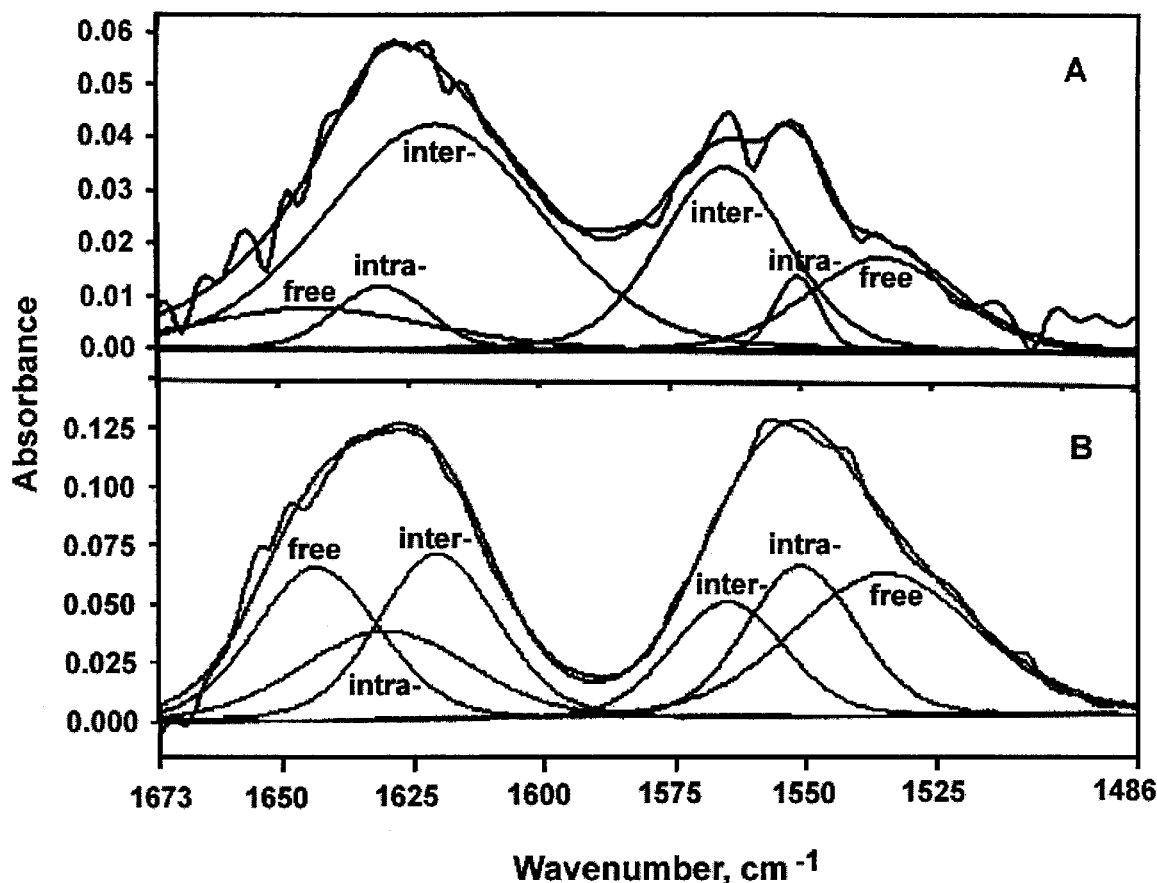


FIGURE 2. Deconvoluted spectra of the amide I and amide II peaks at 25°C (A) and at 40°C (B) in a PNIPA–water system, showing the inter-, intra- and free components of each band.

containing Na_2SO_4 or K_2SO_4 revealed the same gross features. Here, the phase separation point was observed at $28 \pm 1^\circ\text{C}$ and $31 \pm 1^\circ\text{C}$ in the presence of Na_2SO_4 (0.18 M) and K_2SO_4 (0.18 M), respectively.

It is well-known that the band contour of the amide I and the amide II peaks are conformationally sensitive [9]. Following the treatment of Lin *et al.* [21], the amide I peak (mostly C=O stretch) in the subtraction spectra was deconvoluted into three sub-bands: C=O \cdots NH H-bonded sub-band at 1630 cm^{-1} (to be regarded as the intramolecular H-bonded sub-band); C=O \cdots HOH H-bonded sub-band at 1620 cm^{-1} (termed the intermolecular H-bonded sub-band) and a free form of non-H-bonded sub-band at 1643 cm^{-1} . Likewise, the amide II band (mostly N–H deformation) was deconvoluted into intramolecular H-bonded sub-band at 1551 cm^{-1} ; intermolecular H-bonded sub-band at 1565 cm^{-1} and a free form of non-H-bonded sub-band at 1535 cm^{-1} . These wavenumbers are in line with the notion that H-bonding tends to make steeper the potential surface of deformational modes while flattening the potential surface of stretching modes [40]. In the deconvolution, peak positions and peak shapes (20% Lorentzian and 80% Gaussian) were taken as constant parameters, while the intensity and the width of the

amide I, II sub-bands were regarded as free variables.

Figure 2 presents the deconvoluted IR spectra of the amide I and amide II peaks of a 2.5% PNIPA solution at 25°C and at 40°C. The difference in the peaks' shape, representing a decrease in the relative population of polymer–water (intermolecular) H-bonds and an increase in the relative population of polymer–polymer (intramolecular) H-bonds is clearly observed. A detailed, quantitative examination of the temperature-dependence of the relative area of each of the amide II sub-peaks in the water–PNIPA system is presented in Figure 3(A). The results of the same treatment done on a water–PNIPA– Na_2SO_4 (0.18 M) system are depicted in Figure 3(B). It is noteworthy that the deconvolution of the amide II peak in water–PNIPA– K_2SO_4 system gave the same results as in Figure 3(B) and that the PNIPA concentration did not have a significant influence on the deconvolution results. The water–PNIPA system (Figure 3(A)) was characterized by four different stages:

- (I) Below 31°C : raising the temperature causes a moderate decrease in the intermolecular H-bonds and a moderate increase in the free amide population. The intramolecular H-bond population hardly changes.

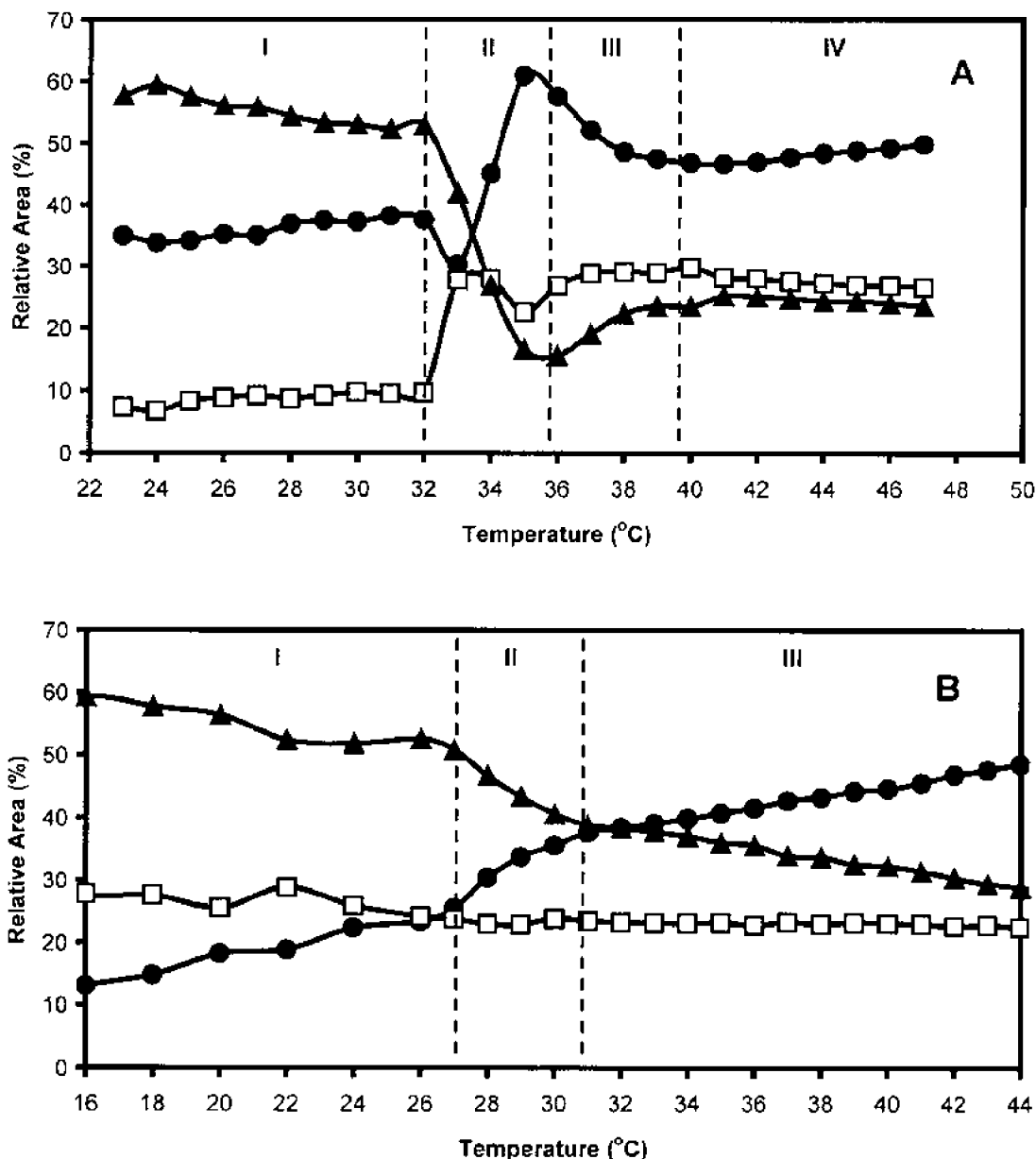


FIGURE 3. Changes in the three sub-bands of the amide II peak as a function of temperature for an 2.5% aqueous solution of PNIPA (A), and for a 2% aqueous solution of PNIPA in the presence of 0.18 m Na₂SO₄ (B): ▲, intermolecular H-bonding sub-band; ●, "free" amide groups; □, intramolecular H-bonding sub-band.

- (II) 32–35°C: an abrupt decrease in the intermolecular H-bonds population, and an abrupt increase in the intramolecular H-bond and the free amide populations. This region was characterized also in an abrupt increase in the width of the free amide sub-band.
- (III) 36–39°C: An increase in the relative amount of intermolecular H-bond population and a decrease in the integrated absorbance and width of the free amide sub-band.
- (IV) Above 40°C: a moderate increase in the relative population of the free amide, coupled with a moderate decrease in the intramolecular H-bond and intermolecular H-bond populations.

Apparently, the temperature-dependency of the amide II sub-bands in the water–PNIPA–Na₂SO₄ system seems to be similar to that in the water–PNIPA system. For example, in both systems raising the temperature of the solution causes a decrease in the relative amount of intermolecular H-bonds and an increase in the relative amount of the "free" amide groups. Likewise, the phase separation stage in both systems is manifested by a sharp increase in the slope. At higher temperatures, far enough from the critical point, these changes with temperature become again moderate, having values that resemble the values observed far below the critical point.

A more thorough comparison between the two

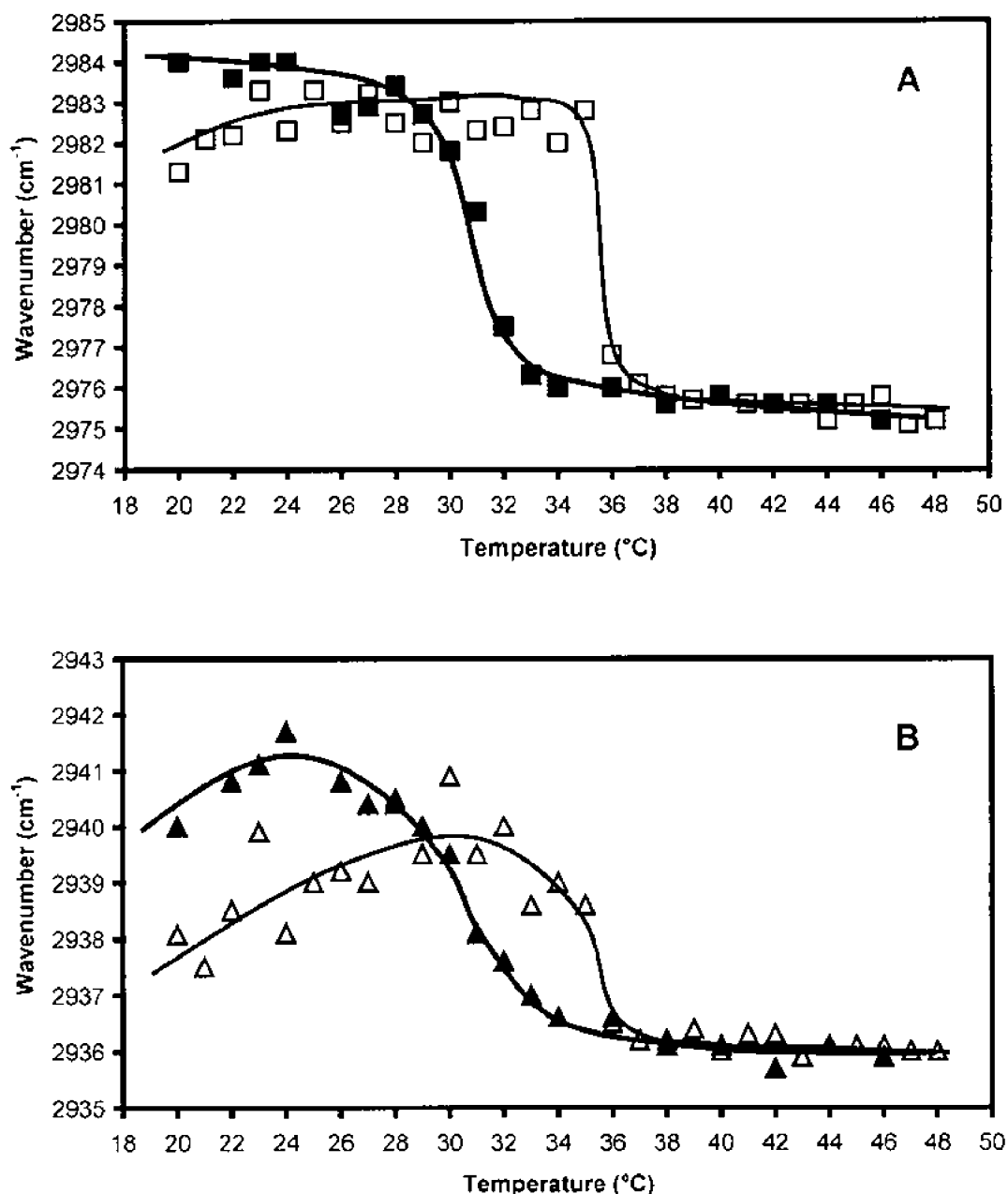


FIGURE 4. Shift in the wavenumber values of CH₃ (A) and CH₂ (B) asymmetric stretching modes as a function of temperature: empty symbols correspond to PNIPA–water system, filled symbols correspond to PNIPA–water–0.18 M K₂SO₄ system.

graphs reveals a number of distinct differences. The most striking differences are the relatively high extent of intramolecular (amide–amide) H-bonds in the water–PNIPA–Na₂SO₄ system even at low temperatures, far from the phase separation point, and the fact that the relative area of this sub-band in the co-solute-added system does not change with the temperature. This is in contrast to the situation in the water–PNIPA system, where the amount of intermolecular H-bonds is very low at low temperatures and increases abruptly a few degrees below the phase separation temperature. Another difference is the absence of a post-phase transition rearrangement stage in the co-solute

added system. Hence, the temperature-induced phase separation in the ternary system consists of three stages only. The differences outlined above suggest that the salting-out salts (Na₂SO₄ and K₂SO₄) act to “smooth” the phase separation process. Moreover, it suggests that the solvated configuration of the polymer in the ternary system is closer to its post-transition configuration in comparison with the situation in the water–PNIPA system.

As observed by others [20], the transition between the solvated configuration and the aggregated configuration is manifested also by a shift towards lower energies in IR peaks assigned to the

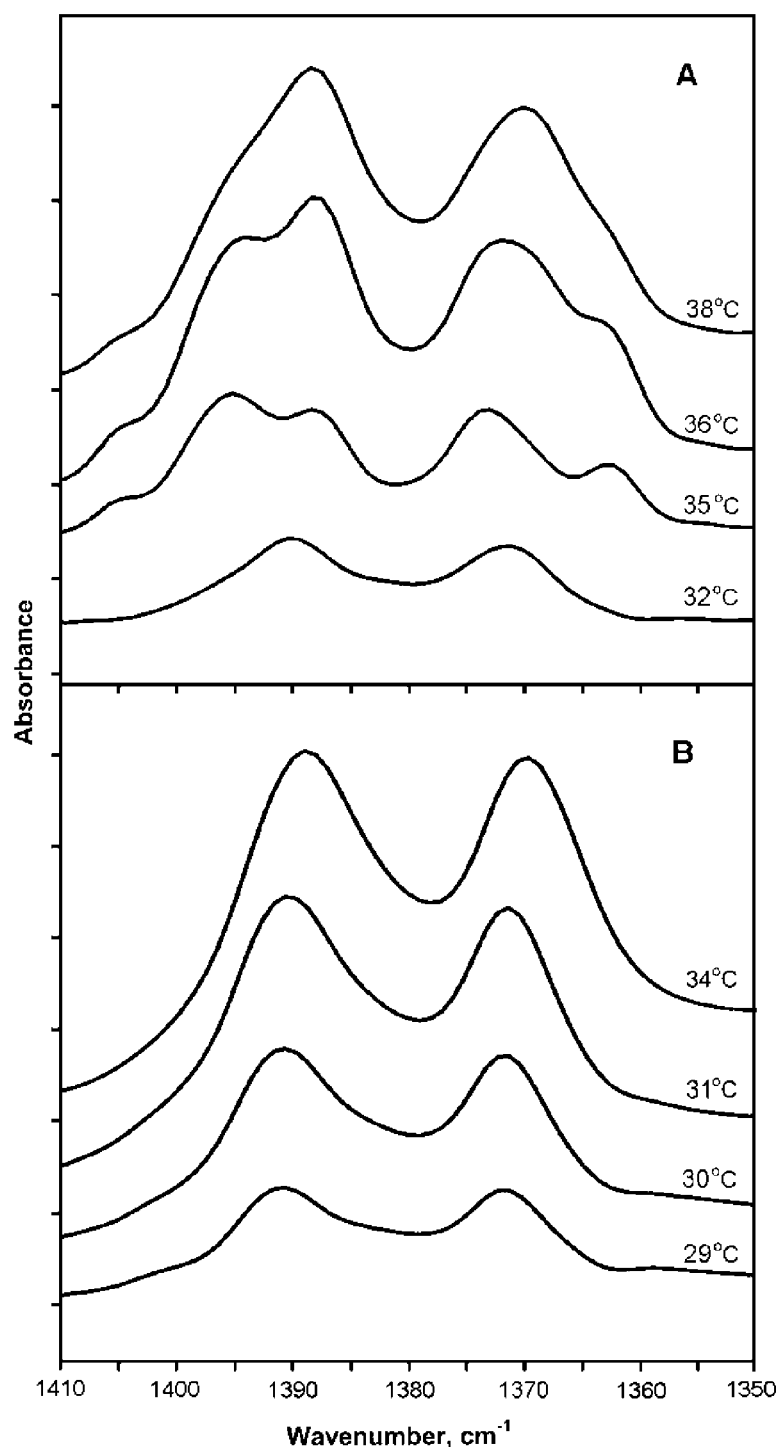


FIGURE 5. Spectra of isopropyl peaks of PNIPA aqueous solution in the absence (A) and in the presence (B) of 0.18 M K_2SO_4 , at four different temperatures around the phase separation point.

CH_2 , CH_3 asymmetric stretch. Figure 4 presents the wavenumbers values of these peaks, showing that the hydrophobic moieties' peaks in both the PNIPA-water system and the PNIPA-water- K_2SO_4 system shift towards lower wavenumber values at the phase transition. The wavenumber values at temperatures higher than the phase separation point were identical for both systems,

i.e. 2936 cm^{-1} and 2975.5 cm^{-1} for the $CH_2(a)$ and $CH_3(a)$ peaks, respectively. Nevertheless, the values at temperatures below the critical points were higher by two to three wavenumbers in the ternary system than in the binary system. This difference is more pronounced for the CH_3 peak (isopropyl) than for the CH_2 peak. Although the overall change in the peak's location is larger for

the ternary system, its derivative with respect to temperature during the phase transition point seems to be more gradual in comparison with the binary system, in agreement with the trend observed by deconvoluting the amide II peak. Here, again, no difference was observed between ternary systems containing K_2SO_4 and ternary systems containing Na_2SO_4 .

Another set of hydrophobic peaks consisted the twinned isopropyl peaks of the polymer at 1370 cm^{-1} and at 1390 cm^{-1} . Here, the change in the location upon phase separation was found to be very mild (from 1371.6 to 1369.2 cm^{-1} , and from 1391.1 to 1388.7 cm^{-1}) for the PNIPA-water system as well as for the sulfate-containing systems. Although both systems behaved exactly the same away from the phase transition point, a significant difference between the two systems at the phase separation point was observed. Here, a second set of peaks at 1362.5 cm^{-1} and at 1395 cm^{-1} appeared in the PNIPA-water system, but not in the sulfate-containing systems. These two peaks were found to gradually emerge into the 1371 cm^{-1} and at 1389 cm^{-1} peaks upon further heating, disappearing entirely at a temperature of 37°C (see Figure 5).

DISCUSSION

The phase separation process of the PNIPA-water binary system was recently discussed in detail by the authors of this manuscript [22]. In that work, a scenario based on structural changes in the polymer, driven by H-bonding forces related to the amide groups, was proposed for the phase separation process. According to this explanation, as the temperature is raised, more and more water-amide H-bonds are broken. As a consequence, the amount of "free" amide groups is increased. Once a critical number of free amide groups is achieved, the polymer chain becomes flexible enough to change its conformation in a manner that promotes the formation of intramolecular (i.e. amide-amide) H-bonding. Once intramolecular H-bonds are formed (at some expense of flexibility), hydrophobic interactions begin to play a more significant role, leading to the collapse of the chains, which is immediately followed by aggregation due to hydrophobic interactions. The collapsed, aggregated polymer tends to adhere to the ATR crystal, hence an increase in the intensity of all polymer-related peaks was observed, together with a decrease in the intensity of all the water-related peaks. This scenario is followed by a rearrangement stage, which reflects the formation of hydrogen bonds between some free amide moieties and water that was entrapped within the collapsed network.

The results presented in this paper, regarding the sulfate-containing system, reflect a scenario which is different from the one described for the PNIPA-water system. Here, the most striking result is the relatively high amount of intramolecu-

lar amide hydrogen bonds far below the pre-transition stage (a relative peak area of 30% for the ternary system versus approximately 10% in the binary system). In the PNIPA-water system the amount of intramolecular H-bonds increases abruptly to 30% at the onset of the phase transition. In contrast, the relative amount of intramolecular H-bonds in the sulfate-containing system remains constant (at *ca.* 30%) throughout the whole process. Hence, it is suggested that the high percentage of intramolecular bonds in the sulfate-containing system even at low temperatures represent a more compact polymer conformation, which is thermodynamically stable, similar to the "crumpled coil" state, proposed by Wu and his collaborators [41, 42], based on light-scattering measurements. Strong salting-out anions such as SO_4^{2-} destabilize the hydrogen bonding between amides and water, by acting on the positive NH part of the amide group [43]. If this explanation holds, then the fact that the salt effects were clearer in the amide II peak than in the amide I peak might be due not only to the partial overlap between the HOH bending and the amide I peak, but also to the anion acting on the NH group (bearing in mind that the amide II reflects mostly N-H deformation while the amide I mode reflects mostly C=O stretch).

Since the effect of the sulfate ions is manifested by compacting the polymer even at temperatures far below the phase transition point, and because the polymer is in a "near onset stage" even at low temperatures, there is no wonder that the phase separation occurs not only at a lower temperature but also in a more gradual, "smoother" manner (Figure 3(B), Figure 4), which does not require the rearrangement stage found in the PNIPA-water system (Figure 3(A)).

Furthermore, the relatively large amide population, engaged in intramolecular H-bonding, should correlate also (since the isopropyl groups are adjacent to the amide groups) with an increased amount of hydrophobic interactions, even at temperatures that are far below the phase separation point. This essentially means that, unlike the PNIPA-water system, hydrophobic interactions may play an important role in the sulfate containing system, already at a very low temperature. Indeed, the collapse and aggregation of the polymer is not echoed in the IR peaks of the isopropyls in the sulfate-containing system, while in the PNIPA-water system a split of the isopropyl assigned peaks is observed during the phase transition, reflecting the co-existence of two populations.

As mentioned before, a shift towards lower wavenumbers was observed in the CH_2 , CH_3 stretch peaks for both the PNIPA-water system and the sulfate-containing system (Figure 4), at the phase separation point. In addition, at temperatures far below the phase transition the methylene peaks in the ternary system (and to a lesser extent also the methyl peaks) were found to be located a few wavenumbers to the blue with respect to the same peaks in the binary PNIPA-water system. It is

claimed that a blue-shift in these peaks may reflect a decrease in degree of order in the polymer (which in general goes together with a decrease in the rigidity) [20, 44] or, as was recently suggested [35, 45], reflects the existence of a strong interaction between hydrophobic moieties and water (the so called "hydrophobic hydration" effect). Since the phase transition is characterized by both a higher compactness (i.e. higher rigidity and order), and at the same time, a lesser amount of interaction between the hydrophobic moieties and water (due to the formation of hydrophobic interactions), both explanations seem to hold for the red-shift observed in both systems upon phase separation. The fact that at low temperatures the CH₃, CH₂ peaks in the ternary system are blue-shifted with respect to the same peaks in the binary system cannot be explained by a lesser amount of hydrophobic interactions in the ternary system, since the ternary system is evidently more compact than the binary system at these temperatures. Hence, one may infer that this difference between the ternary and the binary systems was due to stronger interactions between hydrophobic groups in the polymer and water in the ternary system. In other words, the sulfate, a strong salting-out ion, not only reduces the amount of amide-water H-bonding as shown above, but also stabilizes the hydration around the methyl and methylenic groups, thus making these groups more available for the formation of hydrophobic interactions with other chains.

CONCLUSIONS

This work demonstrates the potential of the ATR-FTIR technique in providing unique information, at a molecular level, on the effect of co-solutes on critical phenomena in aqueous solutions of polymers, in this case the effect of two strong salting-out salts (sodium sulfate and potassium sulfate) on the temperature-induced phase separation in PNIPA. Analysis of the amide II signal as a function of solution temperature suggests that at low temperatures these salts act to increase the amount of amide-amide hydrogen bonds at the expense of the amount of water-amide H-bonds. This, together with the sulfate's effect on the hydrophobic interaction within the polymer chains, leads to a more gradual phase separation process, that does not have to go through a rearrangement stage above the phase separation point, as one observes for systems containing only PNIPA and water. This situation depends on the nature of the co-solute, as will be discussed elsewhere.

ACKNOWLEDGMENTS

This work was performed under contract no. 205-97 of the Israel Science Foundation. The authors would like to thank Professor Rolfe Herber (Hebrew University) for lending the ATR circle cell.

REFERENCES

1. Kubota K, Fujishige S, Ando I. *Polym. J.* 1990; **22**: 15.
2. Schild HG. *Prog. Polym. Sci.* 1992; **17**: 63.
3. Yamamoto I, Iwasaki K, Hirotsu S. *J. Phys. Soc. Jpn* 1989; **58**: 210.
4. Tanford C. *In the Hydrophobic Effect*, 2nd edn. Wiley: New York, 1973: Ch. 1-3.
5. Ulbrich K, Kopecek J. *J. Polym. Sci. Polym. Symp.* 1979; **66**: 209.
6. Ben-Naim A. *In Hydrophobic Interactions*. Plenum Press: New York, 1980.
7. Walker JA, Vause CA. *Sci. Am.* 1987; **253**: 98.
8. Hirotsu S. *J. Phys. Soc. Jpn* 1987; **56**: 233.
9. Schild HG, Tirrell DA. *J. Phys. Chem.* 1990; **94**: 4352.
10. Tiktopulo EL, Bychkova VE, Ricka J, Ptitsyn OB. *Macromolecules* 1994; **27**: 2879.
11. Boutris C, Chatzi EG, Kiparissides C. *Polymer* 1997; **38**: 2567.
12. Fujishige S, Kubota K, Ando I. *J. Phys. Chem.* 1989; **93**: 3311.
13. Winnik FM. *Macromolecules* 1989; **22**: 734.
14. Schild HG, Tirrell DA. *Macromolecules* 1992; **25**: 4553.
15. Armentrout RS, Hu Y, McCormick CL. *Polymer Preprint* 1996; **37**: 659.
16. Skrovanek DJ, Howe SE, Painter PC, Coleman MM. *Macromolecules* 1986; **19**: 1676.
17. Skrovanek DJ, Painter PC, Coleman MM. *Macromolecules* 1986; **19**: 699.
18. Durrani CM, Prystupa DA, Donald AM. *Macromolecules* 1993; **26**: 981.
19. Harris PI, Chapman D. *Trends Int. Biol. Sci.* 1992; **17**: 328.
20. Shibayama M, Marimoto M, Nomura S. *Macromolecules* 1994; **27**: 5060.
21. Lin S-Y, Chen K-S, Run-Chu L. *Polymer* 1999; **40**: 2619.
22. Ramon O, Kesselman E, Bercovici R, Cohen Y, Paz Y. *J. Polym. Sci. Part B: Polym. Phys.* 2001; **39**: 1665.
23. Hofmeister F. *Zur lehre von der wirkung der salze*. *Arch. Exp. Pathol. Pharmacol.* 1888; **24**: 247.
24. von Hippel PH, Schleich T. *In Structure and Stability of Biological Macromolecules*. Timasheff SN, Fasman GD. (eds). Marcel Dekker: New York, 1969: Ch. 6.
25. von Hippel PH, Wong KY. *Science* 1964; **145**: 577.
26. Dhara D, Chatterji PR. *J.M.S.-Rev. Macromol. Chem. Phys.* 2000; **C40**: 51.
27. Annaka M, Motokawa K, Sasaki S, Nakahira T, Kawasaki H, Maeda H, Amo Y, Tominaga Y. *J. Chem. Phys.* 2000; **113**: 5980.
28. Kawasaki H, Mitou T, Sasaki S, Maeda H. *Langmuir* 2000; **16**: 1444.
29. Annaka M, Motokawa K, Sasaki S, Nakahira T, Kawasaki H, Maeda H, Amo Y, Tominaga Y. *Journal of Chemical Physics* 2000; **113**: 5980.
30. Long FA, McDevit WF. *Chem. Rev.* 1952; **51**: 119.
31. Inomata H, Goto S, Otake K, Saito S. *Langmuir* 1992; **8**: 687.
32. von Hippel PH, Peticolas V, Schack L, Karlson L. *Biochemistry* 1973; **12**: 1256.
33. Hamabata H, Von Hippel PH. *Biochemistry* 1973; **12**: 1264.
34. Maeda Y, Higuchi T, Ikeda I. *Langmuir* 2000; **16**: 7503.
35. Maeda Y, Nakamura T, Ikeda I. *Macromolecules* 2001; **34**: 1391.
36. Otake K, Inomata H, Konno M, Saito S. *Macromolecules* 1990; **23**: 283.
37. Libnau FO, Toft J, Ehristry AA, Kvalheim OM. *J. Am. Chem. Soc.* 1994; **116**: 8311.
38. Rahmelow K, Hubner W. *Applied Spectroscopy* 1997; **51**: 160.

39. Elliot A. In *Infra-Red Spectra and Structure of Organic Long-Chain Polymers*. St. Martin's Press: New York, 1969.
40. Vinogradov SN, Linnel RH. *Hydrogen Bonding*. Van Nostrand: New York, 1971.
41. Wu C, Zhou S. *Macromolecules* 1995; **28**: 5388.
42. Wu C, Zhou S. *Macromolecules* 1995; **28**: 8381.
43. von Hippel PH, Schleich T. *Acc. Chem. Res.* 1969; **2**: 257.
44. Precot A, Zhu XX, Lafleur M. *J. Polymer Science* 2000; **38**: 907.
45. Mizuno K, Ochi T, Shindo Y. *J. Chem. Phys.* 1998; **109**: 9502.