ARTICLES

Salt Effects on Poly(*N*-isopropylacrylamide) Phase Transition Thermodynamics from NMR Spectroscopy

Christopher M. Burba,† Shawn M. Carter, Kevin J. Meyer, and Charles V. Rice*

Department of Chemistry and Biochemistry, University of Oklahoma, 620 Parrington Oval, Room 208, Norman, Oklahoma 73019

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NMR spectra were collected for cross-linked poly(*N*-isopropylacrylamide), poly(NIPAM), hydrogels in the presence of NaCl and $CaCl_2$ aqueous solutions. Intensity variations in the ^1H NMR signals of the polymer provide insight into the phase transition process. These data were used to observe a two-stage phase transition process. Thermodynamic quantities were obtained from a van't Hoff analysis of the temperature-dependent equilibrium constants, which were derived from the NMR data. The $\Delta H°$ and $\Delta S°$ values for the hydrogel in D_2O are 3.4 kJ/mol and 11.2 J/mol·K for stage I, which is attributed to the formation of hydrophobic bonds between neighboring isopropyl groups. The formation of hydrogen bonds during stage II yielded $\Delta H°$ and $\Delta S°$ values of 14.8 kJ/mol and 48.4 J/mol·K in D_2O. However, the corresponding $\Delta H°$ values in 150 mM NaCl and 150 mM $CaCl_2$ are reduced to 1.5 and 1.8 kJ/mol for stage I of the dehydration process. This corresponds to the known effect of salts on hydrophobic bond energetics. The value of $\Delta S°$ also decreased to 4.9 and 5.9 J/mol·K in NaCl and $CaCl_2$ solutions, respectively. However, the thermodynamic values during stage II were only slightly affected by the salts. The lower temperatures required to induce spontaneous precipitation implies that $\Delta G°$ of precipitation is reduced. With our measurement of equilibrium thermodynamics, we see that 150 mM NaCl and $CaCl_2$ solutions have a greater effect on hydrophobic bond formation associated with the phase transition process. In this manner, these salts aid in solvent reorganization necessary to form the hydrophobic bond, and this suggests that the formation of hydrophobic bonds is a strong determining factor in the stability of poly(NIPAM) hydrogels in water.

1. Introduction

A tremendous amount of research is devoted to preparing novel thermosensitive polymers for physiological applications such as drug delivery or tissue engineering.[1–9] Among the various families of thermosensitive polymers, alkyl acrylamide-based polymers, especially poly(*N*-isopropylacrylamide) or poly(NIPAM), are among the most heavily investigated for these applications. The good biocompatibility of poly(NIPAM) systems coupled with a lower critical solution temperature (LCST) near 32 °C has contributed much to the success of this family of polymers.[10] At room temperature, poly(NIPAM) is used to form a translucent hydrogel that is completely swellable in water. The hydration shell surrounding the polymer chains is stabilized through extensive hydrogen-bonding interactions between water molecules and the amide groups of the *N*-isopropylacrylamide monomeric units. However, the hydration shell is destabilized when the system is heated to its LCST, leading to rapid dehydration of the polymer and phase separation.

Numerous studies have shown that salts can influence the phase transition temperature of poly(NIPAM). Freitag and Garret-Flaudy suggested that the effect of a given salt on the LCST is a linear function of salt concentration.[11] From a molecular viewpoint, salts were characterized as ordering or disordering the structure of water around the NIPAM polymer. Furthermore, it was shown that anions influence the LCST to a greater extent than cations and can be ranked on a Hoffmeister series.[12] The role of salts in NIPAM dehydration is grouped into three categories: (1) hydrogen bonding to the amide, (2) increasing the surface tension of water in the hydration shell around the hydrophobic groups, and (3) ion binding to the polymer. Undoubtedly the mixture of salts present in the human body will affect the phase transition behavior of poly(NIPAM) in a very complex way, and it is possible that the complex mixtures of salts in a biological fluid are not merely additive. Therefore, the role of different salts in changing the LCST must be characterized as well as various combinations of the salts before a comprehensive investigation of the phase transition behavior of poly(NIPAM) can be elucidated in a biological fluid. Unfortunately, many of the experimental techniques traditionally used to characterize poly(NIPAM) only probe a small region of the sample and may lead to an incomplete picture of the phase transition process.[10,11,13–16] For example, attenuated total reflectance infrared spectroscopy has been successfully used to investigate changes in the local structure of the amide groups of poly(NIPAM) in the presence of Na_2SO_4 salt solutions during the phase transition.[17] Spectral changes were used to identify changes in the hydrogen-bonding network. FTIR has been used to characterize the hydrophilic and hydrophobic portions of the

* Corresponding author. Tel.: 405-325-0449. Fax: 405-325-6111. E-mail: rice@ou.edu.

† Present address: Department of Natural Sciences, Northeastern State University, 600 N. Grand Ave., Tahlequah, OK 74464.

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polymer.[18–22] Upon heating, the dehydration of these functional groups was followed. With low-frequency Raman scattering, Annaka and co-workers found a correlation between the chemical potential of the solvent (pure water or salt water) and the swelling/deswelling behavior of NIPAM gels.[23,24] The ability of salts to reduce the chemical potential of water encourages dehydration of the polymer, leading to hydrogel collapse. Both temperature and salt effects were ascribed to the hydrophobic bonds. However, both infrared and Raman spectroscopy only probe the surface regions of the sample in these experiments, leaving the inner portions of the polymer unexplored. In contrast, nuclear magnetic resonance (NMR) spectroscopy probes the entire sample during the phase transition, so a more complete picture of the phase transition can be obtained.[25] A part of this picture is quantification of the thermodynamic enthalpy and entropy that govern the dehydration process.

In the fully hydrated state (T < LCST), molecular segments of poly(NIPAM) are completely surrounded by water molecules in a stable hydration shell. In the hydrated state, these segments are relatively flexible and any chemical shift anisotropy or dipole−dipole coupling effects that exist in the sample may be removed with high-resolution magic-angle-spinning (HRMAS) NMR spectroscopy.[25–28] However, polymer dehydration over the LCST (typically ranging only 1 or 2 °C) leaves the polymer in a collapsed state. Here, the chemical shift anisotropy and dipole−dipole coupling effects become very strong and solution-state NMR signals are no longer measured for poly(NIPAM). Instead, traditional solid-state NMR techniques must be used to collect well-resolved ^{1}H NMR spectra.[29–31] During the phase transition, poly(NIPAM) exists in an equilibrium between hydrated and collapsed states that is governed by the Gibb's free energy for precipitation. Measuring the proportion of hydrated and dehydrated polymer segments with NMR spectroscopy provides quantitative information concerning the equilibrium constant for this process, and thermodynamic parameters may be evaluated from temperature-dependent measurements of the equilibrium constant.[25] In this work, the relative amount of flexible, hydrated NIPAM segments within the sample are directly measured by following intensity changes of methyl protons from the *N*-isopropylacrylamide substituents with HRMAS ^{1}H NMR spectroscopy, allowing a quantitative assessment of how simple salts such as NaCl or $CaCl_2$ influence the phase transition thermodynamics and kinetics during heating and cooling cycles from the perspective of the NIPAM constituents. This study will form the basis of future NMR spectroscopic analyses of poly(NIPAM) in other salt solutions and simulated biological fluids.

2. Experimental Methods

2.1. Materials. *N*-Isopropylacrylamide (NIPAM), *N*,*N*′-methylene bisacrylamide (MBAAc), *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED), NaCl, and $CaCl_2$ were purchased from Aldrich and used as received. Riboflavin 5′-phosphate was purchased from Spectrum, and deuterium oxide (D_2O) was obtained from Cambridge Isotopes Laboratories. Appropriate quantities of NaCl and $CaCl_2$ were dissolved in D_2O to make 150 mM solutions.

2.2. Polymerization. Poly(NIPAM) hydrogels were prepared by photopolymerization of NIPAM monomer and MBAAc aqueous solutions. In a typical synthesis, 2 mL of NIPAM solution (0.7899 M) is mixed with 127.5 μL of 0.0413 M bisacrylamide. This is followed by the addition of 100 μL of 0.0010 M riboflavin 5′-phosphate and 10 μL of neat TEMED as initiators. The resulting solutions are intimately mixed and neutralized to a pH of 7. Photopolymerization was performed for 30 min using a Spectroline UV Crosslinker Select (Fisher Scientific). The cross-linking density of the resulting hydrogel is approximately 311:1 (NIPAM to MBAAc). The hydrogels were thoroughly washed by heating the gel above the LCST and then rehydrating the gel with doubly distilled H_2O. This process was repeated at least three times to remove excess reagents.

2.3. NMR Spectroscopy. Small quantities (~60 μL) of dehydrated poly(NIPAM) were placed in a ceramic, HRMAS[25] rotor, and the gels were allowed to rehydrate in the presence of neat D_2O, 150 mM NaCl, or 150 mM $CaCl_2$. NMR spectra were collected using a Varian gHX HRMAS NanoProbe and a Mercury VX 300 MHz NMR spectrometer. VnmrJ 1.1D software (Varian, Inc.) was used for data collection and processing. The ^{1}H chemical shift values were referenced to the residual water signal (HOD) at 4.8 ppm. Temperature calibration of the NMR spectrometer was accomplished using the temperature-dependent chemical shift of ethylene glycol. Sample spinning was achieved with a Torlon drive ring, which was used in conjunction with dry air. All samples were spun at a rate of 2000 Hz.

Deuterium lock was maintained throughout data acquisition to control the field frequency ratio over the sample.[28,32,33] Temperature-induced changes in solvent density affect the magnetic susceptibility of the sample, which can change the intensity of the local magnetic field and influence measured chemical shift values. Furthermore, changes in probe temperature also create magnetic inhomogeneities that may result in peak broadening and/or splitting. These effects are removed by sample shimming on the deuterium signal at each temperature interval.

3. Results and Discussion

3.1. Changes in the NMR Spectra upon Heating. Poly(*N*-isopropylacrylamide) hydrogels were analyzed with HRMAS NMR spectroscopy. The ^{1}H spectra collected at different temperatures were used to monitor hydrogel collapse during heating (Figure 1, left). In the fully hydrated state (T < LCST), the molecular units composing the poly(NIPAM) hydrogel are flexible, and any residual chemical shift anisotropy and dipole−dipole coupling effects are removed with the HRMAS technique. At elevated temperatures, when the gel is dehydrated, these effects become very strong and the ^{1}H spins experience very fast T_2 relaxation. Our HRMAS NMR probe cannot deliver the necessary rf power to overcome relaxation effects. Thus, the intensities of the poly(NIPAM) signals in the ^{1}H NMR spectrum decrease during the phase transition. We have used variable temperature ^{1}H HRMAS NMR spectra to determine the phase transition temperature while providing molecular-level information concerning the coil-to-globule transition that occurs in these polymers. Below the LCST, poly(NIPAM) yields four peaks in the ^{1}H spectra (Figure 1); assignments of the peaks are included in the figure. Small changes in peak intensity are observed below the LCST; however, near 32 °C the intensities rapidly decrease and vanish, leaving negligible intensities at elevated temperatures. Over the same temperature range, the sample appearance turns from a translucent gel to an opaque solid, an observation often used to characterize the phase transition. Infrared spectroscopic measurements indicate that the extensive hydrogen-bonding network, which exists between the amide groups of the polymer and water molecules below LCST, is replaced with amide−amide intra- and intermolecular hydrogen bonds in the collapsed state.[17]

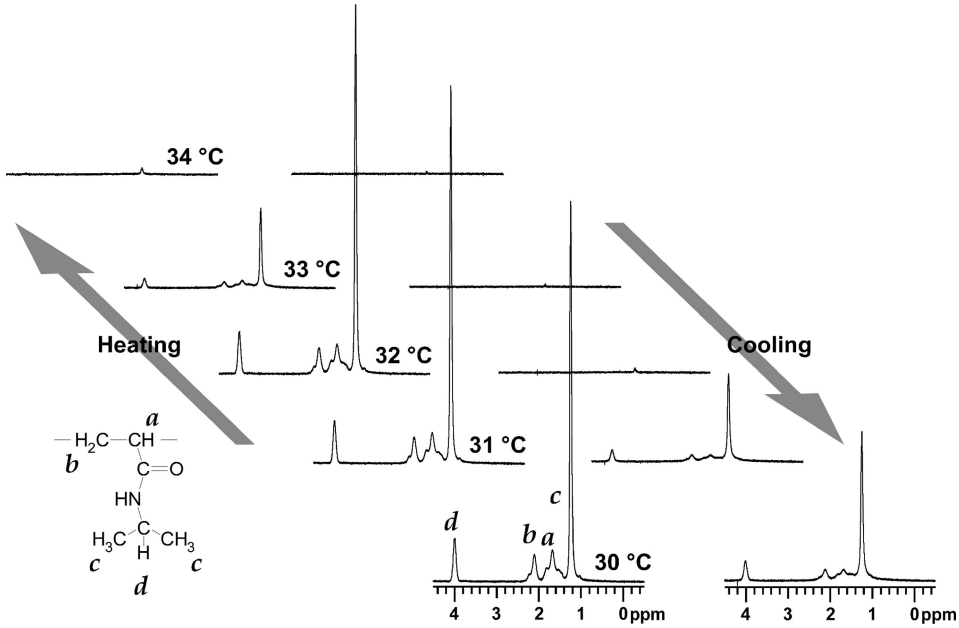

Figure 1. ^{1}H NMR spectrum of poly(*N*-isopropylacrylamide) gives four distinct signals. As a cross-linked gel, HRMAS is needed to increase spectral resolution for accurate measurement of the methyl group peak intensity. To follow the temperature-induced phase transition, the sample was heated (or cooled) in 0.2 °C increments and an NMR spectrum collected after a 5 min dwell time. The above spectra show that, during the heating of a 300:1 poly(NIPAM) gel in D_2O, signal loss occurs after 32 °C. The observed signal represents the flexible portion and provides quantitative information regarding the phase transition. However, the spectra collected during sample cooling show that only 40% of the signal is recovered at 30 °C.

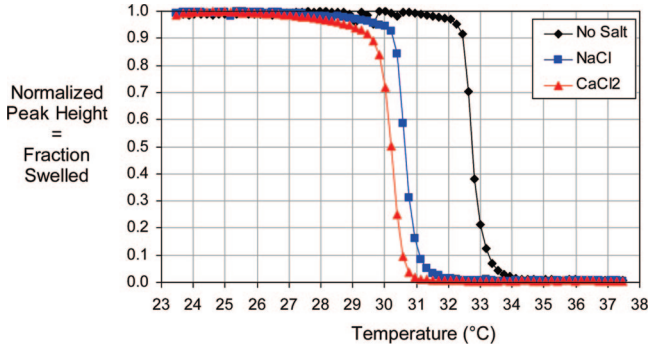

Figure 2. Effect of salts on the phase transition can be seen by plotting the methyl group intensity (peak c, Figure 1) as a function of temperature. The data points are separated by 0.2 °C, and the sample was held at each temperature for 5 min prior to data collection. In NaCl and $CaCl_2$, signal loss occurs at a lower temperature. These data represent the equilibrium fraction of hydrated NIPAM segments.

Figure 2 shows the temperature dependence of the ^{1}H NMR signal of the methyl protons on the isopropyl groups as poly(NIPAM) hydrogels are heated in the presence of neat D_2O, 150 mM NaCl, and 150 mM $CaCl_2$ solutions. Although other protons of the polymer side chain or backbone could be used for analysis, the methyl protons of the isopropyl groups yield the strongest ^{1}H NMR signal of the polymer. Thus, variations in molecular structure associated with the coil-to-globule phase transition may be easily monitored with this peak. NMR measurements indicate the midpoint of the coil-to-globule transition temperature occurs at 32.7 °C for poly(NIPAM) in neat D_2O (Figure 2). Similar transition temperatures have been reported using UV−vis spectrophotometry and light scattering techniques.[10–12,34] The LCST of poly(NIPAM) is lowered to 30.6 and 30.2 °C, respectively, in the presence of 150 mM NaCl and 150 mM $CaCl_2$ solutions. The "salting out" phenomenon (i.e., a decrease in the cloud point temperature) has been observed with a wide variety salts for this polymer.[10–12,34] Anions typically influence the phase transition temperature to a greater extent than cations, and the degree of the temperature shift is a linear function of the salt concentration.[12] Salts may be ranked according to their ability to shrink or swell charged and uncharged macromolecules; the resulting sequence is termed the *Hoffmeister series*. A typical series of anions in order of increased destabilization is as follows: $SO_4^{2-} > Cl^- > Br^- > I^- > SCN^-$. According to this ranking, sulfate anions are expected to have a stronger influence on the LCST of poly(NIPAM) than iodide ions. Chloride anions are relatively high in the series and exert a strong influence on the LCST of poly(NIPAM).

3.2. Thermodynamics of Hydrogel Dehydration. HRMAS NMR spectroscopy can provide additional insight into the phase transition thermodynamics of poly(NIPAM) hydrogels. During the phase transition, poly(NIPAM) exists in an equilibrium between hydrated and collapsed states that is governed by the Gibb's free energy for precipitation. As noted in Figure 1, the polymer chains yield measurable ^{1}H NMR signals when hydrated, but the intensity of the NMR peaks rapidly decreases over the phase transition. At sufficiently high temperatures, no NMR signals are observed from the hydrogel. Because the peak signals are due solely to flexible, hydrated portions of the polymer, the normalized peak intensities may be used to directly measure the equilibrium constant, K_{eq}, for the phase transition. In terms of the NMR signal, denoted as S, the equilibrium constant may be defined as K_{eq} = [collapsed]/[hydrated] = $(S_0 - S)/S = (1 - S)/S$ when the spectra are normalized with $S_0 = 1$ at 25 °C. Moreover, temperature-dependent measurements of the equilibrium constant may be used to calculate the enthalpy and entropy of precipitation with the van't Hoff equation.[35] As shown in Figure 3, the van't Hoff plot reveals the phase transition occurs in two stages. Thermodynamic parameters for each stage are listed in Table 1. The enthalpies of precipitation for stages I and II are endothermic. The value of $\Delta H°$ for stage I is attributed to hydrophobic bond formation energies,[35,36] whereas $\Delta H°$ for stage II is comparable to hydrogen-bond

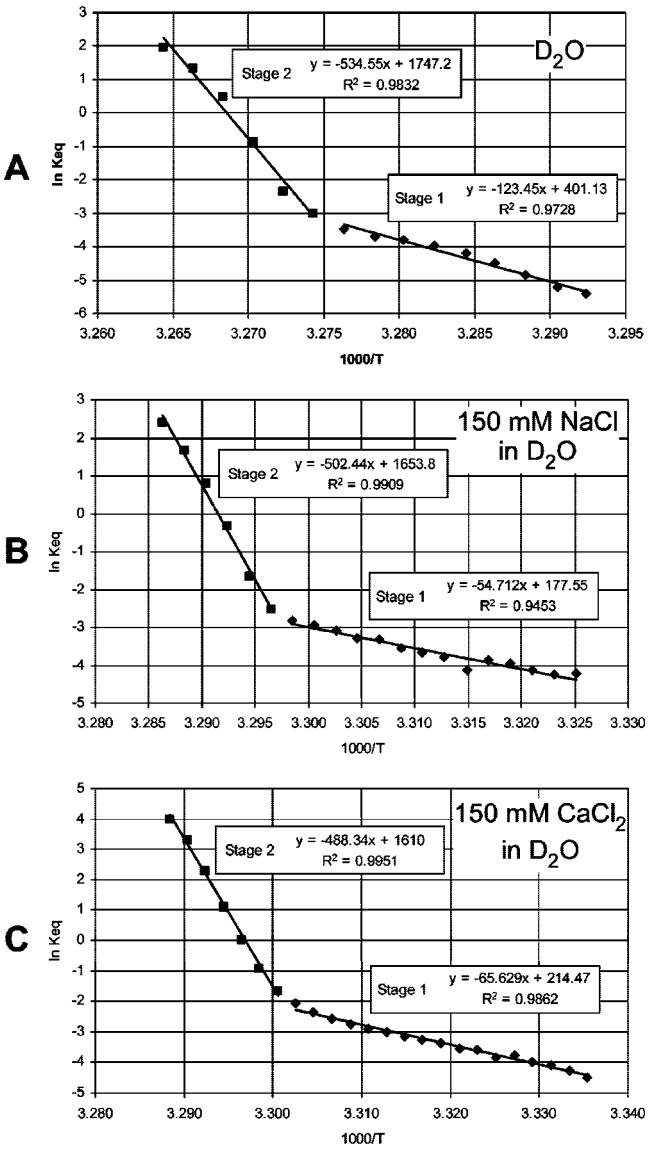

Figure 3. With the use of the NMR data from Figure 2, the temperature-dependent equilibrium constants were used to create van't Hoff plots. Each plot clearly shows a two-stage process, which have very different enthalpic and entropic components. Stage I is shown by filled squares, while filled circles represent stage II. The $\Delta H°$ and $\Delta S°$ from the slope and intercept were divided by 300 to obtain these parameters in the units of kJ/(mol monomer).

TABLE 1: NIPAM Hydrogel Thermodynamic Values

$\Delta H°$ kJ mol^{-1}	no salt	150 mM NaCl	150 mM $CaCl_2$
stage I	3.4 ± 0.2	1.5 ± 0.2	1.8 ± 0.1
stage II	14.8 ± 1.0	13.9 ± 0.7	13.5 ± 0.4
$\Delta S°$ J mol^{-1} K^{-1}	no salt	150 mM NaCl	150 mM $CaCl_2$
stage I	11.2 ± 0.7	4.9 ± 0.4	5.9 ± 0.2
stage II	48.4 ± 4.2	45.8 ± 2.2	44.6 ± 1.4
$\Delta G°$ (298 K) kJ mol^{-1}	no salt	150 mM NaCl	150 mM $CaCl_2$
stage I	0.108	0.049	0.047
stage II	0.331	0.259	0.231

dissociation energies for water molecules interacting with the polymer.[35] Changes in entropy are much larger for stage II than stage I. The small structural changes associated with forming hydrophobic bonds during stage I only slightly increase the entropy of the polymer. However, the release of bound water molecules from the polymer during stage II and the resulting increase in configurational entropy of the collapsed polymer contribute to the larger value of $\Delta S°$ for stage II.

In the early stages of the coil-to-globule phase transition, slight rearrangements of the polymer backbone increase interactions between neighboring hydrophobic segments of the polymer. Further heating disrupts the hydration shell surrounding the hydrophobic portions,[37] leading to stable hydrophobic bonds and ultimately producing rapid dehydration and aggregation of the polymer segments. Nearly 40 years ago, Nemethy and Scheraga calculated the hydrophobic bond thermodynamics for interactions between the different amino acids.[38–40] This work provided crucial insight in the driving force for protein secondary structure. The NIPAM monomeric units of poly(NIPAM) is chemically similar to valine, and $\Delta H°$ and $\Delta S°$ for valine−valine interactions were predicted to be 4.5 kJ/mol and 28.02 J/mol·K.[40] Although enthalpy values are similar to those measured in stage I, we measure an entropy value of about one-half of this value. This discrepancy may be because the entropy calculated by Nemethy and Scheraga accounted for the hydration shell around two valine groups, whereas our measurement is reported for a single NIPAM monomer.

At the concentration studied, essentially no changes in the thermodynamic parameters are found for stage II processes (hydrogen bonding) when poly(NIPAM) is immersed in either salt solution. Although some changes are noted for stage II, the differences are small. The slight increases in $\Delta H°$ and $\Delta S°$ are probably related to subtle rearrangements of the water molecules within hydrogen bonds between polymer segments. For instance, Paz et al. used infrared spectroscopy to identify an increase in the relative number of amide−amide hydrogen bonds for poly(NIPAM) saturated with 180 mM Na_2SO_4.[17] In contrast, stage I thermodynamic parameters differ from neat D_2O when the polymer is placed in either NaCl or $CaCl_2$ solutions. The enthalpy is reduced by a factor of 2.27 for NaCl and 1.89 for $CaCl_2$, whereas $\Delta S°$ is reduced by factors of 2.29 and 1.90 when the hydrogels are immersed in NaCl and $CaCl_2$ solutions, respectively. Salts affect the structure of the water molecules, which alters hydration of hydrophobic molecules and lowers the energy barrier for the formation of hydrophobic bonds.[12,23,36] It was noted by Zhang et al. that strongly hydrating anions influence the phase transition through hydration entropy.[12] However, Cl^- is a weakly hydrating anion and exerts its influence through surface tension effects on the hydration shell around the hydrophobic groups. This work also reproduced the earlier observation of the solvent isotope effect on LCST, which is slightly higher (0.7 °C) in D_2O versus H_2O.[41] Given the role of salts in hydrophobic bonds, we expect salts to have a greater effect on stage I of the phase transition. Although the experiments by Zhang et al. used salt concentrations much higher than our work, the role of Cl^- in the hydrophobic bond formation remains an important explanation of our results. Additional support for the salt effect on hydrophobic bonds is given by the work of Annaka et al.[23] Their Raman scattering work clearly indicated the important of hydrophobic bonds, salts, and the chemical potential of the solvent. Because the chemical potential of the salt−water solvent is lower than that of the hydration shell around the hydrophobic groups, this should also have a strong influence on the rehydration of the NIPAM hydrogel. The hydrophobic groups will become solvated until the chemical potential of the solvent increases, which occurs with lower temperature due to entropy effects. As shown below, our NMR

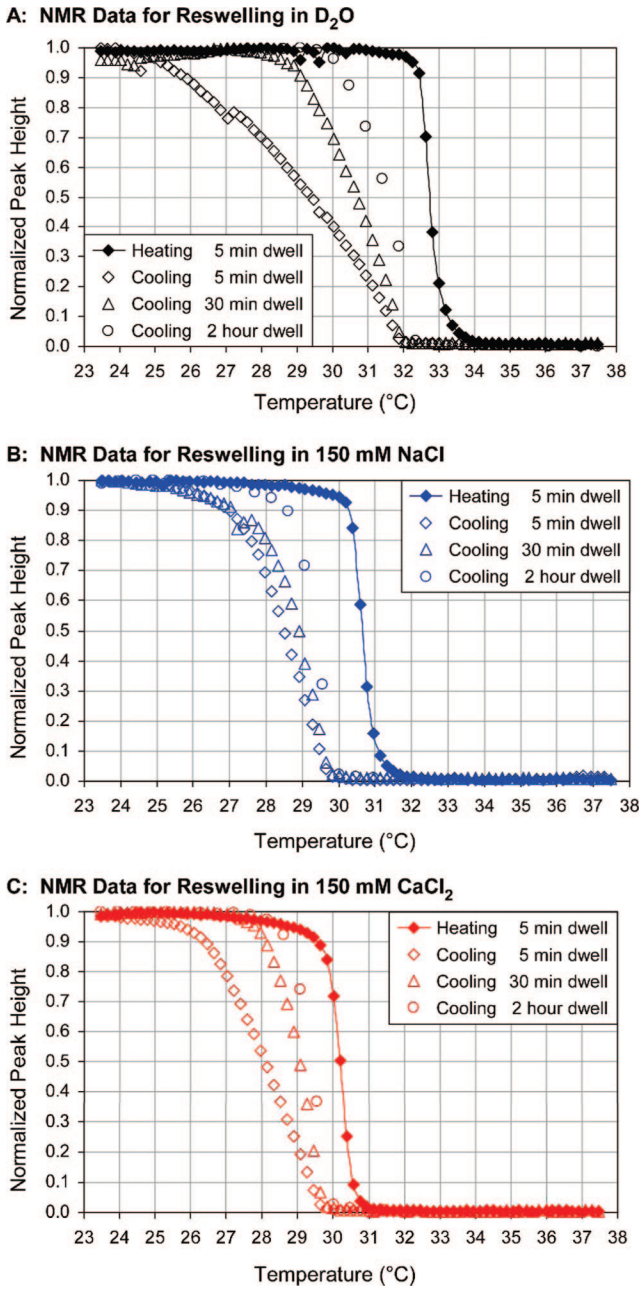

Figure 4. Temperature-dependent NMR spectra in Figure 1 show significant differences between data collected on the heating and cooling cycles. By plotting peak height vs temperature, hysteresis in the data is apparent. The hysteresis is reduced if the dwell time between data points is increased from 5 min to 2 h. However, in each of the different solutions, the signals begin their recovery at the sample temperature point regardless of the dwell time. As described in the text, the hydrophobic bonds must be broken prior to polymer rehydration, which we attribute as the cause of hysteresis.

data clearly show that rehydration does not occur until the temperature drops below the onset of stage I, the formation of hydrophobic bonds.

3.3. Changes in the NMR Spectrum upon Cooling. The reversible nature of the phase transition allows us to follow rehydration of the hydrogel during the cooling cycle (Figure 1, right). While heating, nearly all of the NIPAM ^{1}H signals have disappeared at 34 °C. Recovery of the NMR signal when the hydrogel is cooled below the LCST shows significant hysteresis (Figure 4). Long dwell times (>2 h) are necessary for the poly(NIPAM)−water−salt tertiary system to achieve equilibrium upon cooling. Surprisingly, the two tertiary systems appear to rehydrate faster than the binary poly(NIPAM)−water system.

Furthermore, the cooling curves for all three systems do not overlap the corresponding heating curves. The temperature that the NMR signal is recovered is nearly identical to the onset temperature at the beginning of dehydration stage for all three systems, suggesting that the temperature of the systems must be lower than the onset of stage I (hydrophobic bond formation). Polymer swelling requires the polymer segments to separate and restore their respective hydration spheres. Hydrophobic bonds are important during the phase transition, and unlike dipole−dipole, electrostatic or van der Waals forces, become stronger at higher temperatures.[36,37] Thus, cooling weakens the hydrophobic interaction and when broken allows the polymer to swell and rehydrate. This is coupled to the relative chemical potentials of the solvent and the hydration shell around hydrophobic groups.[23] Hysteresis between heating and cooling is likely due to slow diffusion kinetics of water molecules rehydrating the polymer, and the time scale of our experiments (tens of minutes) is insufficient to establish equilibrium. Long delay times (many hours) are necessary to achieve equilibrium on cooling because water molecules slowly diffuse through the hydrophobic polymer. The hysteresis is mitigated in the salt solutions. Stronger signals show greater reswelling rates in the presence of salts because the structure of the hydration sphere around the poly(NIPAM)−water−salt system is different than the poly(NIPAM)−water system below the LCST. In the presence of a salt solution, infrared spectroscopic measurements indicate a substantial amount of amide−amide hydrogen bonding, whereas there is essentially no amide−amide hydrogen bonding in pure water. The poly(NIPAM)−water system is characterized by a large fraction of amide−water hydrogen bonds. As mentioned above, however, rehydration requires breaking hydrophobic bonds at the gel exterior. Thus, we believe that the rate-limiting step for rehydration is controlled by the diffusion of water molecules into the core of the collapsed polymer. Indeed, visual observation of the rehydration event shows that the outer edges of the globule are hydrated first and a solvent concentration gradient must exist within the polymer.

With the ability to provide a molecular-level insight into the phase transition, one could imagine an NMR-based study of rehydration kinetics. Unfortunately, we found it difficult to reproduce the cooling hysteresis curves with different samples. Often, hydrogel pieces would break and/or crack upon insertion into the NMR rotor. Rehydration is known to depend on sample size, shape, and thickness.[42,43] Effects of hydrogel morphology are minimized by using very small samples,[41,44] which are impractical for timely NMR experiments. Therefore, we do not envision NMR as a suitable tool to measure the kinetics of polymer swelling. Likewise, we do not make significant claims as to the role of salts in rehydration kinetic rate constants. However, we can use NMR to demonstrate that salts reduce hysteresis. In all cases, the restoration of the NMR signal does not occur until the hydrophobic bonds are broken.

4. Conclusions

With NMR spectroscopy, we have been able to measure important thermodynamic parameters for the phase transition of poly(NIPAM) in salt solutions (Figure 5). Dehydration of hydrogels occurs at elevated temperatures in a two-stage process. The first step is the formation of hydrophobic bonds, wherein enthalpy and entropy is reduced in 150 mM NaCl and 150 mM $CaCl_2$. Hydrogen bonds are broken in the second step, and salts have a minimal effect on $\Delta H°$ and $\Delta S°$ for this process. During

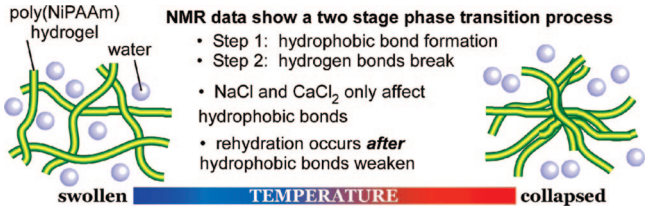

Figure 5. Illustration of the phase transition between the swollen and collapsed states. The two-stage process arises from analysis of the NMR data, which was used to measure the thermodynamics of dehydration. During rehydration, the data clearly show that the hydrophobic bonds must be broken before the water molecules reform the hydration sphere and reswell the polymer.

sample cooling, rehydration of the polymer does not begin until the hydrophobic bonds are broken and their respective hydration shells established. These insights provide important pieces of missing knowledge that compliment decades of prior research. Importantly, we demonstrate that NMR spectroscopy is a powerful analytical tool whose full potential has yet to be realized in the study of hydrogels. For example, systematic studies with kosmotropic and chaotropic salts, concentration dependencies, and pH variables which can be applied to the numerous polymer and copolymer hydrogel systems at the forefront of biomedical engineering and technology should be conducted.

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