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Cation Effects on the Phase Transition of *N*-isopropylacrylamide Hydrogels

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Abstract

Polymers formed from *N*-isopropylacrylamide (NIPAM) are highly water soluble and undergo a temperature-induced phase transition to an insoluble state. The phase behavior is determined by competing hydrophilic and hydrophobic forces. In this report, additional insight regarding the effect soluble metals have on the phase transition process is provided by showing that cation solvation aids with stabilization of hydrophobic forces. This reduces barriers to rehydration and decreases thermodynamic entropy and enthalpy, obtained with variable-temperature ^1H nuclear magnetic resonance spectroscopy of NIPAM hydrogels in D_2O , NaCl , MgCl_2 , and CaCl_2 . For the series of cations studied, it is observed that the order of increasing effect to facilitate the phase transition is $\text{Ca}^{2+} < \text{Mg}^{2+} < \text{Na}^+$. NaCl and MgCl_2 exhibited similar effects on the thermodynamics of the collapsing process. However, significant differences in the phase transition thermodynamics are observed between MgCl_2 and CaCl_2 salt solutions. The influence on Stage 1 enthalpy and entropy values for CaCl_2 solutions is approximately half that of the MgCl_2 solutions. This difference is likely related to their charge density of Ca^{2+} , which is approximately half that of Mg^{2+} .

Graphical Abstract

Keywords

calcium; lower critical solution temperature; magnesium; *N*-isopropylacrylamide; nuclear magnetic resonance spectroscopy; sodium

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Note: Molar units were corrected on May 4, 2015.

1. Introduction

Several biomedical^[1–7] and separations applications^[8–11] have been based upon poly(*N*-isopropylacrylamide) (PNIPAM) and other hydrogel systems. At room temperatures, water completely saturates the polymer matrix forming an extensive hydrogen bonding network between water molecules and amide groups of the polymer side chain. When temperatures are increased above the lower critical solution temperature (LCST), the water hydration shell breaks down and the gel collapses into a hardened opaque substance. PNIPAM hydrogel's good biocompatibility^[4] combined with a LCST slightly lower than body temperatures make it ideal for biomedical applications.^[12] Their reversible phase transition, low cost, and reusability make them attractive for use in various separations processes. These applications often occur in the presence of alkali and alkali earth metal cations, and a more complete understanding of hydrogel chemistry is provided by examining the role of metals in the phase transition LCST.^[13–21]

Tanaka et al. concluded that the degree of ionization of the polymer is an important aspect of the phase transition of ionic gels.^[22] An additional stimulus to affect hydrogel phase transitions is the ionic strength of the solution in which it is immersed. Ohmine et al. investigated the effects of varying salt concentrations of NaCl, CaCl₂, BaCl₂, and MnCl₂ on the phase transition of partially hydrolyzed polyacrylamide gels.^[23] With increasing concentrations of NaCl in water, the gels undergo a continuous transition to a dehydrated state. For NaCl, the phase transition began at 10^{−3} mol L^{−1} concentrations, whereas the phase transition was initiated by much lower concentrations, 10^{−5}–10^{−6} mol L^{−1}, of the divalent salts investigated. The difference between the effectiveness of NaCl and MgCl₂, at inducing the phase transition, was attributed to the fact that only half as many divalent ions are needed to neutralize the ionic polymer network as monovalent ions. Inomata et al. studied the phase transition from two perspectives.^[24] First, temperature was kept constant and the physical state of the PNIPAM hydrogel was monitored as it was immersed in increasingly concentrated salt solutions. The gel collapsed at room temperature in a 0.68 M NaCl solution and 1.1 M NaBr. Second, they used variable temperature experiments to study phase transition of the gel in various salt solutions; they found that increasing concentration decreased the LCST. Zhang et al. suggested that the degree to which anions or cations will affect the phase transition can be ordered according to the Hofmeister series.^[16]

Given the effect of various salts on the phase transition, the behavior of PNIPAM hydrogels for biomedical applications will be significantly altered in biological fluids. Insight regarding how ions affect polymer dynamics is critical to understanding, and predicting, hydrogel properties. This understanding can be obtained through a variety of analytical techniques. Most work has focused on understanding the unique inverse solubility phase transition that PNIPAM exhibits. The first report of studies to understand the LCST of PNIPAM hydrogels used microscopy to follow the phase transition. In a typical experiment, cylinders of hydrogel would be synthesized in glass tubes; the diameter of the tubes would define the initial diameter of the hydrogel. Then the gels would be immersed in a solution (typically water) and allowed to hydrate until they reached equilibrium in the solution. The diameter would be measured again to define a swelling ratio. An experiment, such as a variable temperature investigation or varying solvent composition study can be

accomplished by measuring the equilibrium diameter at each step and then producing a plot of the swelling ratio. Instead of measuring the equilibrium diameter values, an alternative method to determine the swelling ratio uses the weight of the hydrogel.^[25] This swelling ratio can be defined in terms of the weight of the swollen gel and the weight of the dried polymer.

One analytical technique commonly used to study the phase transition of NIPAM hydrogel is Differential Scanning Calorimetry (DSC). PNIPAM will require heat flow to reach a temperature above the LCST. Otake et al. performed most of the initial work studying NIPAM hydrogel with DSC.^[26] The DSC thermograms of PNIPAM gel exhibited an endotherm during the collapsing of the gel and an exotherm during the swelling of the gel. This verified that the phase transition from hydrated to dehydrated is an endothermic process and the opposite transition is an exothermic process.

A different analytical technique used to study PNIPAM hydrogels is dynamic light scattering (DLS). DLS, also known as photon correlation spectroscopy or quasielastic light scattering, provides another method to observe the phase transition in PNIPAM hydrogels. For PNIPAM, variable temperature studies elicit the phase transition causing changes in the scattering intensity. Tanaka et al. used this technique to study the kinetics of the continuous phase transition.^[27] They sealed solutions of PNIPAM hydrogel beads in a microslide and then subjected the solution to sharp temperature changes and observed light scattering response to changing size of the particles. They determined that the kinetics of the phase transition is determined in part by the size and shape of the gel.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) is typically used to study polymers because it allows spectra to be collected for solids and liquids. Ding et al. used ATR-FTIR to study the LCST and phase transition behavior of PNIPAM hydrogel.^[28] They observed LCST values typical of reported values. For PNIPAM, they noticed that the LCST measured from the heating experiments was slightly higher, about 2 °C, than the LCST measured from cooling experiments. This hysteresis is attributed to differing kinetics between the collapsing and swelling processes. After the gel collapses, the surface is a dense hydrophobic layer; as the temperature is decreased, diffusion of water is initially slow until the gel becomes more hydrated and less hydrophobic. They also noted that upon cooling, hydrogels may absorb less water than they originally contained. There are a number of limitations to ATR-FTIR analysis of hydrogels. First, the large amounts of water present exhibits strong IR absorption that interferes with the methylene and amide regions of the spectrum. Therefore, the contribution of water to the ATR-FTIR data must be subtracted before the polymer spectra can be analyzed. Second, ATR-FTIR only probes the surface of the hydrogel and not the entire structure. Importantly, FTIR does not provide quantitative data nor the level of chemical information gained with nuclear magnetic resonance (NMR) spectroscopy.

NMR offers a handful of advantages over other techniques used to study PNIPAM hydrogels. NMR is capable of probing the entire sample, providing qualitative and quantitative chemical structure information. The first NMR study of PNIPAM hydrogels was reported by Badiger et al.^[29] The study used the solid-state magic angle spinning NMR

(MAS-NMR) technique in order to reduce line broadening effects and collect spectra with good resolution. They also observed that as the temperature is increased, the intensities of the proton signals diminish due to increasing line broadening effects. Above LCST, only a residual water peak was observed. They concluded that below LCST, there is a dominance of polymer-solvent interactions while at or above LCST, polymer-polymer interactions are much more significant. The spectra at a given temperature were superimposable, regardless of whether the temperature was reached during a heating or cooling cycle. This observation reinforces the thermoreversible nature of the PNIPAM hydrogel phase transition.

Ohta et al. were the first to report ^{13}C NMR spectra for PNIPAM hydrogels.^[30] The significant finding in their work is the importance of the carbonyl group in the phase transition. They observed a significant reduction in the carbonyl signal above LCST. The carbonyl signal also provides information about hydrogen bonding. When the carbonyl is involved in hydrogen bonding, the signal is shifted downfield. They observed that the signal shifted upfield roughly 1.5 ppm as the temperature was increased, reinforcing the idea that hydrogen bonds are broken during the phase transition. They also observed a slight downfield shift for the other carbon signals (CH_3 , CH_2 , and side chain CH) as the temperature was increased. These were reported to be indicative of the gel collapsing. Diez-Pena et al. continued the study of PNIPAM hydrogels using both ^1H and ^{13}C NMR solid state techniques.^[31,32] They also studied PNIPAM hydrogel using a 2D ^1H - ^1H double quantum (DQ) MAS-NMR. Their findings provided more evidence that hydrogen bonding plays a key role in the phase transition of hydrogels.

When studying the phase transition of PNIPAM hydrogel, temperature-dependent peak intensity data can be used to calculate equilibrium constants which, in turn, can be used to calculate thermodynamic parameters of the phase transition.^[33,34] In previous work, PNIPAM gel swollen in a series of salts with varying anion species was studied with HRMAS-NMR.^[35] While the effect of various alkali metal cations was studied by Annaka et al.,^[36] the effect of divalent cations has yet to be investigated. This work provides new insight into the effect of various cations and their concentration on the phase transition of PNIPAM hydrogel, specifically effects on the LCST, thermodynamics, and hysteresis of the process.

2. Results and Discussion

Poly(*N*-isopropylacrylamide) (PNIPAM) hydrogels with *N,N'*-methylene-bis-acrylamide (MBA) crosslinker were analyzed using variable temperature high-resolution magic angle spinning (HRMAS) NMR spectroscopy. This method has been described previously.^[34,35,37] The samples were heated or cooled in 0.2 °C increments. There was a 5 min delay time at each temperature before the spectrum was collected to allow the sample to equilibrate. HRMAS was used to significantly reduce residual line-broadening effects from chemical shift anisotropy and dipolar coupling. The spectra were used to observe the phase transition of the hydrogel from the swollen to collapsed state and the reswelling process of the hydrogel. The 300 MHz Agilent/Varian Mercury spectrometer used in this work has a low power amplifier to deliver the radio-frequency (rf) pulses for water saturation. NMR pulses for removal of the water signal can lead to sample heating, unless care is taken to reduce

these effects. In the Agilent/Varian software, we selected a saturation power level of “10”, which is 45 dB lower in than the power level of the 90° proton pulse (transmitter power = 55) for data acquisition. Thus, the power level of the saturation pulse is in the sub-mW range and should not cause heating. Even if small heating effects occur, the comparison among the different salts will remain valid. However, rf heating suggests an interesting experiment, where high rf power is used to rapidly heat the sample, and this temperature-jump effect could be used to study dehydration/rehydration kinetics. Studies of this kind are underway.

In order to understand the effect of various anions on the phase transition, PNIPAM hydrogel was heated above LCST after being swollen in the presence of neat D₂O, NaCl, CaCl₂, and MgCl₂ solutions. By keeping the anion (Cl⁻) constant in the experiment, the impact of the three cations studied can be realized. Figure 1 illustrates the different impacts made by the various cations at equal concentrations. Each 100 × 10⁻³ M solution exhibits a similar LCST: 29.6, 29.5, and 29.2 °C for NaCl, CaCl₂, and MgCl₂, respectively. The LCST for the 200 × 10⁻³ M solutions exhibit a slightly wider range: 28, 27.8, and 27.1 °C for NaCl, CaCl₂, and MgCl₂, respectively. The data reveals that the MgCl₂ solution has a slightly larger effect on the phase transition than the CaCl₂ and NaCl solutions.

As seen in Figure 1, the effect of all three salts on the LCST of the phase transition indicate that the hydrated state of the PNIPAM hydrogel is being destabilized. All salt solutions cause a decrease in the LCST of the PNIPAM hydrogel and increases the temperature range over which, the phase transition occurs. Similar to the anion effects investigated, these salt effects can be described in terms of the “salting out” phenomenon that has been observed with various salts for PNIPAM polymer gels.^[16,36,38] The ability of salts to shrink or swell macromolecules can be ordered according to the Hofmeister or Lyotropic series. Like the anion series, the effects of cations can also be ordered according to a Hofmeister series. A typical order of cations is: NH₄⁺ > K⁺ > Na⁺ > Li⁺ > Mg²⁺ > Ca²⁺ > guanidinium. The cations on the left side of the series decrease the solubility of nonpolar molecules and tend to strengthen hydrophobic interactions, whereas cations on the right side of the series tend to increase the solubility of nonpolar molecules and weaken hydrophobic interactions. For the cations studied, the data show that indeed the Hofmeister series is applicable to the effects of cations on the PNIPAM phase transition.

It has been proposed that the effect of cations on the LCST of the phase transition is linearly dependent with respect to concentration.^[36] In order to better understand the effects of each salt, studied series of different salt concentrations were studied. PNIPAM was studied in solutions of 100 × 10⁻³, 200 × 10⁻³, 300 × 10⁻³, and 400 × 10⁻³ M salt concentrations. The NMR data from our experiments supports the conclusion that salt effects are linearly dependent on concentration. Each salt studied exhibited LCST values that are linearly dependent with respect to salt concentration. Figure 2 shows the temperature dependence of the ¹H NMR signal of the methyl protons, when the PNIPAM hydrogel is swollen in various NaCl solutions. The data in Figure 2 illustrate that the LCST is dependent on salt concentration. The change in LCST from no salt to 100 × 10⁻³ M solutions was 2.1 °C, the delta between 100 × 10⁻³ and 200 × 10⁻³ M solutions was 1.7 °C, between 200 × 10⁻³ and 300 × 10⁻³ M solutions was 1.4 °C, and between 300 × 10⁻³ and 400 × 10⁻³ M solutions was 2.3 °C. A graphical representation of the linear trend is provided in Figure 5.

Figure 3 shows the effect of various MgCl_2 concentrations on the phase transition of the PNIPAM gel. This data also illustrates a dependence of the LCST on salt concentration. The change in LCST between no salt and $100 \times 10^{-3} \text{ M}$ MgCl_2 is 2.6°C , the delta between 100×10^{-3} and $200 \times 10^{-3} \text{ M}$ is 2.0°C , the change between 200×10^{-3} and $300 \times 10^{-3} \text{ M}$ is 2.2°C , and the delta between 300×10^{-3} and $400 \times 10^{-3} \text{ M}$ is 2.7°C . This is consistent with the order of cations in the Hofmeister series. This effect is more evident at the higher concentration values, as seen in Figure 5.

The final cation studied was calcium which, according to the Hofmeister series, should have less effect on the phase transition than sodium and magnesium. Indeed, as shown in Figure 4, the CaCl_2 solutions had the least impact on the LCST of the PNIPAM phase transition. The comparison between calcium and magnesium is simple: at every concentration the LCST of the magnesium containing solutions was lower. Calcium also demonstrates a dependence of the LCST on salt concentration. The change in LCST from no salt to $100 \times 10^{-3} \text{ M}$ solutions was 2.2°C , the delta between 100×10^{-3} and $200 \times 10^{-3} \text{ M}$ solutions was 1.8°C , between 200×10^{-3} and $300 \times 10^{-3} \text{ M}$ solutions was 1.9°C , and between 300×10^{-3} and $400 \times 10^{-3} \text{ M}$ solutions was 2.3°C (Figure 5). The $400 \times 10^{-3} \text{ M}$ CaCl_2 solution exhibits a noticeably different slope during heating indicating that the thermodynamics of the phase transition are significantly affected.

Comparisons between the divalent cations and Na^+ are a little more difficult. It must be taken into consideration that at each concentration, the NaCl solution contains half the chloride concentration compared with the same molar MgCl_2 and CaCl_2 solutions. It has been reported that anions impact the phase transition to a greater extent than cations.^[16,24,38] Assuming cation effects to be equal, the lower amount of chloride anions in $100 \times 10^{-3} \text{ M}$ NaCl should cause a smaller drop in LCST than similar concentrations MgCl_2 or CaCl_2 . This is confirmed by the data in Figure 5, which show that the NaCl solutions have a slightly higher LCST than MgCl_2 or CaCl_2 . Upon closer inspection, it is apparent that the cation effects are not equal. If the effect of $\text{Na}^+ = \text{Mg}^{2+} = \text{Ca}^{2+}$, the LCST of $100 \times 10^{-3} \text{ M}$ MgCl_2 and $100 \times 10^{-3} \text{ M}$ CaCl_2 should be much lower, $\sim 28^\circ\text{C}$ because they contain $200 \times 10^{-3} \text{ M}$ of Cl^- ions. Instead, the LCST is $\sim 29^\circ\text{C}$ because the Mg^{2+} and Ca^{2+} allow for NIPAM hydration to remain stable and counteract destabilization from the chloride anions.^[35] In this manner, Mg^{2+} and Ca^{2+} ions are functioning as kosmotropic ions to stabilize the hydrated NIPAM polymer network. Likewise, the sodium cation must be having a weaker influence on NIPAM hydration stability than magnesium and calcium cations allowing the LCST to drop to values seen with MgCl_2 and CaCl_2 even though the NaCl solutions have a reduced Cl^- concentration.^[35]

Solvation of NIPAM in water is a balance of chemical potential manifested in the hydrophobic and hydrophilic forces. By comparing solutions of equivalent total ion concentration, for example $200 \times 10^{-3} \text{ M}$ MgCl_2 and $300 \times 10^{-3} \text{ M}$ NaCl , the NaCl solution exhibits a lower LCST value (26.5°C) than MgCl_2 (27.1°C). This highlights the enhanced ability of magnesium cations to maintain NIPAM solubility even though the MgCl_2 solution contains more chloride anions. The lowering of LCST caused by $300 \times 10^{-3} \text{ M}$ Na^+ and $300 \times 10^{-3} \text{ M}$ Cl^- ions is greater than $200 \times 10^{-3} \text{ M}$ Mg^{2+} and $400 \times 10^{-3} \text{ M}$ Cl^- ions. The expected destabilization from significantly more chloride anions does not arise, thus anion

effects are counter balanced by the magnesium cations, allowing water molecules to maintain a hydration sphere around the polymer.

Within our samples, the LCST values have a linear relationship with salt concentration (Figure 5). This agrees with the Hofmeister series and also gives the ability to predict LCST values from the equations in Figure 5, developed from our quantitative NMR data. Compared with our previous work,^[35] the trends in LCST show that cations do not impact the PNIPAM phase transition as greatly as anions. Altering cations at a given concentration results in smaller ranges of LCST values than varying anions. For example, at 200×10^{-3} M salt concentrations, the range of LCST values for the cations studied is only 0.9 °C, whereas the range of LCST values for the anions studied was 3.4 °C.^[35] This observation holds true for each salt concentration studied; varying anions exhibit a wider range of LCST values. This observation has been reported previously^[16,24,38] and the NMR data supports the validity of applying the Hofmeister series to the effects of cations on the PNIPAM hydrogel phase transition. A better picture can be gained by examining thermodynamic measurements.

2.1. Cation Effects on Phase Transition Thermodynamics

Using the HRMAS-NMR data and a van't Hoff plot, the thermodynamics of the phase transition can be determined.^[33,34,39] The van't Hoff plots reveal that the phase transition occurs in two stages; these data for the PNIPAM hydrogel in each solution are provided in the Supporting Information. Figures S1–S14 (Supporting Information) show the van't Hoff plots for the PNIPAM hydrogel in each solution of pure D₂O (S1), MgCl₂ (S2–S5), CaCl₂ (S6–S9), and NaCl (S10–S13). Thermodynamic values were calculated using these van't Hoff plots and the parameters are listed in Table 1.

From a van't Hoff analysis of the temperature-dependent equilibrium constant, we see that the phase transition has distinct multiple stages in D₂O and aqueous salt solutions. Evaluation of the slope and intercept yield thermodynamic parameters. Both enthalpy and entropy values were divided by 300 to obtain the thermodynamic quantities in units of kJ per mol monomer.^[33–35,37] The enthalpies of the phase transition are endothermic for Stage 1 and Stage 2, regardless of the salt solution in which PNIPAM was hydrated. The enthalpy values of Stage 1 have been attributed to intramolecular hydrophobic bond formation energies and the enthalpy values of Stage 2 have been compared with hydrogen bond dissociation energies for water interacting with the polymer side chains.^[33] The entropy values for Stage 1 are much smaller than those of Stage 2. The formation of intramolecular hydrophobic bonds causes small increases in entropy. During Stage 2, the organized water shell is completely broken down, water is expelled from the polymer matrix, and the gel completely collapses. These significant structural changes cause a large increase in entropy.^[33]

Lower concentrations of CaCl₂ have a smaller impact on enthalpy and entropy values for both Stages 1 and 2. For the NaCl solutions studied, there is very little change in the enthalpy and entropy terms as the concentration is increased, with the exception of the 400×10^{-3} M NaCl solution. Despite the relatively similar enthalpy and entropy values, the Gibbs' free energy gradually decreases as the NaCl concentration is increased; as would be

expected based on LCST data. At 400×10^{-3} M, G° is negative indicating that the phase transition is spontaneous at room temperature and indeed the LCST in 400×10^{-3} M NaCl is 24.2°C . When comparing solutions with equivalent anion concentration, the MgCl_2 salt solution's effect on the Stage 1 enthalpy and entropy values of the phase transition was similar to the NaCl salt solutions. For example, the 400×10^{-3} M NaCl Stage 1 enthalpy and entropy terms are 1.38 kJ mol^{-1} and $4.64 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively; the Stage 1 enthalpy and entropy terms for 200×10^{-3} M MgCl_2 are 1.39 kJ mol^{-1} and $4.62 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively. Unlike the NaCl and CaCl_2 data for Stage 1, increasing MgCl_2 concentration steadily increased the enthalpy and entropy thermodynamic parameters. Likewise, the Gibb's free energy steadily decreases as MgCl_2 concentration is increased. The phase transition at 400×10^{-3} M concentration is spontaneous at room temperature according to the calculated Gibb's free energy and indeed the LCST is 22.2°C . Comparing the Gibb's free energy of 200×10^{-3} M NaCl and 100×10^{-3} M MgCl_2 solutions as well as 400×10^{-3} M NaCl and 200×10^{-3} M MgCl_2 solutions, we see in both cases that NaCl is affecting the phase transition to a greater extent, lowering the Gibb's free energy more than the MgCl_2 salt solution.

The CaCl_2 salt solutions exhibited the smallest changes in the thermodynamic parameters of the phase transition compared with all other salts studied. The values are relatively consistent for 100×10^{-3} , 200×10^{-3} , and 300×10^{-3} M concentrations. At 400×10^{-3} M, the values decrease noticeably as observed in Figure 4. In agreement with the LCST trend observed, the Gibb's free energy steadily decreases as CaCl_2 concentration is increased. The phase transition at 400×10^{-3} M concentration is spontaneous at room temperature according to the calculated Gibb's free energy and indeed the LCST is 23.5°C . The significant difference between the magnesium and calcium thermodynamic parameters could be related to their considerably different charge densities. Looking at the Stage 1 G° s, the effect of the lower concentration CaCl_2 solutions is roughly half that of the same concentration MgCl_2 solutions. For example, at 100×10^{-3} M, the MgCl_2 solution decreased the entropy and enthalpy by factors of 2.23 and 2.21, respectively, whereas the CaCl_2 solution decreased the entropy and enthalpy by factors of 1.19 and 1.18, respectively. Interestingly, the charge density of Ca^{2+} , the amount of electrical charge in the volume of the atom, is 52 C mm^{-3} , which is a little less than half that of the Mg^{2+} charge density, which is 120 C mm^{-3} .^[40] This possible correlation warrants further study using other divalent cations.

Direct comparison between MgCl_2 and CaCl_2 is simpler than comparisons including NaCl. However, comparing values from solutions with the same anion concentration shows that order of increasing effect is $\text{Ca}^{2+} < \text{Mg}^{2+} < \text{Na}^+$. Similar to our previous study with anion effects^[35], the salt solutions have a significant initial impact on the thermodynamic values but after that initial drop, the changes become much less drastic as the salt solution concentrations are increased.

2.2. Cation Effects on the Reswelling Process

During the heating cycle, the NIPAM undergoes a transition from a water soluble to an insoluble state. As described above, salts enable breakdown of the water hydration shell and

thus, lower the LCST values. Upon cooling, the salts also affect disruption of hydrophobic interactions that precede polymer rehydration. When the temperature is lowered, water begins to rehydrate the polymer in a process that must break the hydrophobic bonds. Hydrophobic bonds, unlike dipole–dipole, electrostatic or van der Waals bonds, become stronger at higher temperatures. Thus, cooling weakens the hydrophobic interaction and, when broken, allows the polymer to swell and rehydrate. Because hydrophobic bonds form in the early stages of the phase transition, the temperature must fall below the point at which the hydrophobic bond is stable. The result is hysteresis between the heating and cooling curves (see Supporting Information). The hysteresis in the data increases in the presence of salt solutions and is between 2.7 and 3.3 °C for most samples. However, the 100×10^{-3} M CaCl_2 solution shows a difference of 3.2 °C (Figure S19, Supporting Information) between the heating and cooling curves while the 200×10^{-3} and 300×10^{-3} M CaCl_2 solutions show a difference of 2.5 and 1.9 °C, respectively (Figures S20,S21, Supporting Information).

Above the LCST, the hydrophilic nature of the ions retains water in a hydration shell and prevents the solvent molecules from forming around the hydrophobic NIPAM domains. Upon cooling, these ions also affect the resolution of hydrophobic domains. NaCl caused negligible changes in the hysteresis of the rehydrating process, at most an increase or decrease of 0.1 °C. This shows that sodium is not significantly impacting the strength of hydrophobic interactions. These data also reinforce the placement of Na^+ and Cl^- in the middle of the Hofmeister series, where they should have little effect on protein solubility. Similarly, lower concentrations of MgCl_2 caused only small changes in the hysteresis, whereas the affect is magnified with the 400×10^{-3} M MgCl_2 solution. Hysteresis increased from 2.7 to 3.3 °C, highlighting the role of Mg^{2+} in destabilizing polymer solubility by making it harder to rehydrate the collapsed hydrogel. This is not unexpected as magnesium ions are kosmotropic and form strong ion-water bonds and hinder the formation of water-water bonds required for a stable water shell to form around macromolecules.^[41] However, CaCl_2 solutions showed the opposite trend, reduced hysteresis at higher concentration. The CaCl_2 ions weaken the hydrophobic interactions, allow water to break up the domains, and facilitate polymer solvation. In this study, calcium ions are acting as a chaotropic agent^[42] during the cooling cycle, destabilizing hydrophobic domains, and increases solubility. This reduces hysteresis by making it easier to reform the hydration shell around NIPAM. This behavior, contrary to MgCl_2 , seems to indicate that the additional calcium ions may be decreasing the strength of hydrophobic interactions compared with the water itself. Thus, the hydrophobic forces will be weakened earlier in the cooling cycle and the hydration shell will organize earlier than in MgCl_2 solution. This trend has been observed in the denaturation of proteins and the role of CaCl_2 that weakened the hydrogen bonding network.^[42] Additionally, differences between Mg^{2+} and Ca^{2+} are likely to arise from a combination of complex phenomena, such as change in surface tension^[14,18] and free energy of hydration. The observed trends in the hysteresis of the reswelling process demonstrate that NMR spectroscopy can be an important analytical tool for tailoring hydrogels with desired dehydration/rehydration properties.

3. Experimental Section

3.1. Materials

N-isopropylacrylamide (NIPAM) was purchased from Aldrich and recrystallized from *N*-hexane prior to use. Azobisisobutyronitrile (AIBN) was purchased from Aldrich and recrystallized from methanol prior to use. *N,N'*-methylene-bis-acrylamide (MBA), NaCl, MgCl₂, and CaCl₂ were purchased from Aldrich and used as received. D₂O was obtained from Cambridge Isotope Laboratories. The necessary amounts of all the salts were dissolved in D₂O to prepare 100×10^{-3} , 200×10^{-3} , 300×10^{-3} , and 400×10^{-3} M salt solutions.

3.2. Hydrogel Preparation

Poly(*N*-isopropylacrylamide) (PNIPAM) hydrogels were prepared via thermally initiated free radical addition polymerization. Typically, 52.3 mg (0.462 mmol) of NIPAM were transferred to a small glass test tube and dissolved in 250 μ L of DMSO. Next, 12 μ L of MBA in DMSO solution (0.1286 M) was added to the solution followed by 15 μ L of AIBN in DMSO solution (0.0925 M). The resulting solution was carefully and thoroughly mixed. The test tube was purged with N₂ gas for approximately 1 min and then sealed with a rubber stopper. The test tube was then placed in a heater block at approximately 60 °C for 12 h. The hydrogels synthesized have a crosslink density of approximately 300:1 (NIPAM:MBA). After polymerization, the hydrogel was repeatedly washed to remove DMSO and any excess reagents. Washing was accomplished by heating the gel above LCST and then rehydrating it in doubly distilled H₂O.

After being thoroughly washed, the hydrogel was cut into smaller pieces that were able to fit in the ceramic HRMAS-NMR rotor. The pieces of gel were dehydrated by heating them above LCST and then were placed in small vials containing 1 mL of D₂O or various salt solutions: 100×10^{-3} , 200×10^{-3} , 300×10^{-3} , and 400×10^{-3} M solutions of NaCl, MgCl₂, and CaCl₂. The gel pieces rehydrated in these various solutions before being transferred to the NMR rotor to be studied.

3.3. HRMAS-NMR Spectroscopy

Small quantities (≈ 50 μ L) of hydrated poly(NIPAM) were placed in a ceramic, high-resolution magic-angle-spinning (HRMAS) rotor. NMR spectra were collected using a Varian 300 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 ¹H chemical shift values were referenced to the residual water signal (HOD) at 4.8 ppm. Temperature calibration of the NMR spectrometer was accomplished with the temperature-dependent chemical shift of ethylene glycol. All samples were spun at a rate of ≈ 2000 Hz. During variable temperature NMR measurements (both heating and cooling cycles), the samples were allowed to rest for 5 min at each temperature prior to acquiring the spectra. Deuterium lock was maintained throughout data acquisition to control the field frequency ratio over the sample. Temperature-induced changes in solvent density affect the magnetic susceptibility of the sample. This can change the intensity of the local magnetic field at the sample, influencing 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.

4. Conclusion

The effect of various alkali and alkaline earth metal chloride salts on the phase transition of PNIPAM hydrogel has been studied with HRMAS-NMR spectroscopy. The effect of each salt on the LCST, thermodynamics, and hysteresis of the thermoreversible phase transition was measured. Decreases in LCST were observed for all three salts at varying concentrations. Additionally, the change in LCST was found to be linearly dependent with respect to salt concentration. Thermodynamic parameters were more impacted by the NaCl and MgCl₂ solutions compared with values measured in CaCl₂ solutions. The hysteresis of the reswelling process was affected by NaCl and MgCl₂ solutions, whereas increasing CaCl₂ concentrations decreased the hysteresis. Overall, the effects of both sodium and magnesium are fairly similar, especially when considering salt solutions with the same anion concentrations. This work demonstrates the applicability of the Hofmeister series to the effects of cations on the PNIPAM hydrogel phase transition. This work also demonstrates that cations do not affect the phase transition to the same extent as anions.^[35] Additionally, the possible correlation between charge density and the degree of change in thermodynamic parameters for divalent cations warrants further studies using other divalent cations. Although other research suggests that metal:polymer interactions are present,^[17,19,43–45] we have not investigated if cations closely associate with the polymer, for example if a cation is drawn toward the electronegative carbonyl oxygen. This question could be resolved through REDOR NMR experiments to determine internuclear distances between a cation and the polymer structure.

Supplementary Material

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Figure 1.

The effect of different cations on the phase transition of PNIPAM hydrogel. The degree of impact on the phase transition for the Ca^{2+} , Mg^{2+} , and Na^{+} is evident and these data can be used to measure the Gibbs free energy.

Figure 2.

The effects of various NaCl concentrations on the phase transition of PNIPAM hydrogel.

The LCST values of the gel in pure D₂O, 100×10^{-3} , 200×10^{-3} , 300×10^{-3} , and 400×10^{-3} M NaCl concentrations are 31.7, 29.6, 27.9, 26.5, and 24.2 °C, respectively.

Figure 3.

The effects of various MgCl_2 concentrations on the phase transition of PNIPAM hydrogel.

The LCST values of the gel in 100×10^{-3} , 200×10^{-3} , 300×10^{-3} , and 400×10^{-3} M

MgCl_2 concentrations are 29.1, 27.1, 24.9, and 22.2 °C, respectively.

Figure 4.

The effects of various CaCl_2 concentrations on the phase transition of PNIPAM hydrogel. The LCST values of the gel in 100×10^{-3} , 200×10^{-3} , 300×10^{-3} , and 400×10^{-3} M CaCl_2 concentrations are 29.5, 27.7, 25.8, and 23.5 °C, respectively.

Figure 5. Summary of LCST values measured with NMR spectroscopy. The values obtained in salt solution form linear trends that converge near 31.7 °C, the phase transition temperature value in pure D₂O.

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Table 1.

Phase transition thermodynamic values.

	Stage 1			Stage 2		
	H° [kJ mol ⁻¹]	S° [J K ⁻¹ mol ⁻¹]	G° (298.15 K) [kJ mol ⁻¹]	H° [kJ mol ⁻¹]	S° [J K ⁻¹ mol ⁻¹]	G° (298.15 K) [kJ mol ⁻¹]
No Salt	2.70 ± 0.15	8.85 ± 0.5	0.061	7.13 ± 0.97	23.4 ± 3.2	0.153
100 × 10 ⁻³ m NaCl	1.59 ± 0.10	5.26 ± 0.34	0.022	5.32 ± 0.40	17.6 ± 1.3	0.073
200 × 10 ⁻³ m NaCl	1.57 ± 0.07	5.22 ± 0.22	0.014	5.24 ± 0.82	17.4 ± 2.7	0.052
300 × 10 ⁻³ m NaCl	1.61 ± 0.08	5.36 ± 0.28	0.012	5.08 ± 0.53	17.0 ± 1.8	0.011
400 × 10 ⁻³ m NaCl	1.38 ± 0.05	4.64 ± 0.16	-0.003	4.12 ± 0.24	14.0 ± 0.9	-0.054
100 × 10 ⁻³ m MgCl ₂	1.21 ± 0.06	4.01 ± 0.22	0.014	4.59 ± 0.27	15.2 ± 0.9	0.058
200 × 10 ⁻³ m MgCl ₂	1.39 ± 0.10	4.62 ± 0.33	0.013	5.87 ± 0.79	19.5 ± 2.6	0.056
300 × 10 ⁻³ m MgCl ₂	1.60 ± 0.07	5.38 ± 0.24	-0.004	4.72 ± 0.39	15.9 ± 1.3	-0.021
400 × 10 ⁻³ m MgCl ₂	1.68 ± 0.12	5.70 ± 0.40	-0.019	5.24 ± 0.54	17.7 ± 1.8	-0.037
100 × 10 ⁻³ m CaCl ₂	2.27 ± 0.10	7.49 ± 0.33	0.037	5.82 ± 0.42	19.2 ± 1.4	0.096
200 × 10 ⁻³ m CaCl ₂	2.20 ± 0.10	7.32 ± 0.32	0.018	6.96 ± 0.93	23.1 ± 3.1	0.073
300 × 10 ⁻³ m CaCl ₂	2.27 ± 0.09	7.58 ± 0.29	0.010	5.74 ± 0.74	19.2 ± 2.5	0.016
400 × 10 ⁻³ m CaCl ₂	1.75 ± 0.06	5.90 ± 0.22	-0.009	4.00 ± 0.37	13.5 ± 1.3	-0.025