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Sodium Chloride-Induced Phase Transition in Nonionic Poly(*N*-isopropylacrylamide) Gel

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ABSTRACT: A nonionic poly(*N*-isopropylacrylamide) hydrogel, which exhibits a temperature-dependent volume phase transition (lower critical solution temperature, LCST) behavior in aqueous solution, demonstrates an unusual sodium chloride-induced phase transition phenomenon as well. The gel collapses sharply at critical sodium chloride concentration, which depends on the temperature. Other salts tested show no such behavior, and typical salting-out behaviors (the gradual gel collapse with increasing their concentration) were observed. It appears that chloride ions play a major role in this phase transition.

Introduction

Synthetic and biological polymers can be strongly influenced by various salt solutions. It has been recognized that conformational transitions of such macromolecules can be induced by different kinds of salts and their concentrations. For example, it has been found empirically that ions can be ranked in order of increasing effectiveness in stabilizing or destabilizing protein molecules.[1-3] A lyotropic series of salts was proposed to explain the different degrees of conformational transition in macromolecules. Increasing the amount of salt in the solution raises the ionic strength of the surrounding medium. Since most of the biopolymers are polyelectrolytes, ionic strength generally influences inter- and intramolecular interactions, mainly ionic interactions, which stabilize protein molecules. As a result, various conformational changes such as salting-out (or salting-in), helix–coil transition, denaturation, and gelation can be observed in proteins. Synthetic polymers have often been used as an analogy of a protein to study the salt effect on the macromolecular conformation due to their relatively simple molecular structure.[4,5]

Hydrogels, which have been defined as a cross-linked hydrophilic polymer network, exhibit different equilibrium degrees of swelling in response to various kinds of salts as well as their concentrations. Thus, they can be used to measure, in a simple way, the effects of the solvent environment on the polymer. Donnan equilibrium has been successfully used to predict the swelling behavior of charged gels in electrolyte solutions.[6-8] A salt-induced phase transition in a hydrolyzed (ionic) polyacrylamide gel was reported in a water–organic solvent mixture.[9] A discrete volume phase transition has been observed in response to mono- or divalent electrolytes only in the region of high acetone concentration. This phase transition behavior in the relatively hydrophilic gel could be caused by van der Waals interactions in the medium of low dielectric constant. In the case of nonionic gels, increased salt concentration in the bulk medium affects inter- and intramolecular hydrogen-bonding and polar interactions as well as hydrophobic interactions associated with water molecules. Thus, it can be anticipated that, even though nonionic gels demonstrate the salting-out (or salting-in) behavior, they normally induce a far weaker salt-induced swelling change compared to the ionic gels.

One of nonionic hydrogels, a lightly cross-linked poly(*N*-isopropylacrylamide, NIPAAm) exhibits a lower critical solution temperature (LCST) phenomenon in aqueous solution.[10,11] This gel collapses and shrinks above the LCST and reswells and expands below that critical temperature. Hydrophobic interaction driven by an entropy gain in water molecules is responsible for that behavior. In this study, swelling equilibria of the poly(NIPAAm) gel were determined in various salt solutions. Surprisingly, the nonionic poly(NIPAAm) gel exhibits a sharp volume phase transition at a critical concentration of sodium chloride in aqueous solution. The critical concentration depends on the temperature. Other salts tested do not cause such an abrupt volume change. The salt-induced phase transition of a nonionic gel in the aqueous solution has not been reported previously.

Materials and Methods

Materials. *N*-Isopropylacrylamide (NIPAAm) from Eastman Kodak was recrystallized with hexane. *N*,*N*′-Methylenebis(acrylamide) (MBAAm), ammonium persulfate (APS), and *N*,*N*,*N*′,*N*′-tetraethylmethylenediamine (TEMED) were purchased from Sigma Co. All other solvents and other chemicals were of analytical grade.

Preparation of the Hydrogel Membrane. A total of 7.68 g of NIPAAm and 0.32 g of MBAAm were dissolved in 40 mL of deionized water, and nitrogen was bubbled to remove residual oxygen. As redox initiators, 10 mg of APS and 0.1 mL of TEMED were added, and the mixture was immediately injected into the space between two glass plates. Polymerization was carried out at room temperature for 3 h. After the polymerization, the gel membrane sheets were removed, extensively washed with deionized water, and then punctured by a #6 cork borer. The gel disks were dried under vacuum overnight.

Determination of the Water Contents. Dry gel disks in triplicate were incubated in various salt solutions for at least 24 h at different temperatures. The swollen gel disks were weighed after blotting excessive surface water with filter paper. Water contents (or swelling ratios) in the gel were then calculated based on the wet weight and the dry weight. Each data point presented is an average value of the three gel disks.

Preparation of Un-Cross-Linked Poly(NIPAAm). A total of 60.7 mmol of NIPAAm was dissolved in 100 mL of a toluene/tetrahydrofuran mixture (75/25, v/v). After removing residual oxygen with nitrogen bubbling, 21 mg of azobis(isobutyronitrile) (AIBN) was added as an initiator. Polymerization was carried out at 50 °C for 24 h under positive nitrogen pressure. After the polymerization, 200 mL of petroleum ether was added to precipitate the polymer out of the reaction mixture, which was

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then glass-filtered and washed with 100 mL of petroleum ether. The polymer obtained was dried *in vacuo*. The molecular weight was estimated by a gel permeation chromatography using Waters μStyragel columns of pore sizes 500, 1000, 10 000, and 100 Å connected in series. As a standard MW probe, polystyrene was used for calibration. The number-average molecular weight of poly(NIPAAm) was 15 000.

Determination of LCST for Un-Cross-Linked Poly(NIPAAm). The cloud point (LCST) of un-cross-linked poly(NIPAAm) at various concentrations of sodium chloride was determined by reading the absorbance at 600 nm using a UV-vis spectrophotometer. Poly(NIPAAm) (1000 ppm) was dissolved in a given concentration of a sodium chloride solution at 4 °C. A cell holder in the spectrophotometer was thermally controlled by using a heating circulator and a cooler. A thermocouple was inserted in the cuvette to obtain an accurate temperature reading. The temperature was gradually raised from 5 to 40 °C by turning on the heater, and the absorbance value was read 1 °C increment of each sample in a cuvette. The LCST of poly(NIPAAm) in a particular sodium chloride solution was defined at the temperature where 50% of the absorbance transition occurs.

Results and Discussion

Swelling equilibria of macromolecules in various electrolyte solutions have been empirically estimated based on the Hofmeister series.[1,2] For example, the influence of anions of ribonuclease conformation is as follows:

helix, native, salting-out coil, denatured, salting-in

$$SO_4^{2-} < HPO_4^{2-} < CH_3COO^- < Cl^- < Br^- < ClO_4^- < SCN^-$$

Although there have been a number of studies to elucidate the mechanism responsible for the salt-induced changes in hydrodynamic properties of nonionic polymer solutions, the mechanism remains unclear.[4] The main reason is that the common denominator of every polymer–salt system involves water as a solvent medium. This three-component system tends to minimize its free energy by dynamically changing the polymer conformation as well as water structure at a particular concentration of the given salt. In general, the increased electrolyte concentration decreases the solubility of the polymer solution due to the salting-out effect, even though there are a few exceptional salts (chaotropic agents), e.g., thiocyanate and iodide, which exhibit opposite behavior, the salting-in. However, this general trend is often dependent on the type of nonionic polymer chosen. For example, poly(acrylamide) exhibits no hydrodynamic response to a wide range of salts,[12] while poly(vinylpyrrolidinone) demonstrates a decrease in the viscosity.[13] According to an empirical equation of the salting-out, the solubility of any particular water-soluble polymer decreases gradually with increasing ionic strength as seen in many cases of proteins. The solubility of a polymer in an aqueous salt solution can be expressed by the empirical Setschenow equation (1);

$$\log \gamma = \log(S_0/S) = K_S C_S$$

where γ is the activity coefficient of the polymer, which is defined as S_0/S, where S_0 is the solubility of the polymer in pure water and S is the solubility in an aqueous salt solution, K_S is the salting-out coefficient for a given salt, and C_s is the salt concentration. From the above equation, it can be easily expected that, in nonionic polymers, salt will not lead to an abrupt change in the solubility at a "critical" concentration, because it is believed that increasing the salt concentration gradually changes either ion–dipole interactions between the ion and the polar group in the polymer or the water structure associated with the nonpolar group. These interactions have been regarded as the most probable driving force in the salt effect on macromolecules.[1,2] When the polymer is cross-linked and formed into a gel, the change in solubility (or change in viscosity) for a nonionic un-cross-linked polymer in the salt solution can be reflected as a change in the equilibrium swelling degree of the gel. The decreased swelling degree directly implies a decrease in the solubility (or viscosity).

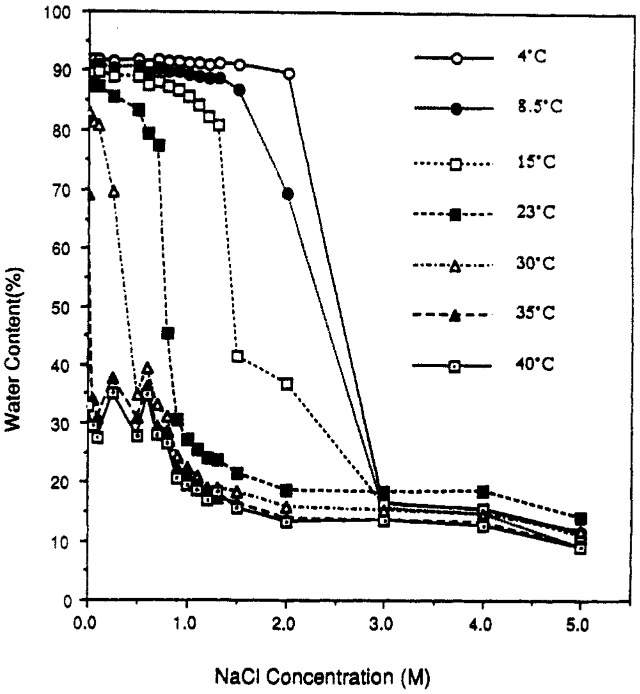

Figure 1. Effect of sodium chloride concentration on the water content of the poly(NIPAAm) gel as a function of temperature. Each data point is an average value of three samples.

The water contents of the poly(NIPAAm) gel plotted against the sodium chloride concentration at seven different temperatures are shown in Figure 1. It can be seen that, at each temperature, the water content of the gel sharply decreases at a certain sodium chloride concentration. As the temperature is raised, the critical concentration of sodium chloride decreases concomitantly. Obviously, this striking phase transition behavior of the nonionic poly(NIPAAm) gel, which is induced by sodium chloride, cannot be explained in terms of either the above-mentioned "salting-out" effect or the osmotic pressure difference between the gel and the bulk medium. Since the poly(NIPAAm) gel has an LCST ca. 32–33 °C,[10] it can also be observed that, above that temperature region, the gel is totally collapsed and the water content decreases slightly with increasing temperature. Below the LCST, however, the water content of the gel is a strong function of the concentration of sodium chloride. The gel phase transition appears to be very sharp only in a narrow temperature range between 15 and 23 °C as seen in Figure 1. The critical concentration of sodium chloride, at which the gel phase transition occurs, is plotted against temperature in Figure 2 (top figure). A good linear relationship can be obtained between the two, indicating that a smaller amount of sodium chloride is needed for the phase transition as the temperature increases. Very similar behavior can be seen for un-cross-linked poly(NIPAAm). Its LCST decreases as the concentration of sodium chloride increases, as shown in Figure 2 (bottom figure). These results suggest that un-cross-linked and cross-linked polymers have the same LCST regardless of their physical state in solution and further imply that the molecular weight of the polymer is not a significant factor in determining the LCST. In cases of nonionic poly(vinyl

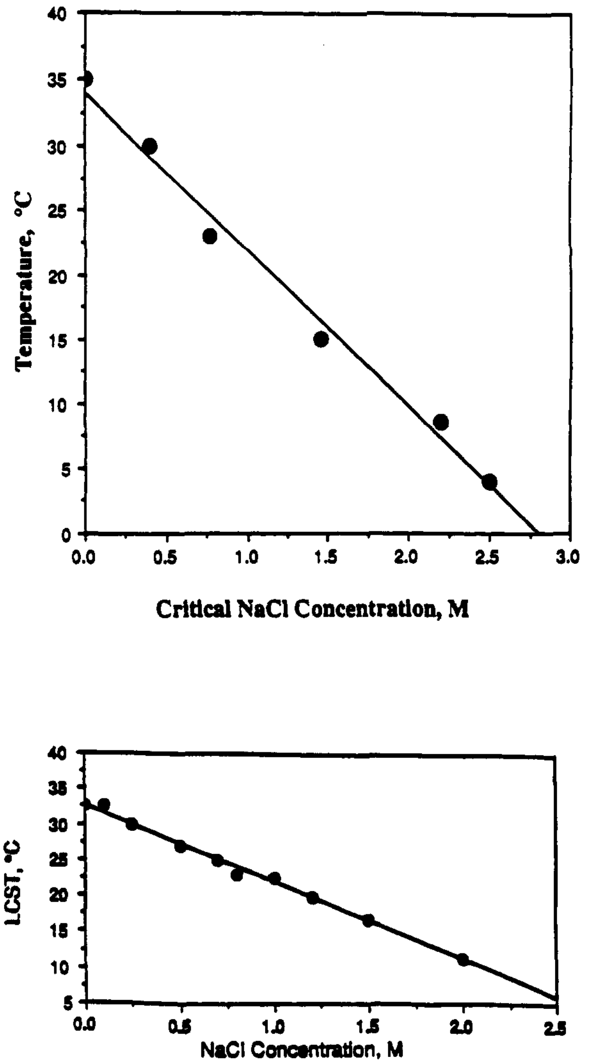

Figure 2. Effect of temperature on the critical sodium chloride concentraton at which the poly(NIPAAm) gel exhibits a phase transition (top). The bottom figure demonstrates the decrease of LCST with increasing sodium chloride concentration, where the LCST was obtained from the midpoint of the absorbance transition.

methyl ether) and poly(ethylene oxide) which exhibit an LCST phenomenon, their LCST is also lowered by the salt.[5,14] More recently, it has been shown that various salts affect the LCST of cross-linked and un-cross-linked poly(NIPAAm).[15,16] The decreasing trend in the transition temperature was correlated with the viscosity B coefficient (a measure of ion–water interaction) of ions, suggesting that water structure-making and -breaking effects by ions may result in the LCST shift. However, there have been no reports so far about the salt-induced phase transition in nonionic gels. Since the two trends in Figure 2 are the same, it can be said that the LCST of the gel and the critical sodium chloride concentration are closely related. Even though the exact role of sodium chloride in lowering the LCST is not clearly understood,[15] it seems that the driving force for the phase transition behavior of the poly(NIPAAm) gel induced by either sodium chloride or temperature, is the same, i.e., via inter- and intramolecular hydrophobic interactions. In order to test whether other salts exhibit similar salt-induced phase transitions as seen, swelling behaviors of the poly(NIPAAm) gel have been measured in solutions of sodium phosphate, sodium sulfate (Figure 3), and sodium thiocyanate (Figure 4). It can be seen that there are no apparent salt-induced phase transitions of the gel in these salt solutions, although gradual decreases in the water content take place. Among the salts tested, the sulfate anion is the strongest salting-out agent which also lowers the LCST with increasing concentration. It is also of interest to note that the chaotropic salt, sodium thiocyanate ions, appears to allow the gel to slightly swell at low concentration ranges, while observing the gel collapse above the concentration, 0.5 M. This may be related to the ability of thiocyanate ions for making water into structured and destructured states depending on its concentration. These data agree well with the previously reported data for un-cross-linked poly(NIPAAm).[15,16] Additional additives such as sodium iodide and urea did not show the phase transition behavior

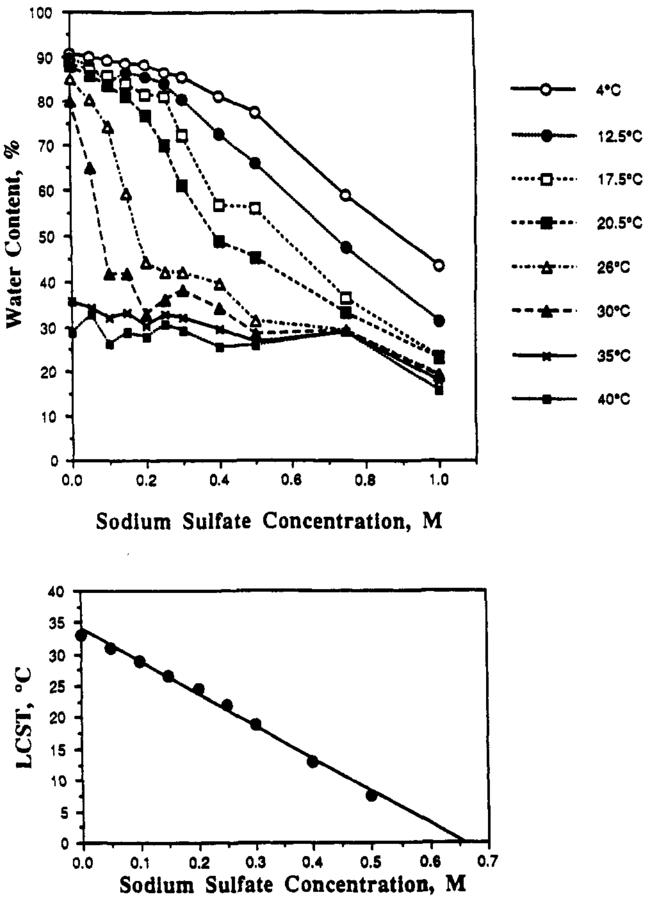

Figure 3. Effect of sodium sulfate concentration on the water content of the poly(NIPAAm) gel as a function of temperature (top) and the effect of sodium sulfate on the LCST of un-cross-linked poly(NIPAAm) (bottom).

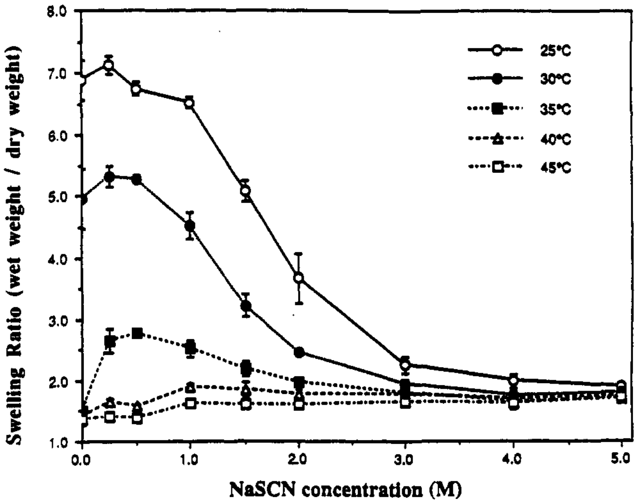

Figure 4. Effect of sodium thiocyanate concentration on the swelling ratio of the poly(NIPAAm) gel as a function of temperature.

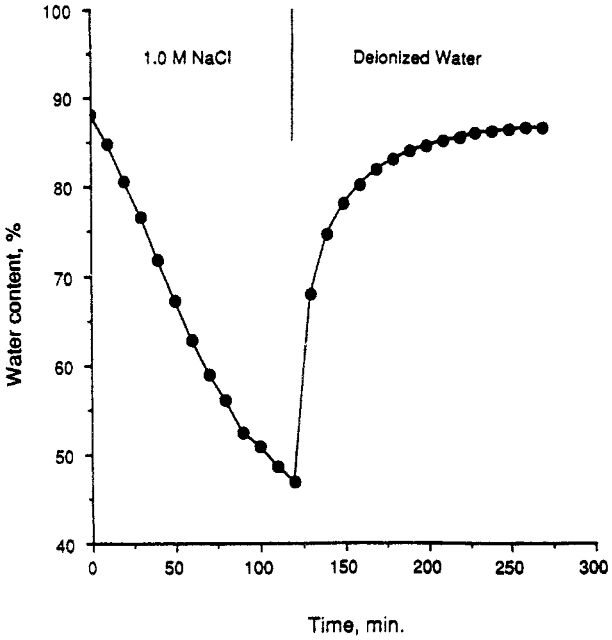

Figure 5. Reversible deswelling and reswelling of the poly(NIPAAm) gel in response to a 1 M sodium chloride solution and deionized water at room temperature.

either.[17] These facts indicate that sodium chloride is a unique salt to induce the phase transition of the poly(NIPAAm) gel. Because the sodium ion is a common cation in all of the salts, the chloride ion is responsible for this behavior. It will be necessary to test a wide range of salts in order to understand this unusual phenomenon. Presumably, chloride ions might bind specifically to certain portions (perhaps polar amide groups) of the polymer segment and/or might interact with hydrated water molecules which are associated with polar or hydrophobic polymer segments. This may lower the LCST of poly(NIPAAm). In fact, it has been shown that a direct interaction between certain anions and the amide dipole results in the solubility change of the uncharged model peptide.[18] However, it is still questionable how only chloride ions elicit such a cooperative, sudden change in the gel over a narrow range of temperature. The chloride ion is ranked in the middle region of the lyotropic series,[1,2] the salting-out constants (K_S),[18] the viscosity B coefficients[19,20] for various salt solutions. A plausible explanation for the abrupt swelling change induced by chloride ions may be related to the "stiffness" of the polymer chains, which has been suggested as one of the requirements for exhibiting the phase transition behavior.[21] The stiffness of the polymer chain is defined as the ratio of the chain persistance length to its hydration thickness. A minimal chain stiffness is necessary for the volume phase transition to be discontinuous. From this point of view, the structure and extent of the hydration sheath around the polymer chain may be adequately matched up by the chloride ion, its concentration, and the temperature, so that the chain stiffness satisfies that requirement for an abrupt phase transition elicited by the small change in chloride ion concentration.

Figure 5 shows the reversible swelling and deswelling of the poly(NIPAAm) gel in response to the incubation between deionized water and a 1.0 M NaCl solution at room temperature. It can be seen that the water content of the gel rapidly decreases in the presence of sodium chloride but regains its swelling capacity when incubating it back in deionized water.

In summary, it has been demonstrated that sodium chloride induces an abrupt volume phase transition in the nonionic poly(NIPAAm) gel, while the LCST of the gel is lowered by increasing the sodium chloride concentration. This unique phase transition behavior may have a wide range of potential applications such as chloride ion-sensitive biosensors.

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