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ARTICLE *in* JOURNAL OF APPLIED POLYMER SCIENCE · DECEMBER 2004

Impact Factor: 1.77 · DOI: 10.1002/app.21000

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Independent Control of Lower Critical Solution Temperature and Swelling Behavior with pH for Poly(*N*-isopropylacrylamide-*co*-maleic acid)

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Received 20 February 2004; accepted 21 May 2004
DOI 10.1002/app.21000
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Polymer solutions that gel in response to changes in temperature and pH are of interest for various forms of drug delivery, and it is desirable to increase swelling for diffusion-controlled release without bringing the lower critical solution temperature (LCST) above 37°C. *N*-isopropylacrylamide (NIP) was polymerized with maleic acid (MAc), a diprotic acid, and acrylic acid (AAc), a monoprotic acid, to compare swelling and temperature response with changes in pH. For samples with equal acid contents and almost identical LCST responses to pH, poly(*N*-isopropylacrylamide-*co*-maleic acid) (pNIP MAc) demonstrated greater swelling than poly(*N*-isopropylacrylamide-*co*-acrylic acid) (pNIP AAc). The LCST increase for MAc occurred at a pH corresponding to the deprotonation of almost all of the first acid groups. Further increases in pH led to the deprotonation of the second —COOH and only served to increase the charge concentration at a given location. These results provide strong support for the theory that LCST results largely from uninterrupted chain lengths of NIP and that swelling results from the actual charge density of acid groups along the chain. Because the use of a diprotic acid copolymer allows swelling to be decoupled from LCST, pNIP MAc may be an appropriate candidate for pH-sensitive drug-delivery applications. J Appl Polym Sci 94: 2110–2116, 2004

Key words: phase behavior; stimuli-sensitive polymers; biomaterials; drug delivery systems

INTRODUCTION

Certain polymer solutions are known to exhibit a lower critical solution temperature (LCST), below which they exist in a hydrophilic, soluble state and above which the polymer chains become hydrophobic and precipitate from solution. At sufficient concentrations, this transitions the fluid into a gel. Polymer gels are also known to shrink or swell with changes in temperature. As the temperature increases above a critical value (at or above the LCST), the gel collapses, expelling water and shrinking in volume. The swelling behavior is reversible when the temperature is lowered below the LCST,[1–3] and a gelled polymer with ionic monomer content can contain thousands of times its dry weight in water when it is above its temperature transition.[4] Polymer gels can be either physically or chemically associated, and the nature of the sol–gel transition is due to various competing interactions (e.g., ionic and hydrophobic interactions, van der Waals forces, and hydrogen bonding) that are functions of both the system composition and the gel's aqueous environment.[5–7]

The addition of an ionizable comonomer introduces pH sensitivity to the temperature transition. Previous studies of acid copolymers in ionic solutions have revealed an S-shaped pH response curve for swelling and LCST. At low pH, the acid groups are protonated, and the polymer is less soluble because of its neutral charge. A higher LCST is observed at higher pH values, when the acid groups become deprotonated and are more hydrophilic. LCST and swelling behavior increase with increasing pH for polymer systems with acid comonomers. The final delivery rate is thus a function of both temperature and pH.[1,3,8,9] The greatest rate of property change with pH is known to occur at the pK_a value for the acid comonomer,[1,10,11] as this pH has the largest impact on the addition of charged groups.

The entanglement of polymer chains acts as a barrier to the movement of molecules, and the gel's volume collapse is known to prevent or slow bioactive agents' diffusion through the network. In particular, polymers can be used to protect drugs from enzymatic and acidic degradation in the low pH of the stomach.[12,13] The release of a trapped drug is controllable through an increase or decrease in the degree of swelling of the polymer as a function of pH, which thus

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Contract grant sponsor: National Institutes of Health; contract grant number: 1R01 GM065917-01A1.

increases or decreases diffusion through the network in environments with different acidic strengths. A collapsed, shrunken gel at low pH protects a delivered agent in the acidic environment of the stomach, whereas a swollen gel at high pH allows for increased diffusion-controlled release at the physiological pH of the upper small intestine. Release also occurs as the edge of the polymer system experiences a local change in pH, which leads to the surface erosion of the gel. This technique has been previously investigated for the oral delivery of insulin and biologically active substances.[12,14–16] A further application of this material may be injectable delivery. A polymer that is not chemically crosslinked will remain in solution at a lower pH and at room temperature until it is injected into a body temperature of 37°C, where it will gel. Gelation would be followed by the degradation and release of the drug as the pH of the edges of the polymer increase. Increased swelling may be desirable for such a system because it would lead to quicker release of the active agent. A greater increase in swelling in response to a small change in pH would be a highly favorable feature,[10] particularly if the polymer could be injected in a soluble state at a physiologically safe pH of about 6.

The copolymerization of *N*-isopropylacrylamide (NIP) with acrylic acid (AAc) produces a pH/temperature-dependent polymer, with an increase in LCST observed around the pK_a value.[10] Small increases in the acid content for random NIP/AAc copolymers increase the LCST of the polymer above physiologically relevant temperatures for relevant pH values,[17,18] which places constraints on the degree of swelling that poly(*N*-isopropylacrylamide-*co*-acrylic acid) (pNIP AAc) can undergo for a given LCST response. It is, therefore, desirable to develop a material in which the swelling properties can be improved independently of the LCST. Maleic acid (MAc) is a diprotic acid that we copolymerized with NIP to obtain additional benefits over pNIP AAc for application in pH-sensitive drug delivery. Because each MAc acid group corresponds two —COOH groups, the use of MAc as a copolymer increases the amount of charge at a given location. LCST behavior largely corresponds to the deprotonation of the first —COOH group, where a single charge is found at each MAc location between uninterrupted NIP backbone lengths. The deprotonation of the second charged group increases charge density, which in turn, increases swelling without impacting LCST. The LCST transition, therefore, occurs at a lower pH value than the swelling transition. This decoupling allows the polymer to reach a stable LCST value around 37°C after the first pK_a value, even though it will still pass through the various stages of the swelling transition as the pH is increased through the second pK_a value.

For this study, we aimed to characterize the swelling and LCST response of poly(*N*-isopropylacrylamide-*co*-maleic acid) (pNIP MAc), a diprotic copolymer and compare it to pNIP AAc, a monoprotic polymer. It is advantageous to develop a system that is characterized by a greater degree of swelling for a desired LCST of about 37°C.

EXPERIMENTAL

Materials

All materials were reagent grade and were obtained from Aldrich & Co., St. Louis, MO unless otherwise noted. NIP was dissolved in *N*-hexane (10 g per 100 mL at 40°C) and then recrystallized at room temperature. AAc was purified by vacuum distillation (40°C/10 mmHg). MAc was used as received. Anhydrous tetrahydrofuran (THF) was used as received as the polymerization solvent. Azobis(2-methylpropionitrile) (AIBN) was recrystallized from methanol (1 g/20 mL) by dissolution at room temperature and precipitation at −20°C. THF and phosphate buffered solution (PBS) with pH values of approximately 7.4 were used as the solvents for static light-scattering (MiniDawn, Wyatt Technology Corp., Santa Barbara, CA)/high-performance liquid chromatography (HPLC; Shimadzu Corp., Columbia, MD) and multicell differential scanning calorimetry (DSC; Calorimetry Sciences Corp., Lindon, UT), respectively.

Synthesis and characterization of pNIP MAc and pNIP AAc

We performed the copolymerizations of NIP with MAc by varying the feed amounts of molar ratios of 95:5, 96:4, and 99:1. These polymers were synthesized in THF (10 wt %) with AIBN as the initiator (7×10^{-3} mol of AIBN/mol of monomer). Dry nitrogen was bubbled through each solution of about 100 mL before synthesis. Free-radical polymerizations were performed at 60°C for up to 16 h under a dry N_2 atmosphere and well-stirred conditions. The THF was decanted, and the solutions were filtered *in vacuo*. These purified solutions were dropped into 20× excess anhydrous ether; the precipitate was collected, vacuum-filtered, and vacuum-dried overnight. A similar procedure was followed for NIP with AAc in molar ratios of 98:2 and 92:8.

Titrations were completed with an automatic titrator obtained from SANDA Corp., Philadelphia, PA Solutions of pNIP MAc (95:5, 0.1 wt %) were dissolved in water. Titrations were run at 0.5 mL/min with 0.101*N* NaOH as the base. Further titrations were manually performed for pNIP MAc (99:1, 95:5, and 96:4) and pNIP AAc (98:2 and 92:8) with phenolphthalein as the indicator. These titrations were conducted

TABLE I
HPLC/Light-Scattering and Titration Characterization Results for the Free-Radical Polymerizations of pNIP MAc and pNIP AAc in THF

Polymer	Molar ratio	M_n (kg/mol)	M_w (kg/mol)	Acid content (%)
1% pNIP MAc	99:1	16 ± 5	12 ± 5	0.895
2% pNIP MAc	96:4	10 ± 2	13 ± 1	2.48
5% pNIP MAc a	95:5	15 ± 5	20 ± 7	4.48
5% pNIP MAc b	95:5	18 ± 1	25 ± 8	5.71
2% pNIP AAc	98:2	10 ± 1	13 ± 1	2.27
4% pNIP AAc	92:8	12 ± 1	17 ± 2	4.44

M_n = number-average molecular weight; M_w = weight-average molecular-weight. Standard deviations are reported from multiple sample runs; n=3.

with 1 wt % solutions of the polymer in water with 0.101*N* NaOH as the titrant. Static light scattering was performed in THF to determine the molecular weight, with three 30-μL scans run for each polymer sample.

DSC

Solution DSC scans were completed with 5 wt % solutions of 95:5 pNIP MAc in 0.1*M* PBS adjusted with 1.01*N* HCl and NaOH to obtain various pH values from 1.3 to 10.4. Three samples were run per scan; the samples were heated from 0 to 80°C with the reference ampule containing PBS. Additional DSC scans measured LCSTs for pNIP MAc (99:1 and 96:4) and pNIP AAc (98:2 and 92:8) for pH values of 2.7, 6.5, and 10.2 from 0 to 80°C. Peak locations were determined by the maximum vertical distance with reference to PBS baselines. Scans were run at 1°/min.

Swelling

Swelling studies were completed on pNIP MAc (99:1 and 96:4) and pNIP AAc (98:2 and 92:8). Each polymer (25 wt % solutions) was dissolved in PBS adjusted to pH values of 2.7, 6.5, and 10.2. Samples were placed in a water bath at 37°. Each sample was checked at equilibrium (~14 days). Qualitative swelling comparisons were based on previous work,[19] and their criteria are provided in the Results and Discussion.

RESULTS AND DISCUSSION

Characterization

The molecular weights and polymerization results are provided in Table I. Polymer weights ranged from 15 to 35 kg/mol. A large dispersity was observed in the raw molecular weight data because of high polydispersities and molecular weights as low as 2 kg/mol. However, high-molecular-weight polymer chains were also present, which allowed these polymers to form cohesive gels in the range of 10–20 wt % solutions. The acid contents were unexpected on the basis of the intended polymerization results. Molar ratios of NIP with MAc (99:1) and AAc (98:2) resulted in expected acid contents of 1 and 2%, respectively; however, the ratios of NIP with MAc (96:4) and AAc (92:8) resulted in one-half of the expected acid content (2 and 4%, respectively). Because MAc is diprotic, 1% pNIP MAc/2% pNIP AAc and 2% pNIP MAc/4% pNIP AAc both contained the same amount of charge; 2% pNIP MAc and 2% pNIP AAc were characterized by a similar distance between charges.

DSC behavior

A graph of pH versus LCST for two polymerizations of 5% pNIP MAc is provided in Figure 1. The pH–LCST responses of these two polymers are comparable, as would be expected with their similar compositions. As evident by the plateaus located above and below a gradual increase in LCST, pNIP MAc demonstrated pH-dependent LCST behavior. The LCST increase was observed around a pH of 4. Only one definite increase was observed, even though MAc possesses two pK_a values at 1.9 and 6.08.[20]

The lack of a double increase provided insight into the polymer composition. Sen's group concluded that a double increase was observed for experimental swelling behavior collected for pH values between 2 and 8,[20] as confirmed by modeling predictions.[21] However, the actual data from this group suggests that such a double increase was subtle, if present at all. Previous studies have reported a single property change increase in the cumulative release of terbin-

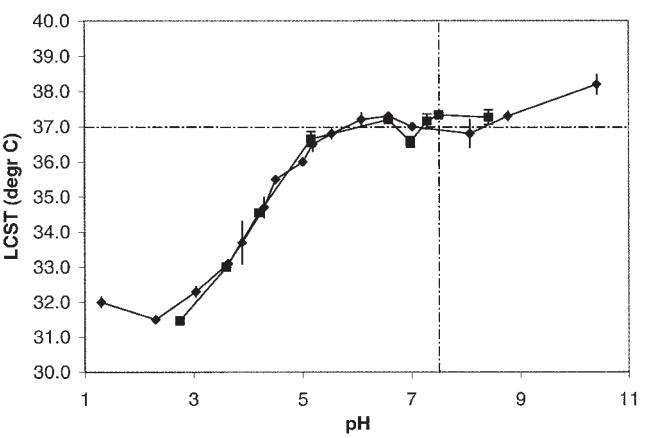


Figure 1 LCST characterization for the two polymerizations of 5 mol % pNIP MAc as a function of pH (5 wt % solutions in PBS adjusted by 1.01*N* NaOH/HCl for pH values of 1.3–10.4). Dashed lines indicate physiological temperature and pH. Scans were run at 1°/min from 0 to 80°C. Standard deviations are reported from multiple sample runs: n=3; (■) 5% pNIP MAc a and (♦) 5% pNIP MAc b.

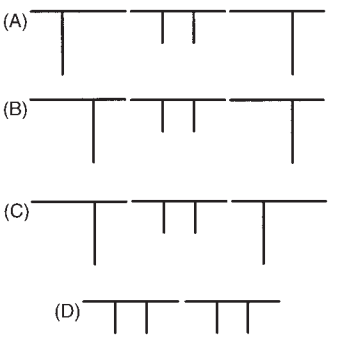


Figure 2 Propagation orientations possible for NIP with MAc resulting in a variance of pK_a along the polymer chain (NIP is indicate by a single leg, and MAc is indicated with two legs, each representing a charged group): (A–C) a single MAc with an oriented NIP monomer on either side and (D) two MAc monomers found side by side. Other combinations were possible as the number of monomer units under consideration increased.

afine hydrochloride,[22] invertase adsorption,[23] α-amylase adsorption,[24] and bovine serum albumin adsorption[25] by poly(acrylamide/maleic acid) gels for a pH range of 5–6, with data lacking for pH values lower than 2. A single property increase was unexpected because MAc possesses two acid groups on each molecule.

It is likely that the single observable transition for MAc copolymers was the result of random polymerization. It is known that random copolymers are not homogeneously distributed.[2] The location and amount of MAc along each NIP chain was variable; although the total acid content of a solution can be known, local amounts of acid (and thus charge) on a chain will vary, causing further variance in the thermal properties. In addition, individual charged groups on MAc encounter differing local environments depending on each —COOH group's proximity to other molecules. Figure 2 demonstrates various propagation orientations (greater variability is possible as more monomer units are considered). Each of the schemes presented in Figure 2 would be expected to result in individual pK_a values for each chain, and summed together, a diprotic random copolymer could exhibit heterogeneous pK_a values. This phenomenon became evident when 5% pNIP MAc was titrated (Fig. 3). Analysis of the titration curve indicated at least two pK_a values because there were multiple plateaus on the first derivative curve; however, the fact that the transitions were not obvious on the raw data suggests that the pK_a values were distributed for a range of values.

Modeling efforts reported by Sen's group concluded that as diprotic pK_a values approach each other, the swelling response with pH begins to look like that of a monoprotic acid. When the two pK_a values of MAc were modeled at values of 3.85 and 5.45 with acrylamide, a double increase in swelling disappeared completely.[21] Monomers with differing transition temperatures that are grafted onto a single backbone are already known to produce a copolymer with multiple LCSTs.[26] The lack of a double LCST increase in the experiments conducted for this study copolymers was likely due to the continuous, heterogeneous distribution observed for pK_a (and thus individual LCSTs) along the polymer chains. Such a phenomenon would explain to the muted increase observed on pH-dependent thermal data (Fig. 1) and the

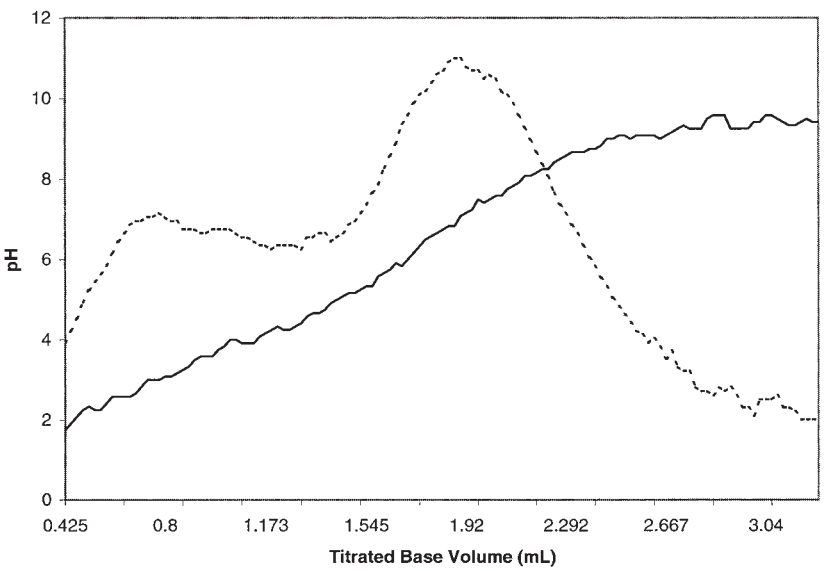


Figure 3 Sample titration results for a 0.1 wt % solution of 5 mol % pNIP MAc in H_2O. The titrant was 0.101*N* NaOH. The dashed line, indicating the first derivative of the raw data yielded two inflection points, indicating the presence of at least two pK_a's.

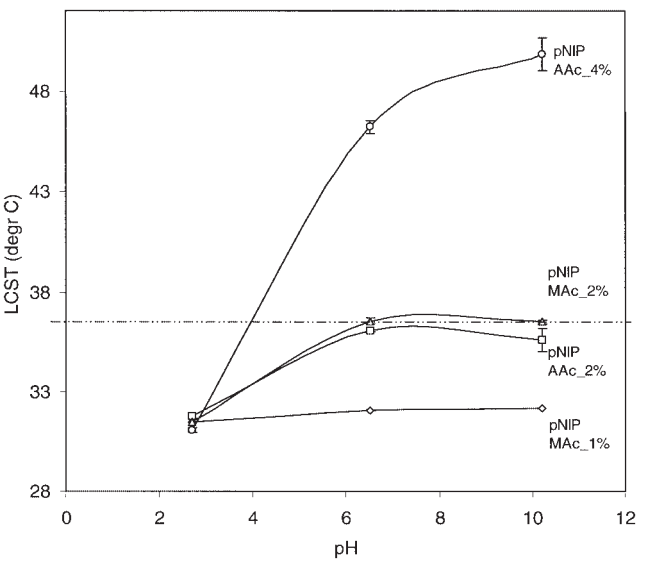


Figure 4 LCST data collected for varying pH values and acid contents of MAc and AAc copolymers of NIP. The solutions were 5 wt % solutions in PBS with pH values adjusted between 2.7 and 10.2 with 1.01*N* HCl and NaOH. Scans were run at 1°/min from 0 to 80°C. A dashed line indicates physiological temperature. Standard deviations are reported from multiple sample runs; n=3.

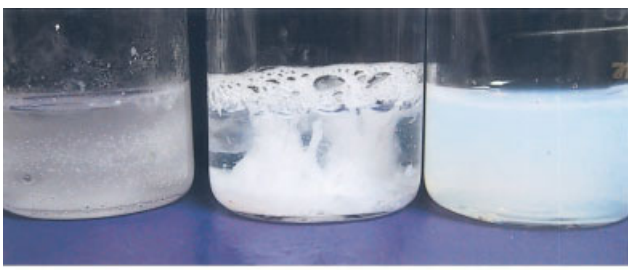
lack of double property increases present in the current literature for MAc copolymers.

In further studies, we tested the LCST response as a function of acid content. Acid composition relates to the location of charged groups along the polymer chain. A 1% AAc polymer and a 1% MAc polymer would both be characterized by similar distances between charged groups; however, the MAc polymer would have double the actual amount of charge. The final DSC data collected compared the pH response of copolymers of 1% pNIP MAc, 2% pNIP MAc, 2% pNIP AAc, and 4% pNIP AAc (Fig. 4) for low, medium, and high pH (2.7, 6.5, and 10.2, respectively). As expected, increasing acid content and pH yielded increasing LCSTs. Almost identical thermal transition behaviors were observed for samples of 2% pNIP AAc and 2% pNIP MAc. Both sets of polymers were characterized by the same distance between charged groups that resulted in the same LCST value for each pH. The increase in charge concentration seen for MAc as compared to AAc did not affect the LCST behavior.

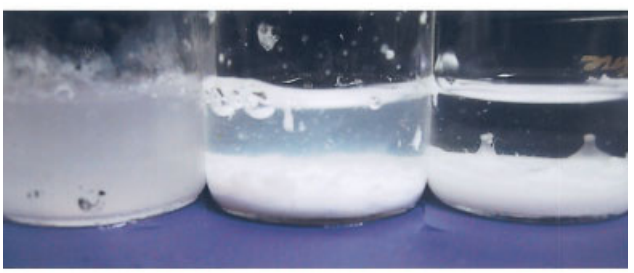
Swelling characteristics

Gels that shrink are known to expel increasing amounts of water as the temperature increases. Previous work has identified several phases of a polymer transition, including clear solution, opaque (milky) solution, and shrunken and swollen phases (which are both a two-phase state where the solution exists as a layer on top of a formed gel).[19] As the temperature is increased, polymer solutions have been observed to pass through all or some of these phases. The phase differences have been attributed to varying degrees of chain precipitation due to heterogeneous LCSTs for the individual polymer chains. The precipitation of a low concentration of polymer chains will yield an opaque, milky solution. As the number of precipitated aggregates increases, hydrophobic interactions cause the aggregates to form physical junctions. At sufficient concentrations, the solution will gel. For increasing molecular weight, chain entanglement increases, and the solution forms a more cohesive gel. Therefore, gelation may be indicated by a precipitated, cloudy state that slowly settles, even if a full gel is not physically formed.

The swelling results for pNIP MAc as compared to pNIP AAc were completed for acid contents of 1 and 2% and 2 and 4%), respectively. The phase of each polymer was determined by qualitative assessment. Criteria (Fig. 5) included opaque solution, viscous solution, and two-phase gel in varying states of swollen (more water and larger volume) and shrunken (less water and smaller volume) gels. Fully formed two-phase gels were characterized by a solid gel beneath a layer of clear solution. At low pH, the gels expelled greater amounts of water and were significantly re-



(a)



(b)

Figure 5 Solutions (25 wt %) of (a) pNIP MAc (2 mol %) and (b) pNIP AAc (2 mol %) in PBS, altered to pH values of 2.7, 6.5, and 10.2 (from left to right). The leftmost gels (pH = 2.7) existed in a shrunken gel phase with clear polymer clinging to the side of the vial. The middle gels (pH = 6.5) were both in the swollen gel phase with slightly cloudy solutions. The rightmost gel (a) (2% MAc, pH = 10.2) was in a viscous solution phase, whereas the rightmost gel (b) (2% AAc, pH = 10.2) was in a stage of moderate swelling.

	pH=2.7	pH=6.5	pH=10.2
pNIP MAc_1%	Shrunk Gel Clear Solution	Swollen Gel Clear Solution	Swollen Gel Clear Solution
pNIP AAc_2%	Shrunk Gel Clear Solution	Swollen Gel Clear Solution	Swollen Gel Clear Solution
pNIP MAc_2%	Shrunk Gel Clear Solution	Swollen Gel Clear Solution	Viscous
pNIP AAc_4%	Shrunk Gel Clear Solution	Swollen Gel* Clear Solution	Swollen Gel* Clear Solution

Figure 6 Qualitative phase judgments for 25 wt % gels in PBS swollen at 37°C to equilibrium. The pH values were adjusted to 2.7, 6.5, and 10.2 for pNIP MAc (1 and 2 mol %) and pNIP AAc (2 and 4 mol %). The increasing darkness of squares indicates increased swelling. *Although the LCST for this polymer was well above 37°C, it exhibited a very small amount of precipitated gel because a percentage of polymer chains had LCSTs at or below 37°C.

duced in volume, turning from opaque to clear in color. Such gels have been termed *shrunken*.[19] All instances of opaque solutions were observed to become two-phase gels after time passed, indicative of kinetic effects. The case of viscous solution did not precipitate or collect at the bottom of the vial for a time span of about 2 months. It was visually distinct from an opaque solution, with a translucent color that signified precipitated polymer chains, a lack of colloids or particles, and a qualitatively higher viscosity. The viscous solution that was found for a high pH of 2% pNIP MAc is promising for application purposes: it demonstrated infinite swelling. Any addition of water was completely incorporated into the matrix without passing through a two-phase state. The material remained homogeneous, swollen, and fluid, regardless of the amount of water added. Materials would degrade and release a drug significantly quicker in this case because, in a viscous state, the polymer chains are more mobile to facilitate diffusion and more accessible to degrade from the polymer matrix.

Shrunken gels were observed for all of the samples at low pH (2.7). The remainder of polymers formed two-phase states, with qualitative swelling increases observed for increasing acid content and pH (summarized in Fig. 6). The 2% pNIP MAc and 2% pNIP AAc samples demonstrated almost identical LCST behavior (Fig. 4); however, pNIP MAc exhibited greater swelling: the MAc polymer demonstrated behavior characteristic of infinite swelling, whereas the AAc existed in a two-phase sol–gel state. This swelling phenomenon is visually evident in Figure 5; for the same pH at the same temperature and for the same LCST value, 2% pNIP MAc existed as a viscous solution, and 2% pNIP AAc existed as a swollen gel.

The difference between the 2% pNIP MAc and 2% pNIP AAc samples was charge concentration. Chen and Hoffman demonstrated that for AAc grafted onto acrylamide backbones, it was the quantity of AAc (charge) on each graft location that largely governed swelling behavior.[17] It may be useful to think of a MAc as two AAc monomers on a single graft location. The double charge found with MAc localized hydrophilic interactions with the surrounding environment, leading to a greater uptake of water, and in at least one case, infinite swelling. Thus, a MAc copolymer can be characterized by the same LCST as an AAc copolymer and still swell to a greater extent. At a high pH, pNIP MAc and pNIP AAc had both passed through their LCST transitions; however, pNIP MAc continued to change swelling as pH increased because its second pK_a was at a higher pH value than the single pK_a of pNIP AAc (pH $\approx$ 4).[12] It was, therefore, possible to partly decouple LCST from swelling through the use of a diprotic acid copolymer.

Observations of independent LCST and swelling behavior provide insight into transition mechanisms. The LCST increase for pNIP MAc occurred at lower pH values, distributed around 4. This location corresponded to the deprotonation of almost all of the first acid groups; deprotonation of any more acid groups would not have impacted the average distance between charges, and LCST was, therefore, observed to plateau after a pH of about 6. In this range, LCST remained constant at physiological temperature. Further increases in pH led to the deprotonation of the second —COOH and only served to increase the charge concentration at a given location. Increased swelling was observed for pNIP MAc after a pH of 6, and this swelling could be improved and adjusted without affecting the LCST response. For the same LCST response, at the same pH, and at a temperature of 37°C, 2% pNIP MAc demonstrated significantly increased swelling over both 2% pNIP AAc and 4% pNIP AAc. These observations provided strong support for the theory that LCST behavior results largely from uninterrupted lengths of backbone and that swelling behavior results largely from actual charge concentration at a given location. We propose that pNIP MAc is a more suitable candidate for pH-sensitive drug delivery when increased diffusion controlled release and erosion is desired. This transition between LCST effect and swelling effect with pH occurred at a pH of about 6, which may be ideal for safe and effective pH-sensitive drug delivery.

CONCLUSIONS

The localization of charges seen for a diprotic acid caused an increase in hydrophilic interactions that, in turn, increase swelling. We demonstrated that multiple swelling stages were available for a single LCST; the LCST response could be partly decoupled from the swelling response through the use of a diprotic acid.

After a certain pH, increasing ion content only increased swelling without impacting the LCST, providing support for the theory that LCST is largely controlled by the average chain length between charges and the volume transition is largely controlled by ion concentration. For the same LCST response, pNIP MAc would deliver an active drug quicker than pNIP AAc and would be injectable at a higher (and thus safer) pH's than pNIP AAc. Although these results present pNIP MAc as a promising material for pH-tailored drug delivery (desirable over pNIP AAc) with a favorable LCST at physiological temperature, the use of a diprotic acid compared to a monoprotic acid also provides insight into hydrogel structure and behavior.

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