

# Thermally Programmable pH Buffers

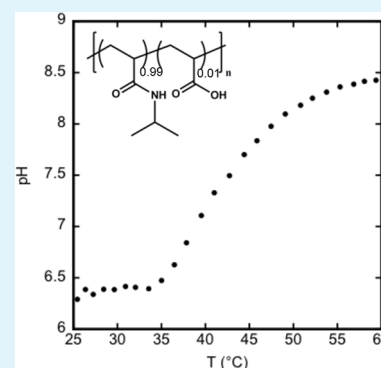
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## S Supporting Information

**ABSTRACT:** Many reactions in both chemistry and biology rely on the ability to precisely control and fix the solution concentrations of either protons or hydroxide ions. In this report, we describe the behavior of thermally programmable pH buffer systems based on the copolymerization of varying amounts of acrylic acid (AA) groups into *N*-isopropylacrylamide polymers. Because the copolymers undergo phase transitions upon heating and cooling, the local environment around the AA groups can be reversibly switched between hydrophobic and hydrophilic states affecting the ionization behavior of the acids. Results show that moderate temperature variations can be used to change the solution pH by two units. However, results also indicate that the nature of the transition and its impact on the pH values are highly dependent on the AA content and the degree of neutralization.

**KEYWORDS:** PNIPAM, buffer, programmable, thermally responsive, pH, copolymer



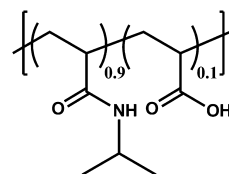
## INTRODUCTION

A wide range of reactions that occur in aqueous solution are heavily dependent on the concentrations of available protons or hydroxide ions. The ability to control the solution pH is required for many reactions in biochemistry, synthesis and processing, and colloid chemistry, as well as for a myriad of aqueous technological processes. Because many of the reactions of interest consume or release either protons or hydroxide ions, reagents that can buffer the solution pH to specific values are often required. When a given process or reaction only requires a single pH value, standard pH buffers are adequate. However, some processes require the ability to easily switch from one pH to another without introducing external acids or bases, for example, pH-triggered mineralizations in confined environments or small volumes<sup>1,2</sup> or programmable drug-delivery applications.<sup>3</sup> The ability to control the pH as a function of an external stimulus would be important, enabling the capability for applications such as these.

We have developed a thermally programmable buffer system based on the introduction of acidic groups into the thermally programmable polymer poly(*N*-isopropylacrylamide) (PNIPAM). At room temperature, PNIPAM is water-soluble, highly swollen, and hydrophilic. At the lower critical solution temperature (LCST) of approximately 30 °C, this polymer undergoes a phase transition to produce a polymer that collapses, is more hydrophobic, and becomes less water-soluble.<sup>4,5</sup> Previously, we utilized the PNIPAM phase transition for processes such as the reversible capture and release of proteins in microfluidic devices<sup>6</sup> and a PNIPAM-acrylic acid (AA) copolymer to modulate the electrolyte solution properties.<sup>7</sup> Other groups have explored the properties of linear

PNIPAM-AA copolymers<sup>8,9</sup> and microgels.<sup>10–13</sup> However, the focus of the present work is on the effect of the polymers on the solution properties. In this paper, we report on the properties of model thermally programmable pH buffers, that is, copolymers containing between 1 and 20 mol % AA in PNIPAM (Scheme 1). In the remainder of this paper, we will refer to this polymer system as PNIPAM-AA.

**Scheme 1. Chemical Structure of a Programmable pH Buffer PNIPAM-AA Comprised of 90 mol % NIPAM and 10 mol % AA**



The PNIPAM-AA buffer system relies on switching of the hydrophilic/hydrophobic character of the environment within which the carboxylic acid groups reside. Below the PNIPAM transition temperature, the polymer-bound AA groups reside in a water-rich, hydrophilic environment. In this environment, these AA groups exhibit acid-base properties that are similar to those exhibited by carboxylic acids dissolved in water, with a  $pK_a$  of around 5. The  $pK_a$  and pH are given by

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$$\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-; \quad K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}; \quad \text{p}K_a = -\log K_a \quad (1)$$

$$[\text{H}^-] = \frac{K_a[\text{HA}]}{[\text{A}^-]}; \quad \text{pH} = -\log[\text{H}^+] = -\log\left(\frac{K_a[\text{HA}]}{[\text{A}^-]}\right) \quad (2)$$

Here, HA represents a protonated AA group, A<sup>-</sup> is the deprotonated form, and K<sub>a</sub> is the acid dissociation constant. Above the thermally activated LCST phase transition, the polymer collapses, surrounding the AA component within a hydrophobic environment that suppresses dissociation of the protons from AA. This suppression is reflected in a dramatic increase in the pK<sub>a</sub> of the AA group.<sup>14</sup> On the basis of eq 2, it is evident that the solution pH is controlled by K<sub>a</sub> (pK<sub>a</sub>) and the number of acid sites present.

The properties of interest for programmable pH buffers include (1) the capacity of the buffer, which is controlled by the number of equivalents of polymer-bound acid groups that can be dissolved in, or exposed to, the solution, (2) the pH range buffered by the copolymer, and (3) the transition temperature at which switching occurs, which is controlled via the polymer composition.<sup>15,16</sup> In principle, it should be possible to create a programmable pH buffer to meet almost any application because it is known that the switching temperature,<sup>15,17</sup> the pK<sub>a</sub> range,<sup>11,18</sup> and the pK<sub>a</sub> midpoint can be controlled via the composition. In practice, optimizing the properties of the buffer can be somewhat challenging because compositional changes that mediate one property affect all properties.

In this paper, we report on the thermal and ionic properties associated with a series of model programmable pH buffers (Table 1). By varying the relative ratios of the protonated and

**Table 1. Physical Properties of PNIPAM-AA Copolymer Buffers**

copolymer	M <sub>n</sub> (g/mol)	target AA content (mol %)	PDI <sup>a</sup>	measured AA content (mol %)
PNIPAM-1AA	2.34 × 10 <sup>4</sup>	1	2.1	1.5
PNIPAM-10AA	3.23 × 10 <sup>4</sup>	10	2.5	9
PNIPAM-20AA	5.81 × 10 <sup>4</sup>	20	1.7	17.3

<sup>a</sup>PDI = polydispersity index.

anionic forms of the AA groups (i.e., [HA] and [A<sup>-</sup>]), we demonstrate how different polymer properties vary as a function of the polymer composition and/or buffer capacity. The results suggest that thermally programmable pH buffers allow for significant and reversible programming of the solution pH.

## EXPERIMENTAL SECTION

**Materials.** N-Isopropylacrylamide (NIPAM), acrylic acid (AA), *n*-butyl acrylate (nBA), tetrahydrofuran (THF), hexanes, methanol, and α,α'-azoisobutyronitrile (AIBN) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) was purchased from Fisher Scientific.

**Polymer Synthesis.** The copolymers studied in this work were prepared using a standard thermally initiated radical polymerization. AA and nBA were vacuum distilled, and NIPAM was recrystallized from hexanes. The monomers were dissolved in inhibitor-free THF at the appropriate mole ratios with AIBN. In a typical synthesis, a copolymer comprised 10 mol % AA and 90 mol % NIPAM utilized 90

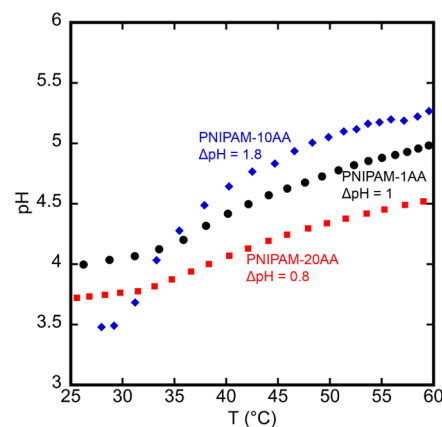
mg of AA, 1280 mg of NIPAM, and 18.8 mg of AIBN in 60 mL of THF. This solution was degassed using five freeze-pump-thaw cycles. Polymerization was performed at 70 °C with vigorous stirring under a N<sub>2</sub> atmosphere and allowed to proceed overnight. The solvent was removed by vacuum, and the products were dissolved in methanol. A solvent extraction was then performed with hexanes three times. The polymer product was then dried by rotary evaporation and left under vacuum at 60 °C overnight to remove remnant methanol.

**Polymer Characterization.** The approximate molecular weights of the polymers were determined against polystyrene standards using gel permeation chromatography in *N,N*-dimethylformamide. The polymer composition was verified by Fourier transform infrared (FTIR; Varian 2000; see Supporting Information, Figure S1), and the acid content was verified by titration against 0.1 M NaOH. Aqueous solutions (1% by weight) were prepared for each polymer in order to study the transition temperature and pH response to the temperature. The transition temperature of each solution was determined as a function of AA neutralization; 0, 1, 10, 50, 70, and 100% neutralizations (determined at room temperature) of the AA groups were measured in a melting point apparatus. The pH response to the temperature of each polymer was evaluated at 0 and 10% AA neutralization using a Radiometer Analytical TitrLab 856 Stat Titration Workstation equipped with a combination red rod electrode (PHC2001) and a temperature sensor (T201). The pH meter was calibrated using standard IUPAC buffer solutions of pH 4, 7, and 10 (Hach Chemical Co.). The heating rate for the measurements presented in this manuscript was ca. 5 °C/min; no change in the thermal response of the copolymers was observed at heating rates down to 1 °C/min.

## RESULTS AND DISCUSSION

The buffering capacities of the copolymers depend on the number of AA groups present in solution (eq 2). Thermally programmable pH buffers based on NIPAM-AA copolymers were synthesized with 1, 10, and 20 mol % AA groups and with the incorporation of hydrophobic monomers by thermally initiated radical polymerization. Table 1 shows the compositions, molecular weights, and measured AA contents of the copolymers studied. The molecular weights (M<sub>n</sub>) are reported as polystyrene-equivalent molecular weights. The acid content of each polymer was determined by titration against NaOH at room temperature.

Figure 1 shows the pH response as a function of the temperature for the copolymers with varying AA contents between 1 and 20 mol %. The copolymers provide thermally programmable buffering over a pH range of ca. 3.5 to 5.5 within

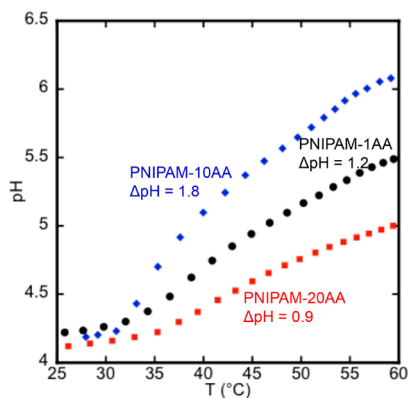


**Figure 1.** Increase in the pH as a function of the temperature for 1 wt % solutions of PNIPAM-1AA (1 mol % AA), PNIPAM-10AA (10 mol % AA), and PNIPAM-20AA (20 mol % AA).

a 35 °C window. Neither homopolymer, PNIPAM nor PAA, displayed a significant change in the pH with temperature (not shown). The pH of each copolymer studied remains fairly constant below the transition temperature because the acid groups reside in aqueous conditions at these temperatures. In this environment, the AA groups are expected to have  $pK_a$ 's similar to those of carboxylic acids in water;<sup>14</sup> see Supporting Information, Figure S2. The phase transitions for the three copolymers were observed between 30 and 35 °C. Above the transition temperature, PNIPAM becomes hydrophobic and precipitates, leading to the sequestration of acid groups from the water, where the more hydrophobic environment suppresses acid dissociation. This results in an increase in the  $pK_a$  and a corresponding increase in the solution pH.<sup>14</sup>

As stated above, the maximum potential change in the pH is limited by the concentration of AA in solution (between 1 and 20 mM in a 1 wt % copolymer solution). PNIPAM-1AA displays a pH change from ca. 4 to 5 over the 35 °C range studied. PNIPAM-10AA, however, shows a more significant change in the pH, from 3.5 to ca. 5.3, with most of the pH change occurring right above the transition temperature between 30 and 45 °C. The initial pH values of PNIPAM-1AA and PNIPAM-10AA agree with the calculated initial pH values of ca. 4 and 3.5, respectively (eq 2). PNIPAM-20AA buffers the solution pH between 3.7 and 4.5, which deviates from the expected trend of increasing pH change with increasing acid content. Though the mechanisms behind this deviation are not entirely clear, the effect is likely attributable to the more hydrophobic nature of protonated acid groups compared to dissociated acid groups. The protonated acid groups decrease the solubility of the copolymer in water (ca. 93.5% of the AA groups are protonated in PNIPAM-20AA; this is calculated with eq 2 assuming a  $pK_a$  of 5) similar to the effect seen with copolymerization with nBA (Figure 4). This increased hydrophobicity would certainly affect proton diffusion and polymer accessibility. Importantly, however, these data illustrate the compositional dependence of the buffering capacity in these copolymer systems. Increasing the AA content is effective only at lower AA concentrations, when the hydrophobic effects of the protonated AA groups do not dramatically affect the buffering function of the copolymer.

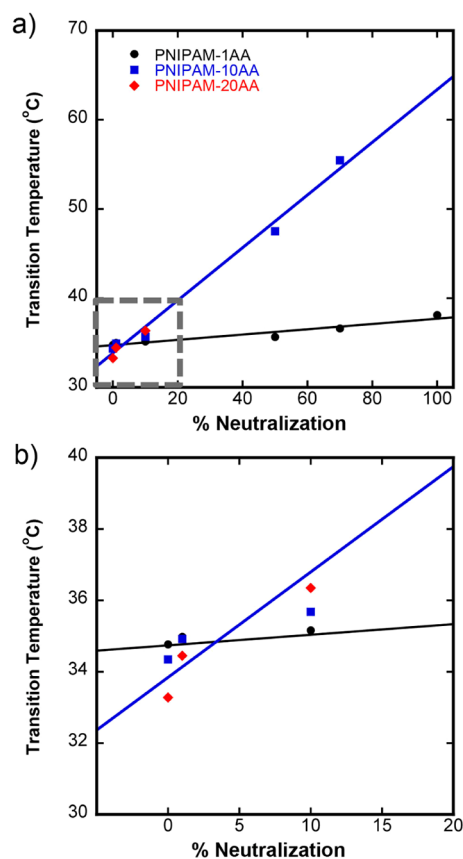
The addition of NaOH to neutralize 10% of the AA groups raises the pH range buffered by the copolymers (Figure 2). The



**Figure 2.** Increase in the pH as a function of the temperature for 1 wt % solutions of PNIPAM-1AA (1 mol % AA), PNIPAM-10AA (10 mol % AA), and PNIPAM-20AA (20 mol % AA) at 10% acid neutralization.

degree of neutralization is determined from the acid content of each copolymer. Below the transition temperature, the pH values for all three copolymers are ca. 4.1, as predicted by eq 2. At 10% neutralization, PNIPAM-1AA undergoes a slightly increased pH change with temperature (from 1 to 1.2 pH units) despite a lower  $pK_a$  change (see the Supporting Information, Figure S3). PNIPAM-10AA was able to buffer over a window of ca. 1.8 pH units, while the accessible pH window shifted to more basic pH values, between ca. 4.3 and 6.1. Notably, the low-temperature pH of PNIPAM-20AA agreed with the values of the other two copolymers at 10% neutralization. This is attributed to the increased solubility of the copolymer due to the higher concentration of neutralized acid groups. However, the extent to which the pH was changed with temperature was still limited to less than 1 pH unit.

The transition temperature of each copolymer is dependent on the degree of ionization (Figure 3). A melting point



**Figure 3.** (a) Variation of the transition temperature for PNIPAM-1AA (1 mol % AA), PNIPAM-10AA (10 mol % AA), and PNIPAM-20AA (20 mol % AA) plotted as a function of the percent neutralization in 1 wt % aqueous solution. (b) Magnified view of the boxed region in part a. Note: Transition temperatures were not determined for PNIPAM-10AA at 100% AA neutralization and PNIPAM-20AA above 10% neutralization because the water boiled before the complete light-scattering curve could be obtained.

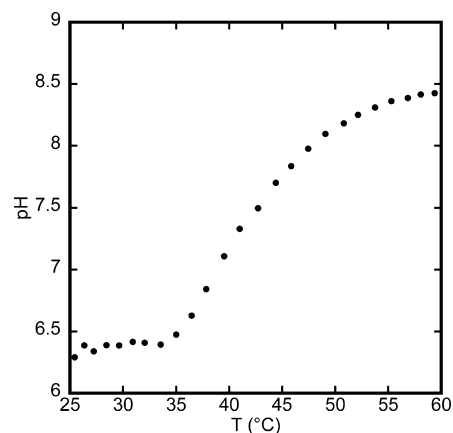
apparatus was used to further investigate the effect of AA neutralization on the copolymer transition temperature. Figure 3 plots the transition temperature (cloud point) of each copolymer as a function of the percent neutralization determined from the transition midpoint. At 0% neutralization, PNIPAM-1AA has the highest transition temperature, followed by PNIPAM-10AA and then PNIPAM-20AA. This trend is

consistent with the expected decrease in the transition temperature with increasing AA content at low pH. When 10% of the AA groups are neutralized by the addition of NaOH, the trend is reversed because deprotonated carboxylic acid groups are known to be extremely hydrophilic and to increase the transition temperature of the copolymers.<sup>15</sup> Above 10% neutralization, the transition temperature was indeterminate for PNIPAM-20AA, while a linear increase in the transition temperatures of PNIPAM-1AA and PNIPAM-10AA was observed. This linear increase in the transition temperature with increasing negative charge allows us to use the degree of neutralization to control the transition temperature.

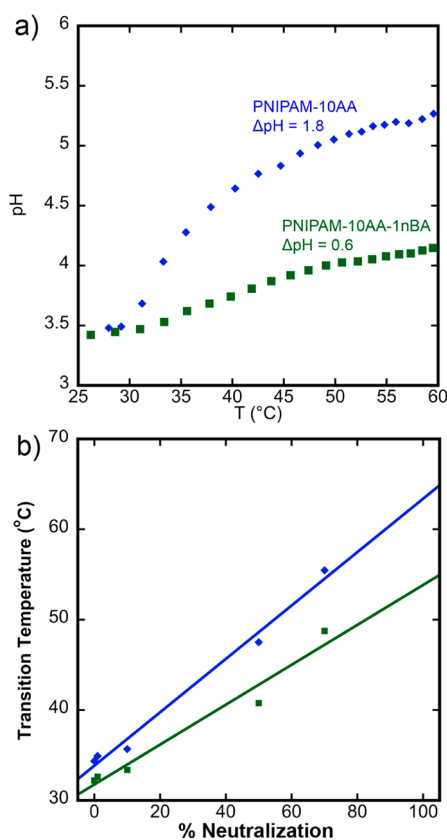
Previously, the addition of hydrophobic groups into PNIPAM was shown to modify the transition temperature.<sup>16,17</sup> The addition of 1% nBA to a copolymer containing 10 mol % AA (PNIPAM-10AA-1nBA) was expected to reduce the transition temperature of the copolymer while maintaining a wide pH window. The transition temperature as a function of the acid neutralization is noticeably suppressed with the incorporation of only 1 mol % nBA (Figure 4). However, PNIPAM-10AA-1nBA displays a reduced change in the pH with the temperature (from ca. 3.5 to 4.2) compared to PNIPAM-10AA (ca. 3.5 to 5.3).

Programming the solution pH around neutral at temperatures ranging from 25 to 60 °C is attractive for biological applications. It is apparent in Figure 3 that no significant change in the transition temperature of PNIPAM-1AA was

observed up to 100% neutralization of acid groups. Combined with the slight increase in the accessible range over which the pH can be controlled with temperature through acid neutralization, these results led us to hypothesize that this copolymer may display buffering ability at elevated pH values over biologically relevant temperatures. Figure 5 shows the pH



**Figure 5.** pH response of PNIPAM-1AA (1 mol % AA) as a function of the temperature with the initial pH adjusted to 6.3 in 1 wt % aqueous solution.



**Figure 4.** (a) pH response of PNIPAM-10AA (10 mol % AA) and PNIPAM-10AA-1nBA (10 mol % AA and 1 mol % nBA) in 1 wt % aqueous solutions. (b) Transition temperatures for PNIPAM-10AA and PNIPAM-10AA-1nBA plotted as a function of the percent neutralization. Note: Transition temperatures were indeterminate for both copolymers at 100% AA neutralization.

versus temperature response of PNIPAM-1AA with the initial solution pH adjusted to 6.3. By comparison, the higher acid content copolymers showed a significantly less dramatic pH change with temperature, less than 0.5 pH units, and no clear transition was observed.

## CONCLUSIONS

In this work, we showed the pH response with temperature for a series of model thermally programmable pH buffers comprised of random copolymers containing AA and NIPAM in aqueous solution. The buffer capacity and pH window accessible by the copolymer buffers was directly impacted by the AA content. PNIPAM-10AA (10 mol % AA) was found to buffer the pH over a wider range of concentrations than copolymers containing 1 and 20 mol % AA, while the transition temperature of the copolymers decreased with increasing AA content. The transition temperature of the copolymers increased with the amount of neutralized groups present. Additionally, the introduction of a hydrophobic monomer (nBA) was found to decrease the transition temperature. The combination of the AA content, neutralization, and addition of hydrophobic monomers may provide a strategy for turning the temperature and pH ranges for programmable pH buffers. The effectiveness of thermally programmable pH buffers for mineralization and condensation and the effect of multivalent salts is currently under study.

## ASSOCIATED CONTENT

### Supporting Information

FTIR spectra for the copolymer samples, plots of the effect of temperature on  $pK_a$  for PNIPAM-1AA, PNIPAM-10AA, and PNIPAM-20AA in water and with 10% AA groups neutralized, and the effect of acid neutralization on  $pK_a$ . This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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