Engineering a Sharp Physiological Transition State for Poly(*n*-isopropylacrylamide) Through Structural Control

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ABSTRACT: Poly(*N*-isopropylacrylamide) (pNIPAAm), a well-studied, biologically inert polymer that undergoes a sharp aqueous thermal transition at 32 °C, has been a subject of widespread interest for possible biological applications. A major hindrance to its successful application is due to the difficulty of maintaining a sharp transition when the polymer is modified for a physiological transition temperature, especially in isotonic solutions. Current copolymer blends raise the transition temperature but also make the transition significantly broader. We have combined the use of reversible addition-fragmentation chain transfer (RAFT) polymerization with tacticity control to synthesize well-defined pNIPAAm that demonstrates sharp transitions under physiological conditions. By selecting a RAFT agent with appropriate end groups, controlling molecular weight, and increasing the racemo diad content, we were able to increase the thermal transition temperature of pure pNIPAAm to a sharp transition at 37.6 °C under isotonic conditions. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 50: 976–985, 2012

KEYWORDS: reversible addition fragmentation chain transfer (RAFT); stimuli-sensitive polymers; structure-property relations; syndiotactic

INTRODUCTION New developments in biomedical diagnostics, theranostics, and sensing applications increasingly rely upon "smart" materials, materials which have properties that can be triggered to change upon exposure to an external stimulus. One of the most well-studied polymers in this class is poly(*N*-isopropylacrylamide) (pNIPAAm), a biologically inert polymer[1] that exhibits an aqueous lower critical solution temperature (LCST) of 32 °C.[2] Because of this well-defined transition, pNIPAAm has been extensively studied by many groups for applications in biotechnology, ranging from protein purification[3] to drug delivery[4,5] to biosensing.[6] One of the many requirements for successful application in biological systems is an LCST at physiological or higher temperatures.[3,5,7,8] For instance, although current technology enables the use of pNIPAAm systems in protein purification from acellular systems,[3] increasing the LCST to physiological temperatures would make it possible to use pNIPAAm to extract hydrophilic proteins in the context of a continuing cell culture without extreme stresses on the cells. In addition, pNIPAAm-based controlled drug delivery systems also require LCSTs at least as high as physiological temperatures to use these properties as drug release triggers. Because these applications must operate within very narrow temperature ranges, the ability to manipulate the LCST to higher temperatures without sacrificing the sharpness of the transition is essential. Although the current trend in research on pNIPAAm-based thermoresponsive polymers lies in the preparation of amphiphilic random, block, graft, or star-shaped copolymers for biomedical applications, through a combination of simple LCST modification techniques, we have synthesized a pure pNIPAAm that can be manipulated to an LCST at physiological temperature under isotonic conditions. These polymers, and the ease with which the transition temperature can be modified using these synthesis techniques we describe, present a novel strategy for the formation of polymer systems with highly homogeneous properties that can be used in applications in biotechnology.

The LCST of pNIPAAm is influenced by a variety of factors, and there are several methods used to modify it. The most common is to copolymerize with a small amount of hydrophilic comonomer. Although several copolymer blends exist to raise the LCST with sharp transitions,[9,10] some of the most popular blends for biological applications such as acrylic acid (AAc) have a widening effect on the LCST. Copolymerization with AAc has the effect of widening the LCST from a transition that occurs over <0.5 °C to a transition that takes place over a range of 5–10 °C or even larger depending on the desired LCST.[2,7,11] This increase in transition range is due to the inhibition of water exclusion and is less than ideal for any application requiring a sharply defined response.[12] Nevertheless, copolymerizing as a

Additional Supporting Information may be found in the online version of this article.

method of raising LCST is widely used, and prior research has examined the use of comonomers precisely designed to change the LCST under certain conditions.[13] This concept has recently extended into attaching specific end groups to the polymer, which can significantly affect lower molecular weight (MW) pNIPAAm although its utility is inversely proportional to the MW.[14]

Another method of modifying the LCST is through changing the polymer architecture. Branching, for example, has been shown to lower the LCST of pNIPAAm, whereas stereospecific polymerization and control over the tacticity of the polymer have been shown to increase or decrease the LCST, depending on whether the polymer is syndiotactic or isotactic.[15–22] For the purposes of raising the LCST, selectively polymerizing in the racemo conformation can be a useful tool.[19] This method modifies the rotational energy required to orient the polymer such that it undergoes the cooperative dehydration that is observed macroscopically as the LCST.[23,24] pNIPAAm with a majority of meso diads exhibits a lower LCST, whereas pNIPAAm with a majority of racemo diads exhibits a higher LCST.[19,21,22] The difference in LCST seen using this method is usually on the order of 3–5 °C, a significant but limited enhancement.

In addition to the intrinsic properties of the polymer, solvent properties play a large role in the observed transition temperature. Certain cosolvents such as methanol can significantly reduce the LCST,[25] whereas pH can have a mild effect[11] and salts can have a large effect on the LCST.[26] This is especially important in biological applications because biological systems require certain osmolarity and salt concentrations, without which cells will undergo apoptosis. The cumulative result of these LCST-depressing effects renders the polymer all but useless for biological applications in its native form.

Secondary to the issue of LCST manipulation is the need for well-defined polymers, a problem that is easily rectified using a living radical polymerization scheme, in this case, reversible addition-fragmentation chain transfer (RAFT) polymerization. RAFT polymerization controls the polydispersity index (PDI, defined as the weight-average MW, M_w, divided by the number-average MW, M_n) of a polymer by introducing a chain transfer agent (CTA).[27,28] The CTA is reversibly reactive toward growing polymer chains and forms a dynamic equilibrium between the actively growing chains and the dormant chains. This reduces the number of actively polymerizing chains and in effect increases the polymerization time.[29] The CTA chosen for this study was *S*,*S*′-bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (**1**), a well-documented symmetric CTA[30–35] that has been previously shown to be very versatile and produce good results with pNIPAAm.[31,34,35] By incorporating RAFT polymerization along with the LCST manipulation principles outlined above, we have successfully synthesized well-defined pNIPAAm with a sharp LCST of 37.6 °C in phosphate-buffered saline (PBS) without the use of copolymerization, thereby introducing another method to optimize pNIPAAm synthesis for biological applications. Furthermore, the incorporation of this living process allows for additional chain extension polymerization, which can be used in the synthesis of hydrogels or for the addition of functionalities through subsequent polymerizations and conjugations.

EXPERIMENTAL

N-Isopropylacrylamide was purchased from TCI America and recrystallized in a 9:1 ratio of hexanes:benzene. Carbon disulfide, tetrabutylammonium hydrogen sulfate, mineral spirits, 1,4 dioxane, Aliquat 336, and 3-methyl-3-pentanol (3Me3PenOH) were purchased from Sigma Aldrich and used without further purification. Chloroform and acetone were purchased from BDH Chemicals and used without further purification.

S,*S*′-Bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (1) Synthesis

Synthesis of **1** was done similarly to the procedure set forth by Lai et al.[30] A total of 6.62 mL (0.1 mol) acetone was reacted with 7.26 mL (0.1 mol) chloroform, 2.16 mL (0.04 mol) carbon disulfide, and 0.241 g (0.7 mmol) tetrabutylammonium hydrogen sulfate in 12 mL of mineral spirits. The reaction mixture was purged with nitrogen for 5 min and run in a water bath at room temperature. Ten milliliters of 50% NaOH was added dropwise over 90 min and the reaction was left to run overnight. Ninety milliliters of water was then added, followed by 42 mL of 6 N HCl. The reaction mixture was then purged under nitrogen for half an hour and filtered. The resulting product was recrystallized in acetone to yield 4 g of product. Synthesis of **1** was confirmed by electrospray mass spectrometry (see Supporting Information).

Polymerization

Polymerization of NIPAAm was carried out under six different conditions. High transition temperature pNIPAAm was synthesized using a "temperature shock" treatment in which the reaction was thermally initiated at 65 °C for 1 h and immediately placed into a room temperature bath to react at room temperature for the rest of the polymerization time, typically 95 h. The purpose of this method was to slow the reaction kinetics to allow for better tacticity control. It also served as a way to control for MW. Typically, a 3.2 g mixture of 100:1:0.5 ratio of NIPAAm:**1**:azobisisobutyronitrile (AIBN) was placed in a sealed 25-mL round-bottom flask equipped with a magnetic stir bar. The mixture was purged with nitrogen for 10 min and 20 mL of nitrogen-purged 1,4 dioxane was added. The solution was reacted at 65 °C for 1 h and at room temperature for 95 h.

To test the effects of majority of racemo diads, 3Me3PenOH was added to the reaction mixture. Accordingly, a 3.2 g mixture of 100:1:0.5 ratio of NIPAAm:**1**:AIBN was placed in a sealed 50-mL round-bottom flask equipped with a magnetic stir bar. A total of 6.7 mL of 3Me3PenOH was added to the reaction mixture. The mixture was purged with nitrogen for 10 min and 20 mL of nitrogen-purged 1,4 dioxane was added. The solution was reacted at 65 °C for 1 or 1.5 h to

initiate polymerization and at room temperature for up to 95 h thereafter.

Control polymers were synthesized using typical RAFT polymerization techniques with **1**. Briefly, a 3.2 g mixture of 100:1:0.5 ratio of NIPAAm:**1**:AIBN was placed in a sealed 25-mL round-bottom flask equipped with a magnetic stir bar. A total of 6.7 mL of 3Me3PenOH was added to the reaction mixture for a control polymer with majority of racemo diads, whereas this step was omitted for the atactic polymer control. The mixture was purged with nitrogen for 10 min and 20 mL of nitrogen-purged 1,4 dioxane was added. The solution was reacted at 65 °C for 48 h.

For radical-polymerized pNIPAAm controls, a 3.2 g mixture of 100:1 ratio of NIPAAm:AIBN was placed in a sealed 25-mL round-bottom flask equipped with a magnetic stir bar. The mixture was purged with nitrogen for 10 min and 20 mL of nitrogen-purged 1,4 dioxane was added. The solution was reacted at 65 °C for 48 h.

A "temperature shock" radical polymerization control experiment was also conducted in which a 3.2 g mixture of 100:1 ratio of NIPAAm:AIBN was placed in a sealed 25-mL round-bottom flask equipped with a magnetic stir bar. The mixture was purged with nitrogen for 10 min and 20 mL of nitrogen-purged 1,4 dioxane was added. The solution was reacted at 65 °C for 1 h as a thermal initiation or temperature shock and then removed from the heat to react at room temperature for 95 h.

A copolymer with 4% AAc, pNIPAAm-*co*-AAc, was also synthesized to compare with the results. This was done by reacting 1.5 g of NIPAAm with 37.9 μL of AAc and 2.18 g of AIBN in a sealed 25-mL round-bottom flask equipped with a magnetic stir bar. The mixture was purged with nitrogen for 10 min and 20 mL of nitrogen-purged 1,4 dioxane was added. The solution was reacted for various lengths of time at 65 °C. An 8% AAc copolymer was also synthesized using the same method and incorporated 75.8 μL of AAc.

Upon completion of reactions, all pNIPAAm samples were precipitated in anhydrous diethyl ether and collected via filtration. The samples were then dissolved in nanopure water and dialyzed with a 2000 MWCO membrane. The water was changed at 1 h, 3 h, and overnight. The samples were then frozen and lyophilized. pNIPAAm and majority of syndiotactic pNIPAAm polymerized by this method are hereafter denoted as pNIPAAm-**1** and pNIPAAm-**1**s, respectively.

Characterization

Polymers were characterized using gel permeation chromatography (GPC), NMR, matrix-assisted laser desorption ionization mass spectrometry (MALDI), and ultraviolet (UV)–visible spectrometry. GPC was conducted on a PL-GPC 50 with UV, refractive index (RI), and evaporative light scattering (ELS) detectors (Agilent) equipped with two Plgel 3-μm MIXED-E columns. Filtered stabilized tetrahydrofuran was used as the polymer solvent and eluent at a flow rate of 1 mL/min. Chromatograms were compared with those of polystyrene standards (Agilent). ^{1}H NMR was conducted on a Varian Mercury Vx 400 spectrometer using DMSO-d_6 as a solvent at 90 °C. The high temperature was used to resolve the methine backbone peaks.[18,36,37] Mass spectrometry was run on an Applied Biosystems 4700 Proteomics Analyzer with a 200 Hz Nd:YAG laser using CHCA matrix and reflecting detector. UV–vis spectrometry was conducted using a Cary 50 UV–Vis Spectrophotometer (Agilent) with the single cell Peltier thermostatted cell holder and accessory for temperature control. Temperature was ramped at a rate of 0.5 °C/min and data points were taken every 0.1 °C.

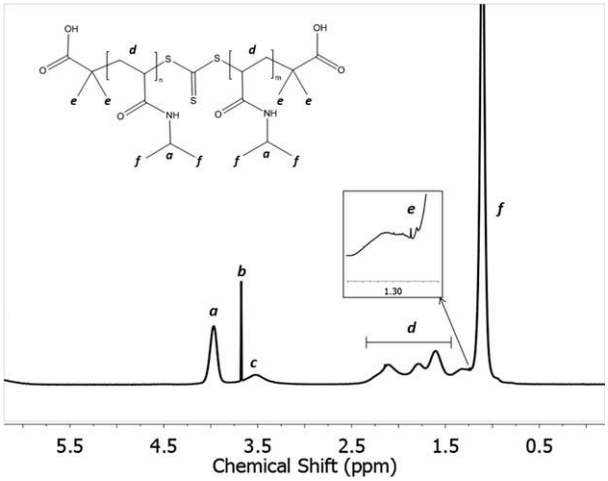

FIGURE 1 H[1] NMR spectra of pNIPAAm synthesized using **1** in chloroform-*d*. This polymer was synthesized at 65 °C for 48 h (pNIPAAm-**1**-HT; GPC M_n = 7541, PDI = 1.23). Peaks ***b*** and ***c*** correspond to residual solvent peaks of 1,4 dioxane and water, respectively. The peaks shown in ***d*** correspond to polymer backbone peaks in various configurations.

RESULTS AND DISCUSSION

MW Control

pNIPAAm was synthesized under various reaction times at room temperature. The initial reaction temperature of all polymers was 65 °C to thermally initiate the reactions. This was maintained for 1 h, and the reactions were then placed in room temperature baths to slowly polymerize over the course of 7 days. This "temperature shock" treatment was used to form well-controlled low-molecular-weight pNIPAAm. The primary goal of using this method, rather than control using feed concentrations, is to allow for better tacticity control because it has been previously shown that reducing polymerization temperature increases the efficacy of bulky alcohols and Lewis bases as syndiotacticity-inducing agents.[18,38] pNIPAAm synthesis was confirmed using ^{1}H NMR as shown in Figure 1.

GPC was conducted on pNIPAAm-**1** at various reaction times using a RI detector; traces are shown in Figure 2. Clearly, the MW continues to increase with time, indicating continued polymerization after reaching room temperature. The MWs and PDIs for the polymers are shown in Table 1. As expected for RAFT polymerization, the PDIs exhibited by the polymers are low, on the order of 1.1. This indicates good

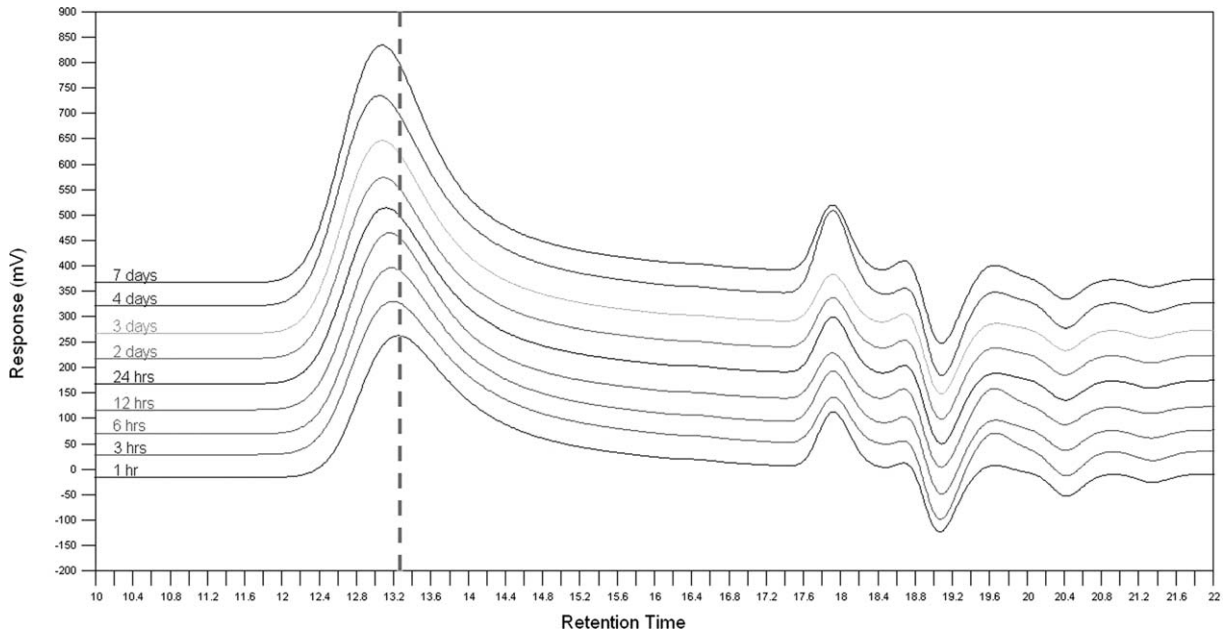

FIGURE 2 pNIPAAm-**1** polymerized for various lengths of time using a 100:1:0.5 ratio of NIPAAm:**1**:AIBN. The time indicated is the total reaction time with 1 h signifying a reaction for 1 h at 65 °C, 3 h signifying reaction for 1 h at 65 °C, 2 h at room temperature, and so forth. As seen in the figure, molecular weight increases with increasing time with the dotted blue line representing the peak retention time for 1 h of polymerization.

TABLE 1 The Molecular Weights and PDIs for the Polymers

	Feed Molar Ratio (NIPAAm:**1**:AIBN)	Total Polymerization Time (h)	M_n (NMR)[a]	M_w (GPC)	M_n (GPC)	PDI[b]	Conversion (%)
pNIPAAm-**1**-1h	100:1:0.5	1	3,194	3,825	3,483	1.10	55.2
pNIPAAm-**1**-3h	100:1:0.5	3	3,316	4,005	3,665	1.09	57.3
pNIPAAm-**1**-6h	100:1:0.5	6	3,597	4,014	3,655	1.10	62.1
pNIPAAm-**1**-12h	100:1:0.5	12	3,815	4,128	3,686	1.12	65.9
pNIPAAm-**1**-1d	100:1:0.5	24	3,974	4,157	3,776	1.10	68.6
pNIPAAm-**1**-2d	100:1:0.5	48	3,981	4,219	3,838	1.10	68.8
pNIPAAm-**1**-3d	100:1:0.5	72	4,122	4,251	3,866	1.10	71.2
pNIPAAm-**1**-4d	100:1:0.5	96	4,127	4,288	3,885	1.10	71.3
pNIPAAm-**1**-7d	100:1:0.5	168	4,150	4,328	3,961	1.09	71.7
pNIPAAm-**r**-1h	100:0:1	1	10,699	11,333	6,902	1.64	92.4
pNIPAAm-**r**-3h	100:0:1	3	10,732	14,533	9,615	1.51	92.7
pNIPAAm-**r**-6h	100:0:1	6	10,350	15,277	9,231	1.66	89.4
pNIPAAm-**r**-12h	100:0:1	12	10,330	16,333	9,242	1.77	89.2
pNIPAAm-**r**-1d	100:0:1	24	10,668	16,555	9,372	1.77	92.1
pNIPAAm-**r**-2d	100:0:1	48	10,466	19,840	13,231	1.50	90.4
pNIPAAm-**r**-3d	100:0:1	72	9,958	19,752	13,188	1.50	86.0
pNIPAAm-**r**-7d	100:0:1	168	10,946	7,402	4,743	1.56	94.5
pNIPAAm-**1**-HT	100:1:0.5	48	5,762	9,338	7,541	1.23	97.0

pNIPAAm was polymerized for varying periods of time at room temperature using 0.9 M NIPAAm solution in 1,4 dioxane. Thermal initiation was conducted by polymerizing in a preheated 65 °C oil bath for 1 h and subsequent polymerization was conducted at room temperature. A high temperature control polymer (pNIPAAm-**1**-HT) was polymerized at 65 °C for 48 h.

[a] Theoretical molecular weight calculated by multiplying conversion and theoretical maximum molecular weight based on feed ratios.

[b] PDI = M_w(GPC)/M_n(GPC).

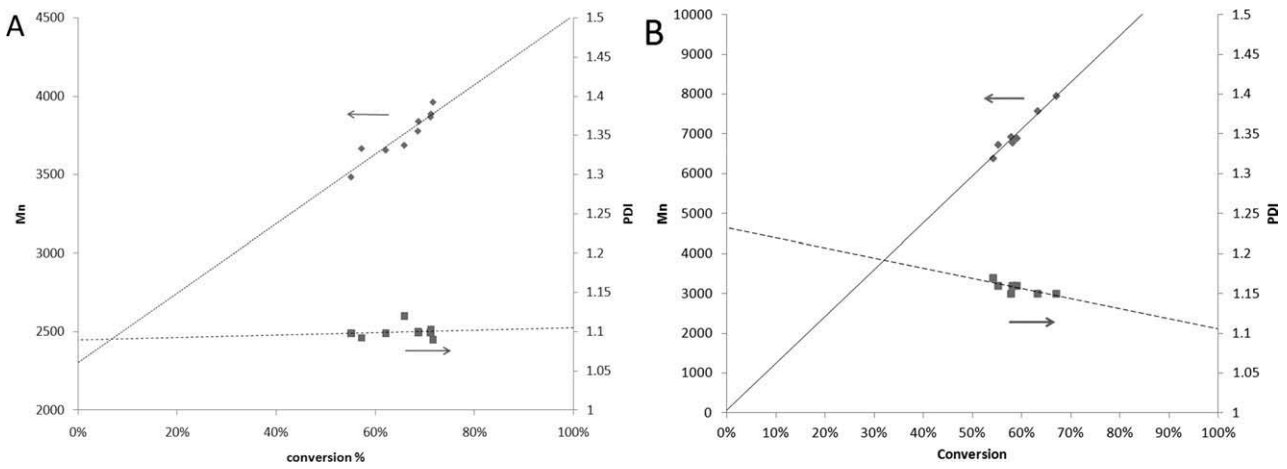

FIGURE 3 Molecular weight versus conversion of (A) pNIPAAm-**1** series and (B) pNIPAAm-**1**s series. The solid line represents theoretical values. Linear correlations between conversion and M_n (R^2 values of 0.86 and 0.96 for pNIPAAm-**1** series and pNIPAAm-**1**s series, respectively) confirm "living" controlled radical polymerization. Nearly constant low PDI indicates that conversion is independent of PDI for these levels of conversion.

control despite the low conversion and overall MW. Free radical polymerization, however, imparts a typically higher PDI (>1.5). The table also indicates relatively consistent MWs regardless of polymerization times for free radical polymerization, indicating that within an hour of polymerization the reaction has already approached completion and further polymerization time at room temperature did not affect conversion or final MW.

When the MW of RAFT-polymerized pNIPAAm (the pNIPAAm-**1** series) was plotted against the conversion, the approximation of a straight line, as seen in Figure 3, further confirms RAFT polymerization. Notably, although pNIPAAm polymerized for varying reaction times at a constant 65 °C reached 97% conversion within 4 h, the level of conversion only reached 71.1% after 7 days of polymerization at room temperature. This is to be expected because of the much higher glass transition temperature (T_g) of pNIPAAm (135 °C) when compared with the reaction temperature.[39] At moderate to high conversions, polymerization slows down considerably because of the vitrification effect.[40–43] This effect is more pronounced when polymerized at room temperature than when polymerized at 65 °C because of the larger temperature difference between T_g and the reaction temperature. Nearly complete conversion such as that seen in the high-temperature polymerization is therefore not expected. Nevertheless, the values shown in Figure 3(A) indicate that reducing the reaction temperature, while slowing the reaction kinetics, did not change the characteristic linear increase of M_n as a function of conversion. It should also be noted that the PDI remained almost completely constant. Therefore, we can conclude that this method of polymerization does not affect the "living" controlled radical polymerization (CRP) while providing finer control over MW.

In a separate experiment, the racemo diad promoting agent 3Me3PenOH was included in the polymerization process to confirm continued CRP behavior when synthesizing a majority of syndiotactic polymer. The results of this experiment are seen in Table 2 and Figure 3(B). As expected, the linear relationship between conversion and M_n continues to be observed. The PDIs of the system (on the order of 1.15) are also within the range of RAFT polymerization although they are slightly higher than those polymerized without the presence of 3Me3PenOH.

Although both polymerization methods exhibited CRP, a direct comparison could not be made because of a longer thermal initiation time for the pNIPAAm-**1**s-a to pNIPAAm-**1**s-g series. Subsequently, a new polymer (pNIPAAm-**1**s-4d, $M_n = 4100$, PDI = 1.15) was synthesized to directly compare pNIPAAm-**1**-4d with a majority of racemo diad version of the same polymer (pNIPAAm-**1**s-4d). Both polymers were polymerized under the same conditions for 4 days, and the GPC traces are shown in Figure 4. An ELS detector was used because of its higher sensitivity to low concentrations of polymer. As seen in Figure 4, pNIPAAm-**1**s-4d is slightly larger than the pNIPAAm-**1**-4d. This is as expected because the bulky alcohol acts as an accelerator during the polymerization process when used in conjunction with free radical polymerization.[19] Therefore, it is not surprising that it has a similar effect in RAFT polymerization. This acceleration may have also contributed to the slightly higher PDI of pNIPAAm-**1**s. In addition to having a larger polymer overall, pNIPAAm-**1**s shows a small peak at 12.5 min into the elution. This peak corresponds to higher MW polymers and/or aggregates (15,200 Da) that may have formed as a result of termination reactions and represent 2.4% of the total polymers. The traces indicate that this sample of pNIPAAm-**1**-4d has a MW of 3700 Da with a PDI of 1.13, whereas pNIPAAm-**1**s-4d has a MW of 4100 Da with a PDI of 1.15.

MW and End Group Control Influence on LCST

The measured cloud point temperature (T_{cp}), indicative of the LCST, is taken in this article to be the temperature at which normalized transmittance drops to 50%. As expected,

TABLE 2 pNIPAAm Polymerization Results in the Presence of 3Me3PenOH

	Polymerization Time at Room Temperature (h)	Feed Molar Ratio (NIPAAm:**1**:AIBN)	M_n (NMR)[a]	M_n (GPC)	PDI[b]	Conversion (%)
pNIPAAm-**1**s-a	0	100:1:0.5	6,269	6,381	1.17	54.1
pNIPAAm-**1**s-b	2	100:1:0.5	6,388	6,728	1.16	55.2
pNIPAAm-**1**s-c	5	100:1:0.5	6,695	6,919	1.15	57.8
pNIPAAm-**1**s-d	23	100:1:0.5	6,736	6,777	1.16	58.2
pNIPAAm-**1**s-e	47	100:1:0.5	6,835	6,888	1.16	59.0
pNIPAAm-**1**s-f	71	100:1:0.5	7,324	7,571	1.15	63.2
pNIPAAm-**1**s-g	95	100:1:0.5	7,760	7,957	1.15	67.0

0.9 M NIPAAm was reacted for varying periods of time in a 2:1 ratio of 3Me3PenOH to NIPAAm. Thermal initiation was conducted at 65 °C for 1.5 h.
[a] Theoretical molecular weight calculated by multiplying conversion and theoretical maximum molecular weight based on feed ratios.
[b] PDI = M_w(GPC)/M_n(GPC).

pNIPAAm polymerized through free radical polymerization shows a sharp T_{cp} at 32 °C in deionized water [Fig. 5(A)]. This is independent of the polymerization conditions and can be seen in long-term low-temperature polymerization as well as short-term high-temperature polymerization. This temperature is shifted to 28 °C when measured in PBS [Fig. 5(A)], the commonly accepted ion concentration and pH for physiological systems. The decrease in temperature is due to the destabilizing effects of the salt ions in aqueous solution.[26] Both sodium chloride and sodium phosphate are known to decrease the LCST and even split it into two or more transitions depending on concentration.[26] Although physiological concentrations of NaCl and NaH_2PO_4 are insufficient to induce the splitting of the LCST, the reduction in LCST is still significant.

With the addition of **1** as the RAFT agent, the T_{cp} is significantly increased [Fig. 5(B)]. The acetic acid end groups (from the RAFT agent) have two significant effects on the polymer. First, these acid groups act in a similar manner to hydrophilic comonomers, especially at low MWs.[14] This is by far the more noticeable of the two effects. Second, these end groups can act as syndiotacticity-inducing agents, hydrogen bonding to the acrylamide group in much the same way that bulky alcohols are reported to do.

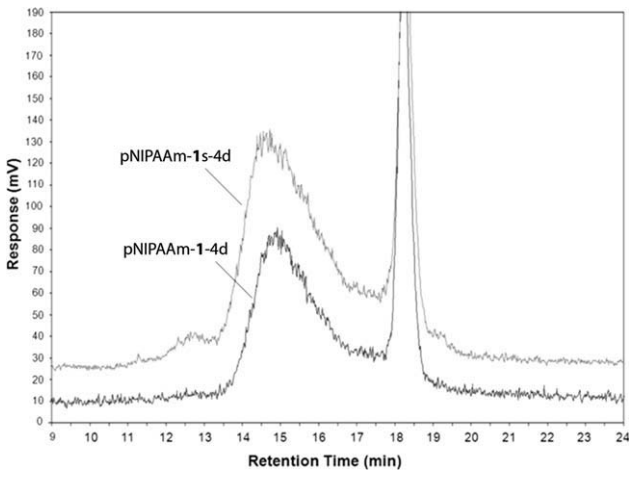

FIGURE 4 GPC traces using ELS detector of pNIPAAm-**1**-4d and pNIPAAm-**1**s-4d (M_n = 3700 and 4100, respectively).

To further analyze the first effect, especially as it related to the MW of the polymer, we compared our sample of pNIPAAm-**1**-4d to pNIPAAm-*co*-AAc copolymers with a target degree of polymerization of 50 and an AAc content of 4%. Such polymers should theoretically have approximately two AAc groups per polymer, similar to the two acid groups from the RAFT polymerization, and comparable MWs. Because of the difficulty in achieving a specific target MW using free radical polymerization, two different samples with number-averaged MWs near that of pNIPAAm-**1**-4d are presented. The T_{cp} of the polymers is shown in Figure 6.

Compared with the copolymers of comparable MWs, pNIPAAm-**1**-4d shows a higher T_{cp} than both the 4900 MW copolymer and the 6100 MW copolymer, with the higher MW copolymer exhibiting a closer T_{cp} curve to pNIPAAm-**1**-4d than the lower MW samples. It is interesting to note that sensitivity of the T_{cp} to MW is not eliminated in the copolymers and is contrary to the MW sensitivity of the RAFT polymers. In addition, the characteristic widening of the T_{cp} curve in these copolymers is small at these low MWs. Because these copolymers were formed through free radical polymerization and have high PDIs, it is likely that many chains contain fewer than the anticipated number of hydrophilic groups. Because of the low number of expected AAc groups per polymer (approximately two), the variation can lead to lower thermal transition temperatures and the still somewhat sharp transitions exhibited.

When polymerized to higher MW (M_n = 11,800, PDI = 1.7) to reduce this effect, as seen in Figure 6(B), the start of the thermal transition is almost identical to that of pNIPAAm-**1**-4d; however, the range of transition for pNIPAAm-**1**-4d was 2.3 °C, whereas the range for high MW pNIPAAm-*co*-AAc was

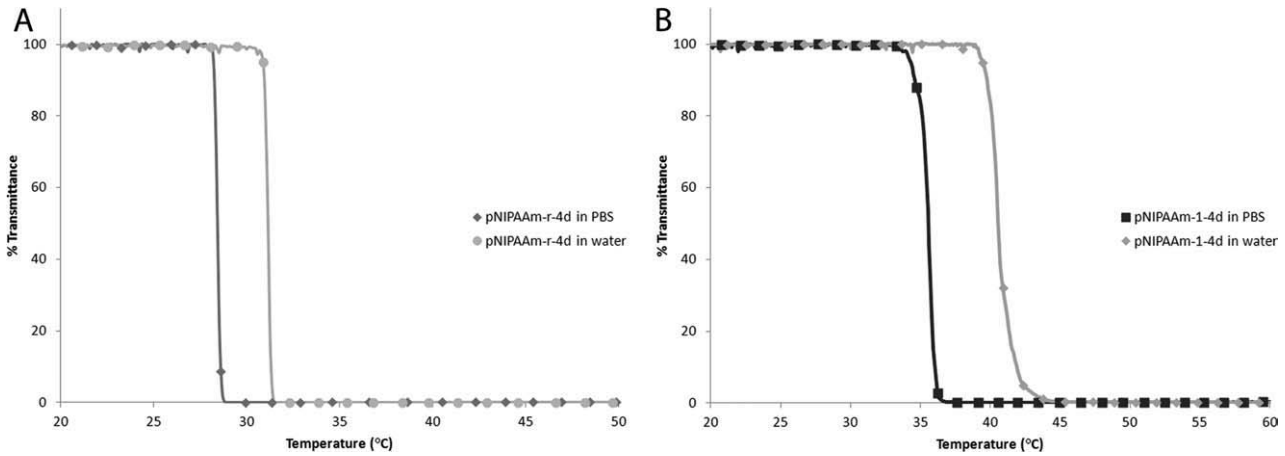

FIGURE 5 Changes in the T_{cp} of polymers assessed in deionized water (18 mΩ) and PBS. (A) pNIPAAm-r-4d and (B) pNIPAAm-**1**-4d. The T_{cp} changes from 32 to 28 °C and 40.5 to 35.5 °C for pNIPAAm-r-3d and pNIPAAm-**1**-4d, respectively.

greater than 10 °C. Furthermore, pNIPAAm-*co*-AAc did not reach a stable transmittance until 26° above the start of the transition. The T_{cp} for the two polymers is also quite different: 44.9 and 40.5 °C for pNIPAAm-*co*-AAc and pNIPAAm-**1**, respectively. The similar starting temperatures for the transitions suggest that comparable ratios of hydrophilic groups were incorporated into both polymers, and the large difference in transition temperature ranges can again be attributed to the less well-defined nature of the copolymer. On the other hand, the exclusion or inclusion of one or two AAc groups per polymer chain will not change the AAc content as drastically as in the case of the lower MW copolymers. In an effort to reproduce both the MW and the thermal transition of pNIPAAm-**1**-4d, an 8% AAc copolymer with an M_n of 5000 was also synthesized [Fig. 6(B)]. As expected, this polymer showed a much higher thermal transition than the comparable 4% AAc copolymers but also a broader transition, indicative of more widespread incorporation of the AAc comonomer. The manipulation required to synthesize copolymers having thermal transition characteristics comparable to our RAFT homopolymer, a more or less iterative process involving multiple variables, confirms the superiority of our method of LCST modification over the traditional copolymerization method.

Tacticity Control Over LCST

A secondary effect of using **1** in the polymerization scheme is the increase of racemo diads in the overall pNIPAAm polymer. Although the number of acetic acid groups is limited to the small amount of RAFT agent available during the polymerization and is inconsequential compared with the concentration of a solvent additive like 3Me3PenOH, the effect is still pronounced, as seen in Figure 7(A,B).

As seen from the methine backbone peaks, there is a slight increase in the percentage of racemo diads when using **1**. The racemo content increases from 54.6% in free radical

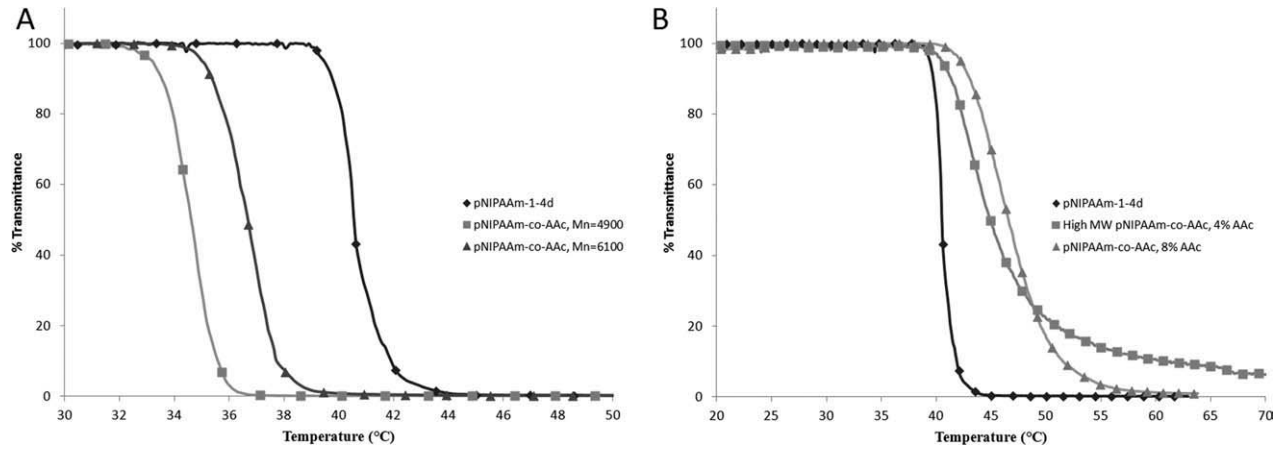

FIGURE 6 Normalized transmittance of 1 wt % aqueous solutions of pNIPAAm-**1**-4d and pNIPAAm-*co*-AAc. (A) pNIPAAm-*co*-AAc with 4% AAc content and comparable molecular weights to pNIPAAm-**1**-4d (M_n of 4900 and 6100 with PDIs of 1.9 and 2.2, respectively) and (B) higher MW 4% AAc content polymer (M_n = 11,800, PDI = 1.7) as well as lower MW 8% AAc polymer (M_n = 5000, PDI = 1.9).

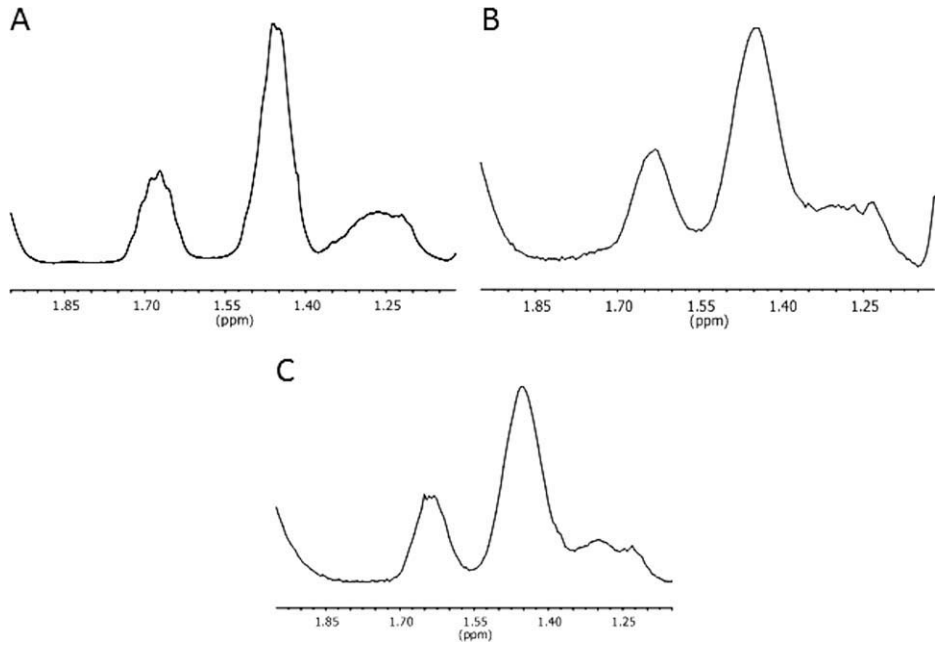

FIGURE 7 Methine backbone peaks of (A) free radical-polymerized pNIPAAm showing 54.6% racemo diads, (B) pNIPAAm-**1**-4d showing 58.6% racemo diads, and (C) pNIPAAm-**1**s-4d showing 61.1% racemo diads. The peaks at 1.67 and 1.27 ppm correspond to meso diads, whereas the peak at 1.46 ppm corresponds to racemo diads.

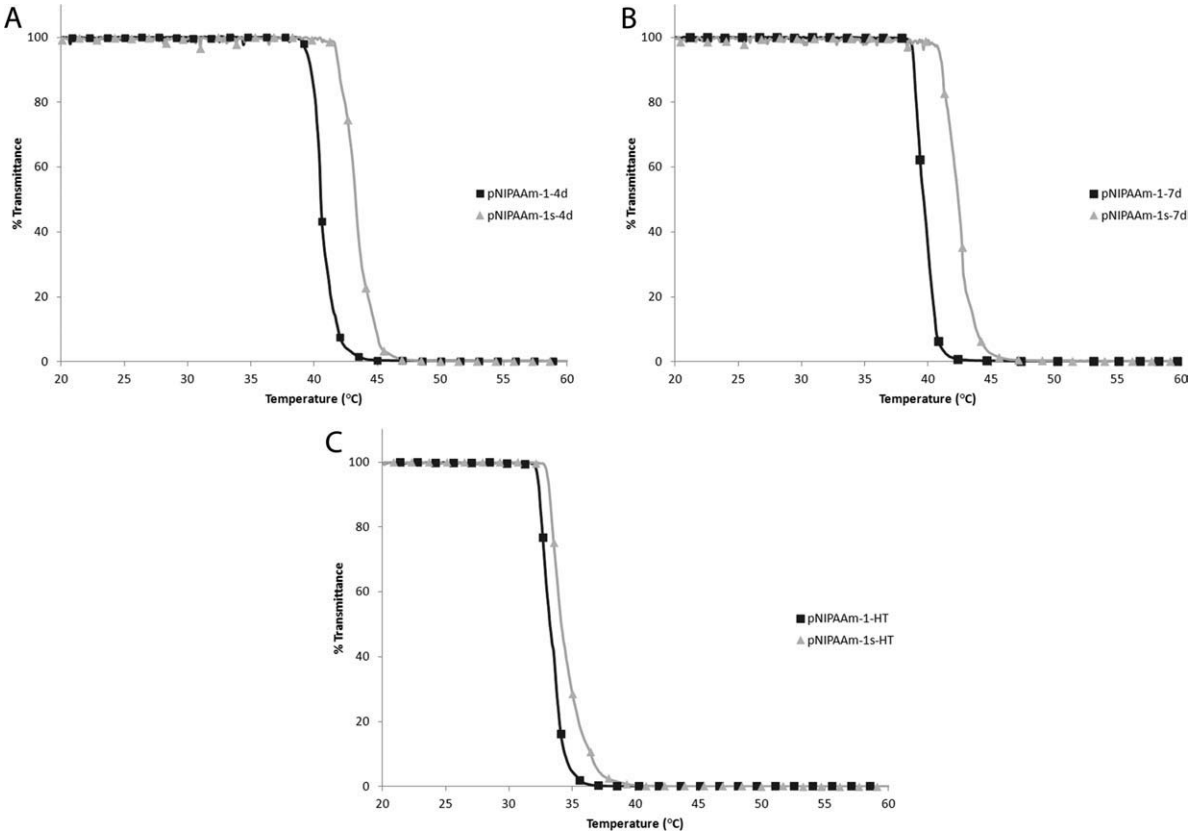

FIGURE 8 Normalized transmittance of 1 wt % aqueous solution of (A) pNIPAAm-**1**-4d and pNIPAAm-**1**s-4d (T_{cp} = 40.5 °C and 43.3 °C respectively), (B) pNIPAAm-**1**-7d and pNIPAAm-**1**s-7d (T_{cp} = 39.7 °C and 42.4 °C respectively), (C) pNIPAAm-**1**-HT polymerized to completion at 65 °C (T_{cp} = 33.7 °C and 35.0 °C for pNIPAAm-**1**-HT and pNIPAAm-**1**s-HT respectively).

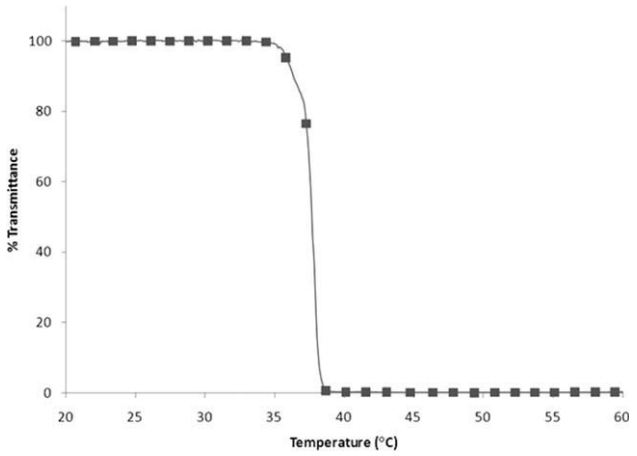

FIGURE 9 Normalized transmittance of 1 wt % solution of pNIPAAm-1-4d polymerized with additional 3Me3PenOH in PBS.

polymerization to 58.4% in polymerization with **1**. When polymerized with 3Me3PenOH, however, the racemo content further increases to 61.1% [Fig. 7(C)]. Previous studies on the relationship between diad tacticity and racemo diad promoting agent properties have shown an inverse relationship between pK_a and racemo diad content.[44] Therefore, it is not surprising that the RAFT agent has an outsized effect on the tacticity despite its low concentration, because the acetic acid groups on the end of the RAFT agent have a much smaller pK_a than 3Me3PenOH. These tacticity changes, though small, have a significant effect on the observed LCSTs, which is consistent with previous work in which pNIPAAm having a higher percentage of racemo diads displays higher LCSTs.[19]

Figure 8 shows the difference that a small change in tacticity can make in the LCST of pNIPAAm. When polymerized for 4 days, we see a transition temperature increase from 40.5 to 43.3 °C. When polymerized for 7 days at room temperature, we see a transition temperature increase from 39.7 to 42.4 °C. When polymerized normally at 65 °C for 48 h to ensure completion, pNIPAAm polymerized with **1** has an LCST at 33.7 °C, slightly higher than that of free radical-polymerized pNIPAAm, but not high enough for applications in biotechnology. With the inclusion of 3Me3PenOH to induce stereospecific polymerization, the transition temperature is increased to 35 °C.

From this, we deduce that by polymerizing slowly for a long period of time the bulky alcohol has more opportunity to induce racemo diads. The LCST shift stays constant at ~2.7 °C for both the 4-day and 7-day polymerizations, whereas it decreases to a 1.3 °C difference with faster, 48 h reactions at 65 °C. This is further confirmed using NMR, in which the methine backbone of the higher reaction temperature pNIPAAm with 3Me3PenOH shows a lower racemo diad content of 60% (see Supporting Information).

The slight decrease in LCST between the 4-day and 7-day polymerizations can be attributed to the larger size of the 7-day polymer, having a M_n of 3997 (PDI = 1.07) compared with an M_n of 3456 (PDI = 1.13) for chains polymerized for 4 days. As expected, the higher MW slightly inhibits the effect of the acetic acid end groups.

Because of the further increase in LCST by inducing a larger percentage of racemo diads, and the relative stability of that 2.7 °C increase, we are, therefore, able to combine tacticity control with the properties of **1** to produce a NIPAAm polymer that undergoes a sharp thermal transition temperature at 37.6 °C, exactly within physiological temperature range, in a solution of PBS as seen in Figure 9. This transition takes place 2.1° above the transition temperature of the pNIPAAm synthesized in the presence of 3Me3PenOH and occurs within a span of 2 °C. Such a polymer with its low PDI of 1.15 and lack of comonomers can greatly improve the sensitivity to temperature that pNIPAAm copolymer systems for biological applications currently lack.

CONCLUSIONS

Successful applications of pNIPAAm for biological purposes have been limited in part due to the trade-off between having an LCST that is high enough and having a very sharp transition. We have shown in this article that this problem can be overcome by implementing various polymerization methods and tools. By polymerizing slowly with **1** over the course of 4 days while inducing racemo diad formation, we were able to synthesize well-defined pNIPAAm with a sharp LCST of 37.6 °C in a solution of PBS. This reaction scheme combines tacticity control with RAFT polymerization, MW control, and end group control. Such polymers can be used for more accurate transitions for drug delivery, diagnostics, BioMEMs, and other applications in biotechnology.

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