Thermo- and pH-Induced Self-Assembly of P(AA-*b*-NIPAAm-*b*-AA) Triblock Copolymers Synthesized via RAFT Polymerization

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ABSTRACT: A series of environmentally sensitive ABA triblock copolymers with different block lengths were prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization from acrylic acid (AA) and *N*-isopropylacrylamide (NIPAAm). The GPC and ^{1}H NMR analyses demonstrated the narrow molecular weight distribution and precise chemical structure of the prepared P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers owing to the controlled/living characteristics of RAFT polymerization. The lower critical solution temperature (LCST) of the triblock copolymers could be tailored by adjusting the length of PAA block and controlled by the pH value. Under heating, the triblock copolymers underwent self-assemble in dilute aqueous solution and formed nanoparticles revealed via TEM images. Physically crosslinked nanogels induced by inter-/intra-hydrogen bonding or core-shell micelle particles thus could be obtained by changing environmental conditions. With a well-defined structure and stimuli-responsive properties, the P(AA-*b*-NIPAAm-*b*-AA) copolymer is expected to be employed as a nanocarrier for biomedical applications in controlled-drug delivery and targeting therapy. © 2015 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2016**, *54*, 1109–1118

KEYWORDS: block copolymer; core-shell particles; nanogels; reversible addition fragmentation chain transfer (RAFT); RAFT polymerization; self-assembly; stimuli–sensitive polymers

INTRODUCTION Amphiphilic block copolymers consisting of both lipophilic and hydrophilic units have received great interest in decades because they could self-assemble into supramolecular structures such as spheres, cylinders, bilayers, and vesicles.[1,2] Therefore, they are very useful especially in anticancer drug delivery,[3] tissue engineering,[4] biotechnology,[5] and so on. Moreover, their structures can be manipulated by altering the chemical composition of polymer backbone and block chain length, in addition to changing the physically environmental conditions such as the solvent, pH, and temperature.[6] In order to obtain well-defined block copolymers having designed composition, controlled molecular weight and specific functional groups, controlled/living radical polymerization (CLRP) is frequently applied as a precise and effective route for synthesis.[7–9] One of the most common method for preparing block copolymers is the reversible addition-fragmentation chain transfer (RAFT) polymerization owing to its tolerance to a wide range of monomers and functional groups.[6,10]

Poly(*N*-isopropylacrylamide) (PNIPAAm) is the most well-known thermo-responsive polymer, which undergoes a reversible coil-to-globule transition in an aqueous system at around 32 °C, that is, the lower critical solution temperature (LCST).[11] Above the LCST, PNIPAAm exhibits a hydrophobic and colloidal structure due to the disruption of intermolecular hydrogen bonding with water and simultaneously the association of PNIPAAm chains.[12] On the other hand, PNIPAAm becomes water-soluble with a random-coil structure below the LCST. For some specific applications, it is necessary to combine other environmentally sensitive properties in addition to the temperature such as pH-sensitivity because many physiological phenomena are accompanied with the change in pH value.[13] Among those potential candidates, poly(acrylic acid) (PAA) is commonly referred as one of the most important pH-sensitive polymers. It can be physical blended or copolymerized with the PNIPAAm to provide its pH-sensitivity. Yet, the LCST of the resulting modified PNIPAAm could be changed due to the existence of the PAA

component. For example, latex particles of P(NIPAAm-*co*-AA) copolymer with an average diameter of about 200 to 500 nm were prepared via surfactant-free emulsion polymerization by Lin et al.[14] They showed that increasing the AA content in the copolymer would increase the LCST of latex particles; and under neutral environment, the copolymer had a smaller particle size than the PNIPAAm homopolymer. Similarly, Liu et al.[15] found that different LCSTs would be obtained for the graft copolymers of P(AA-*g*-NIPAAm) with various compositions, which were also dependent on the pH value of dilute aqueous solution.

Moreover, as NIPAAm copolymerizes with other monomers, its temperature-sensitive property could provide a potentially self-assembly method into various structures. Chiang et al.[16] synthesized two graft copolymers which consisted of acrylic acid (AA) and 2-methacryloylethyl acrylate (MEA) units as the backbone and either PNIPAAm alone or both PNIPAAm and monomethoxypoly(ethylene glycol) (mPEG) chain segments as the grafts. The results showed that they could form hollow nanogel spheres via the spontaneous co-association of two graft copolymers into the vesicle architecture in aqueous phase. Zhang et al.[17] synthesized nonlinear, multihydrophilic block copolymers, poly(*N*-isopropylacrylamide)$_2$-[poly(*N*-vinylpyrrolidone)-*b*-poly(acrylic acid)]$_2$, and poly[*N*-isopropylacrylamide-*b*-(acrylic acid)]$_2$-poly(*N*-vinylpyrrolidone)$_2$ and demonstrated that they tended to self-assemble into three different types of micellar aggregates and unimers which could be controlled by pH value and temperature of aqueous solution. Furthermore, the AB-type diblock copolymer of P(NIPAAm-*b*-AA) was studied by Schilli et al.[18] At higher temperatures above the LCST, they observed the formation of larger aggregates at pH 4.5, yet micelles were found at pH 5 to 7. On the other hand, at a pH value of 5.6, only micelles were observed at 50 °C; whereas larger aggregates and micelles coexisted at lower temperatures. According to the aforementioned statements, hydrogen bonding between PNIPAAm and PAA blocks in the P(NIPAAm-*b*-AA) copolymers strongly influenced their self-assembly behaviors in aqueous solution. Diblock copolymers of P(NIPAAm-*b*-AA) also displayed accelerated thermo-response to the external temperature changes when compared with the PNIPAAm nanogel.[19] In addition, Figg et al.[20] synthesized PNIPAAm block copolymers via RAFT polymerization of NIPAAm from a hydrophilic chain transfer agent composed of *N,N*-dimethylacrylamide and acrylic acid in water at 70 °C. They found that during polymerization the growing PNIPAAm could self-assemble into polymeric nanoparticles with a range of structures (e.g., micelles, worms, and vesicles), where the morphology was a function of the PNIPAAm block length. The dual pH- and thermo-sensitivities of P(NIPAAm-*b*-AA) copolymers thus makes themselves very useful as a controlled drug delivery system.[21]

The LCST of the PNIPAAm can be tailored by varying molecular weight, incorporating hydrophilic or hydrophobic comonomers or even functional end-group moieties. And by changing pH and/or temperature, the modified PNIPAAm could self-assemble into various structures. Though ABA triblock copolymers of P(AA-*b*-NIPAAm-*b*-AA) have been prepared by atom transfer radical polymerization (ATRP)[22] or RAFT polymerization[18,23,24] for various applications such as bone tissue engineering, there are few reports focused on the influence of main-chain composition on the self-assembled morphologies of P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers. Our previous studies indicated that not only telechelic HOOC-PNIPAAm-COOH homopolymer but also P(AA-*b*-NIPAAm-*b*-AA) could self-assemble into spherical nanoparticles under specific pH value and temperature.[25,26] Herein, we systematically design a series of P(AA-*b*-NIPAAm-*b*-AA) copolymers with various molar ratios of NIPAAm to AA unit via RAFT polymerization using a trithiocarbonate as the chain transfer agent (CTA). Moreover, the environmentally responsive properties of the prepared P(AA-*b*-NIPAAm-*b*-AA) block copolymers were investigated by measuring their optical transmittance, zeta potential and particle size at different temperatures and pH values.

EXPERIMENTAL

Materials

Acrylic acid (AA) was purified by distillation under reduced pressure in order to remove the inhibitor before use. *N*-isopropylacrylamide (NIPAAm) was recrystallized from *n*-hexane. The chain transfer agent for RAFT polymerization, *S,S′-bis*(α,α′-dimethyl-α′′-acetic acid)trithiocarbonate (CMP), was synthesized following the protocol proposed by Lai et al.[27] Azobisisobutyronitrile (AIBN) was re-crystallized from methanol and used as the thermal initiator. Deionized water (18.2 MΩ) and methanol were used throughout the work. All other chemicals and solvents were at least analytical grade and used as received.

RAFT Polymerization of NIPAAm and AA

AA monomer at various amounts was dissolved in 20 mL methanol containing 0.12 g of CMP and 0.017 g of AIBN. After all the chemicals were dissolved, the reaction was continued for 3 h at 70 °C with stirring and purged with nitrogen. Subsequently, NIPAAm monomer was dissolved in another 20 mL methanol and then introduced into the system. The reaction was continued for another 14 h at the same temperature to synthesize the triblock copolymer of P(AA-*b*-NIPAAm-*b*-AA). After reaction, the solution was removed for dialysis against methanol in order to remove any unreacted monomers. It was then dried under vacuum at room temperature. The produced PAA macro-CTA in the first stage and the subsequent P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers are designated as A_x and A_xN_y, respectively, where x and y represent the respective molar ratios of AA and NIPAAm to CMP in the feed. The feeding amounts of reactants, initiator and chain transfer agent are summarized in Table 1, and the reaction mechanism is shown in Figure 1. The theoretical number-average molecular weights of the PAA macro-CTA produced in the first-stage and the final P(AA-*b*-NIPAAm-*b*-AA) triblock copolymer were calculated by the following equations with the assumption of 100% conversion:

$$M_{n,PAA} = \left(\frac{[AA]_o}{[CMP]_o}\right) \times M_{AA} + M_{CMP} \quad (1)$$

TABLE 1 Experimental Conditions of the Synthesis of P(AA-*b*-NIPAAm-*b*-AA) Triblock Copolymers via RAFT Copolymerization

Sample Code	AA (g)	NIPAAm (g)	CMP (g)	AIBN (g)	MeOH (mL)
$A_{50}N_{150}$	1.53	7.21	0.12	0.017	40
$A_{100}N_{150}$	3.06				
$A_{150}N_{150}$	4.60				
$A_{150}N_{75}$	4.60	3.60	0.12	0.017	40
$A_{150}N_{150}$		7.21			
$A_{150}N_{225}$		10.8			

$$M_{n,P(AA\text{-}NIPAAm\text{-}AA)} = \left(\frac{[AA]_o}{[CMP]_o}\right) \times M_{AA} + \left(\frac{[NIPAAm]_o}{[CMP]_o}\right) \times M_{NIPAAm} + M_{CMP} \quad (2)$$

where $[AA]_o$, $[NIPAAm]_o$, and $[CMP]_o$ stand for the initial concentrations of AA, NIPAAm, and CMP in the feed, respectively. M_{AA}, M_{NIPAAm}, and M_{CMP} represent the respective molecular weights of AA, NIPAAm, and CMP.

Structure Characterizations

Chemical structure and the block chain length ratio of the synthesized P(AA-*b*-NIPAAm-*b*-AA) copolymers were determined from the nuclear magnetic resonance (^{1}H NMR) spectra which were recorded on a Bruker AV-400 NMR spectrometer. d4-Methanol was used as the solvent. For comparison, NMR spectra of individual homopolymers of PAA and PNIPAAm obtained via RAFT polymerization were also recorded.

Number-average molecular weight (M_n) and molecular weight distribution indicated by polydispersity index (PDI) of the produced P(AA-*b*-NIPAAm-*b*-AA) copolymers were determined by gel permeation chromatography (GPC). A solvent delivery system (model: PU-2080, JASCO) equipped with two columns (Malvern Viscotek T-columns LT5000) and a differential refractometer (RI, model: RI-2031, JASCO) was applied, using 0.05 M sodium sulfate aqueous solution as the eluent at a flow rate of 0.8 mL/min at 25 °C. A dilute solution of sodium hydroxide (0.01 M) was added into the sodium sulfate$_{(aq)}$ to enhance the solubility of triblock copolymers. M_n and PDI were then reported based on poly(methacrylic acid) (PMAA) standards.

LCST of P(AA-*b*-NIPAAm-*b*-AA) Solutions

The lower critical solution temperature (LCST) or the cloud point of various P(AA-*b*-NIPAAm-*b*-AA) solutions was determined by using a UV/Visible spectrophotometer (Thermo Spectronic gamma series). Sample solutions were prepared by dissolving 120 mg triblock copolymer in 4 mL of aqueous solutions The acidity of the aqueous solution was adjusted by the addition of 0.1 N NaOH$_{(aq)}$ or 0.1 N HCl$_{(aq)}$. Finally, the transmittance of the solution at the wavelength of 480 nm was measured at different temperatures using a heating cell. The LCST was then determined at the point while the rapid decline of the optical transmittance occurred.

Zeta Potential of P(AA-*b*-NIPAAm-*b*-AA) in Aqueous Solutions

Sample solutions were prepared at a concentration of 3 wt % in deionized water. Under certain pH values and temperatures, nanoparticles could be formed and their zeta potential was then measured with a Malvern Zetasizer Nano.

Determination of Glass Transition Temperature (T_g)

The glass transition temperature (T_g) of synthesized polymers was determined by differential scanning calorimeter (DSC, TA Q20, USA) at a heating rate of 10 °C /min. All samples were first heated to 200 °C to exclude the influence of thermal history on the sample preparation; their T_gs were then determined on the second heating curves.

Morphology Observation

Sample solutions were further diluted with deionized water to a concentration of 0.2 wt % and dripped onto a copper-grid coated with a collodion. It was then dried at room temperature or in an oven with a preset temperature. The morphology of the dispersed nanoparticles was then observed

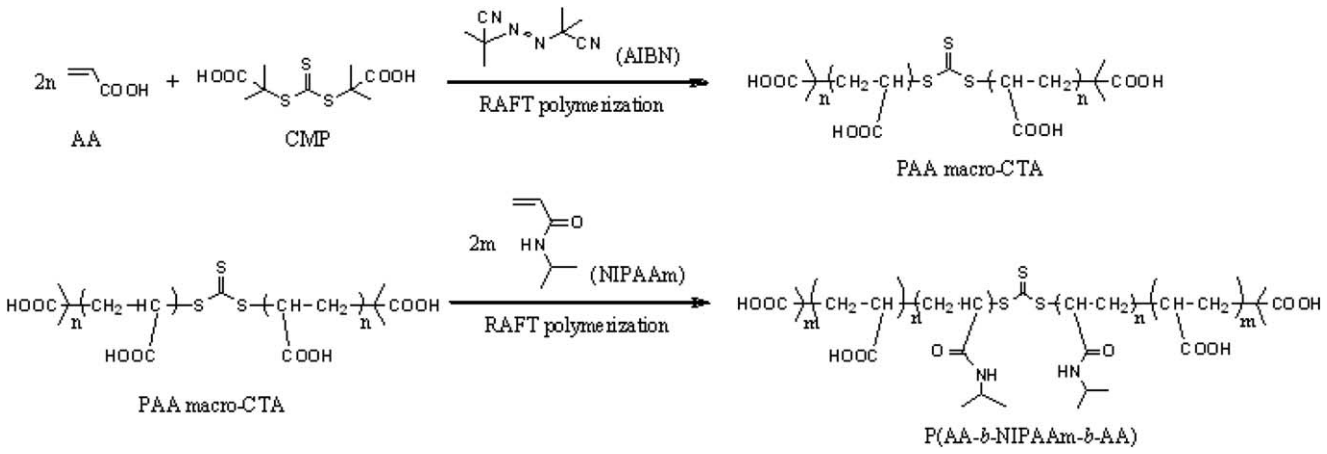

FIGURE 1 RAFT copolymerization of AA and NIPAAm to prepare P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers.

TABLE 2 Structure Characterizations of HOOC-PNIPAAm-COOH, HOOC-PAA-COOH, and P(AA-*b*-NIPAAm-*b*-AA) Triblock Copolymers by RAFT Polymerization Using CMP as the Chain Transfer Agent

Sample	$M_{n,theo}$[a]	$M_{n,exp}$[b]	PDI	$(AA: NIPAAm)_{feed}$	$(AA: NIPAAm)_{exp}$[c]
N_{400}	40,500	39,800	1.28	–	–
A_{50}	3,890	9,440	1.03	–	–
A_{100}	7,490	12,230	1.13	–	–
A_{150}	11,090	14,960	1.15	–	–
$A_{50}N_{150}$	20,600	31,200	1.15	1.0:3.0	1:2.56
$A_{100}N_{150}$	24,200	48,600	1.27	1.0:1.5	1:1.45
$A_{150}N_{150}$	27,800	50,900	1.22	1.0:1.0	1:0.94
$A_{150}N_{75}$	19,300	48,200	1.15	1.0:0.5	1:0.49
$A_{150}N_{225}$	36,300	63,800	1.51	1.0:1.5	1:1.37

[a] The theoretical molecular weight $M_{n,theo}$ was calculated from the feed with the assumption of 100% conversion.
[b] The experimental molecular weight ($M_{n,exp}$) and polydispersity index (PDI) were determined by GPC.
[c] The experimental molar ratio of AA to NIPAAm in the triblock copolymer was determined from NMR spectra.

by using a transmission electron microscope (TEM, JEOL JSM-1230).

RESULTS AND DISCUSSION

Structures of PAA Macro-CTA and P(AA-*b*-NIPAAm-*b*-AA) Triblock Copolymers

In order to synthesize block copolymers, AA monomer was introduced into the reaction system to produce PAA macro-CTA first by RAFT polymerization. The molar ratio of AA to CMP was changed to obtain different chain lengths of PAA block. Subsequently, NIPAAm monomer was added into the system to synthesize P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers. The number-average molecular weight (M_n) and molecular weight distribution indicated by polydispersity index (PDI) of the PAA macro-CTA and P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers are summarized in Table 2. The fact that the experimental value of molecular weight of each copolymer determined from GPC is significantly greater than its theoretical one calculated from eqs 1 and 2 is because it is a relative value based on the PMAA standard. Apparently, the PAA block is more hydrophilic than the PMAA in the aqueous solution of $NaOH_{(aq)}/Na_2SO_{4(aq)}$, making the PAA relatively extended in the solution. Consequently, the measured molecular weight is higher than the expected. Nevertheless, the molecular weight increases with increasing the added amount of AA monomer. In addition, the molecular weight distribution can be kept very narrow for the PAA macro-CTA as its PDI is 1.03 for the A_{50} and slightly increases to 1.15 for the AA_{150}. The subsequent addition of NIPAAm further increases the molecular weight as shown in Table 2, owing to the living characteristics of the PAA macro-CTA. The structure of the produced triblock copolymer is shown in Figure 1. However, the PDI is also increased with the addition of PNIPAAm block. The longer the chain length, the larger the PDI is. In most samples, the PDI values are less than 1.3.

In order to obtain the molar ratio of repeating unit AA to NIPAAm in the triblock copolymer, NMR analysis was employed. As shown in Figure 2, characteristic signals of isopropyl group in the PNIPAAm block can be clearly observed from the ^{1}H NMR spectrum: methine, -C***H***$(CH_3)_2$ (peak *a* at 4.0 ppm) and methyl, -CH(C***H***$_3)_2$ (peak *d* at 1.2 ppm). The molar ratio of AA to NIPAAm could be calculated from the following equation via identifying the peaks at 2.1 ppm (*b*, -CH_2C***H***-) and 1.6 ppm (*c*, C***H***$_2$CH-) in the main chain.

$$\left(\frac{\mathrm{AA}}{\mathrm{NIPAAm}}\right)_{\mathrm{NMR}} = \frac{\frac{(\mathrm{Area_b}+\mathrm{Area_c})}{3} - \mathrm{Area_a}}{\mathrm{Area_a}} \qquad (3)$$

where $Area_a$, $Area_b$, and $Area_c$ are the integrated areas of peak a, b, and c from the spectrum, respectively. It can be found that the molar ratios of AA to NIPAAm in the produced triblock copolymers are all close to their respectively molar ratios in the feed, indicating the high monomer conversion and the effectiveness of RAFT polymerization. This is in agreement with the results from the weight analysis. It was found that the AA conversion was nearly complete in the first stage and the NIPAAm conversion in the second stage was at least 90%. In other words, the structure of P(AA-*b*-NIPAAm-*b*-AA) triblock copolymer, especially the block length ratio, could be simply tuned via manipulating the feeding amount of monomers.

LCST of P(AA-*b*-NIPAAm-*b*-AA) Triblock Copolymer

The hydrophilic AA monomer was introduced into the RAFT copolymerization with the NIPAAm in order to tune the LCST of the thermo-responsive polymer and also to provide the self-assembling ability. Because of the ionization nature of the PAA block (-COOH → -COO^-), it is expected that the LCST of the P(AA-*b*-NIPAAm-*b*-AA) depends on the pH value of aqueous solution. In this study, the LCST of various copolymers at each pH value is determined at the point where the rapid decline of the optical transmittance occurs, as shown in Figure 3(a). The variations of the LCST with the

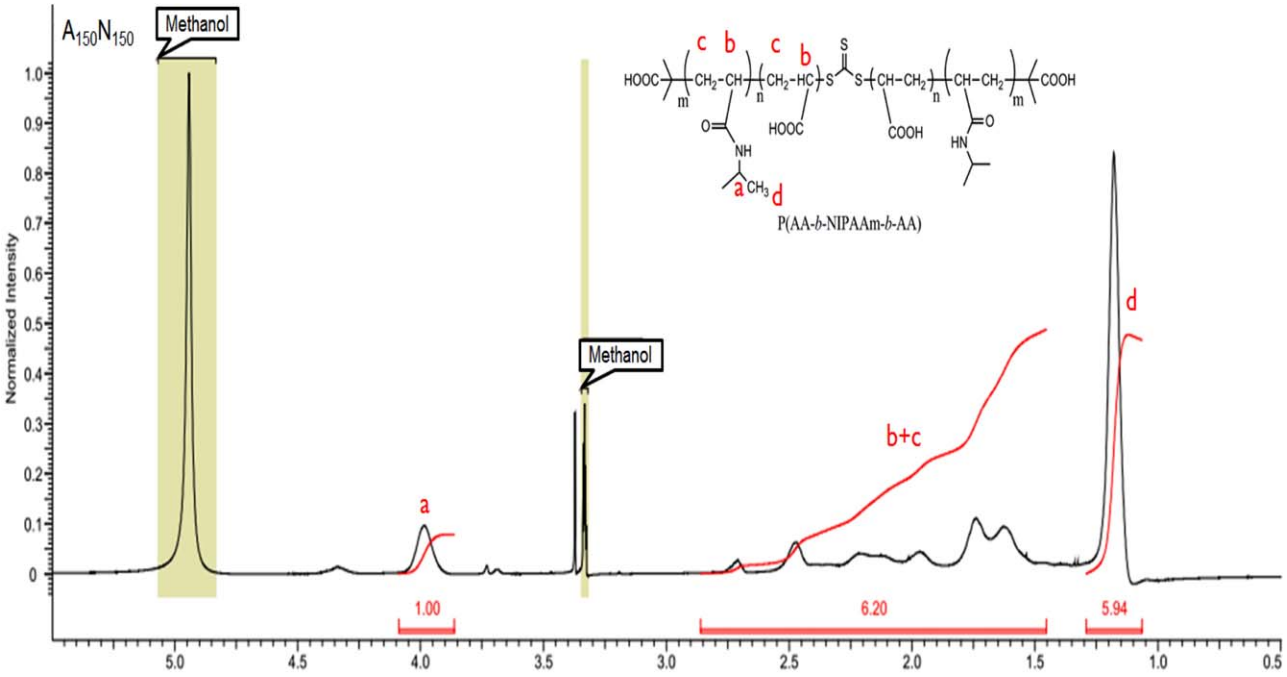

FIGURE 2 Chemical structure and characteristic signals of ^{1}H NMR of P(AA-*b*-NIPAAm-*b*-AA) triblock copolymer, where $A_{150}N_{150}$ was taken as an example. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

pH value for the triblock copolymers of A_xN_{150} series and $A_{150}N_y$ series are summarized in Figure 3(b,c), respectively. Figure 3(b) clearly shows that the length of PAA block in the A_xN_{150} copolymers and also the pH value of aqueous solution affect the LCST; and the pH sensitivity increases with an increment in the length of PAA block. At a pH of 4.45, the LCSTs of these three triblock copolymers are all higher than 32 °C which is the LCST of the neat PNIPAAm homopolymer. The pKa of PAA is known to be around 4.3,[28] and therefore it is reasonably assumed that many carboxylic acid groups in the PAA block are ionized and become negatively charged. Not only the ionized PAA is more hydrophilic, but the repulsive force from the negative charge along the PAA block would make it more extended in the aqueous medium. Consequently, the LCST is raised and increases with increasing the block length of the PAA, i.e. the LCST increases in the order of $A_{50}N_{150}$, $A_{100}N_{150}$, and $A_{150}N_{150}$ at a high pH value. On the contrary, at a low pH value of 4.0, the LCSTs of the $A_{100}N_{150}$ and $A_{150}N_{150}$ are lower than 32 °C. This indicates that the triblock copolymers become more hydrophobic than the neat PNIPAAm once the PAA block is above a certain length. At this pH value, lots of carboxylic acid groups exist in their neutral form, which are less hydrophilic than their ionized counterparts. In addition, it is believed that there are inter/intramolecular interactions among the PAA and PNIPAAm blocks when there are a lot of COOH groups in the PAA.

One of the most important interactions among the PAA and PNIPAAm blocks is hydrogen bonding. This is verified from DSC results as shown in Figure 4. The glass transition temperatures (T_gs) of A_{150}, N_{150}, and $A_{150}N_{150}$ are 95, 125, and 160 °C respectively. Compared with those of neat PNIPAAm and PAA, the T_g of triblock copolymer is much higher, indicating that strong hydrogen bonding among themselves plays an important role in the chain mobility and aggregation behavior of the P(AA-*b*-NIPAAm-*b*-AA) block copolymers. For better understanding the hydrogen bonding in the triblock copolymers, polymer blends of A_{150} and N_{150} were also prepared by direct dissolving them in aqueous or methanol solution and afterwards drying at 70 °C. Their DSC curves are shown as well in Figure 4. A T_g is found at 110 °C which lies between the T_gs of the neat PAA and PNIPAAm, as expected for the miscible polymer blends without strong inter-molecular interactions. However a significant transition temperature occurred at 160 °C for the blends of A_{150} and N_{150} of either prepared from water or from methanol, which strongly supports the existence of hydrogen bonding between the PAA and PNIPAAm, leading to the large increase in the T_g.

However, the $A_{50}N_{150}$ triblock copolymer with the shortest PAA length still has a LCST of 35 °C. It is suspected that the length of its PAA block is not long enough to cause evident hydrogen bonding. Therefore, the chain length and the ionization extent of the PAA block affect the amount of hydrogen bonding among polymer chains. In the intermediate pH values between 4.0 and 4.45, the interplay between the hydrogen bonding and the repulsive ionic force owing to the presence of COOH and COO^- groups determines the exact LCST value of the triblock copolymer. Hydrogen bonding seems to be dominant in the $A_{100}N_{150}$ and $A_{150}N_{150}$, whereas the repulsive ionic force dominates in the $A_{50}N_{150}$.

In addition to the A_xN_{150} series, we also investigated the $A_{150}N_y$ series with the same PAA length. The effects of pH

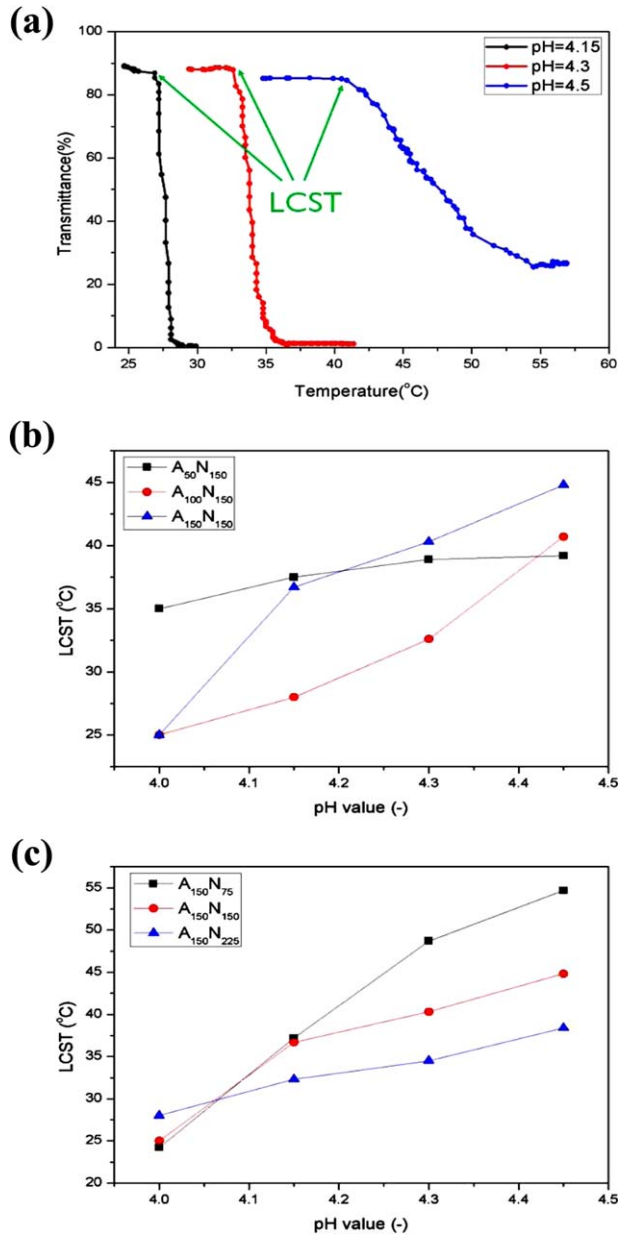

FIGURE 3 (a) The determination of LCST for P(AA-*b*-NIPAAm-*b*-AA) at various pH values. $A_{100}N_{150}$ was taken as an example. The LCSTs of the P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers at various pH values: (b) A_xN_{150} samples with the same length of PNIPAAm block, and (c) $A_{150}N_y$ samples with the same length of PAA block. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

value and the length of PNIPAAm block on the LCST of the $A_{150}N_y$ are shown in Figure 3(c). The pH sensitivity increases with a decrease in the PNIPAAm length, i.e. with an increase in the length ratio of PAA to PNIPAAm, which is apparently similar to the A_xN_{150} series. However, the PAA length in the $A_{150}N_y$ series is long enough that the hydrogen bonding would be dominant for all three copolymers at a low pH value of 4.0. Therefore, the LCSTs of these $A_{150}N_y$ copolymers at pH 4.0 are all lower than 32 °C. As the pH value is increased, the LCST is increased as well, because the ionization extent in the triblock copolymer is increased and thus its hydrophilicity. In addition, at higher pH values above 4.0, the LCST is higher for the triblock copolymer with shorter PNIPAAm length, since the length ratio of PAA to PNIPAAm becomes larger. At a pH value of 4.45, the negatively charged COO^- groups in the PAA block dominate the solution behavior. However, when the pH is increased to a value over 5.0, the P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers become too hydrophilic to measure their LCST from optical transmittance where no apparent phase transition is observed up to 80 °C. This is because most carboxylic acid groups in the PAA segments would dissociate to carboxylate ions

Self-Assembling Behavior and Morphology

By manipulating the pH and temperature of the aqueous solution, it is possible that the P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers could possess both hydrophilic and hydrophobic units for self-assembling under some certain circumstances. Once they are formed, TEM was then employed for monitoring the morphology of self-assembled nanoparticles. Self-assembling conditions and the observed morphologies of the P(AA-*b*-NIPAAm-*b*-AA) copolymers are summarized in Table 3 and will be discussed in the following paragraph.

Thermo-Induced Self-Assembling of HOOC-PNIPAAm-COOH Homopolymer

In our previous study,[25] we synthesized the HOOC-PNIPAAm-COOH homopolymer (N_{400}) by RAFT polymerization using the CMP as the chain transfer agent. The N_{400} was thus composed of PNIPAAm main chain with two terminal carboxylic acid groups. Under a basic medium at pH 10, the carboxylic acid end-groups in N_{400} would dissociate into carboxylate ions which were highly hydrophilic. Therefore, the

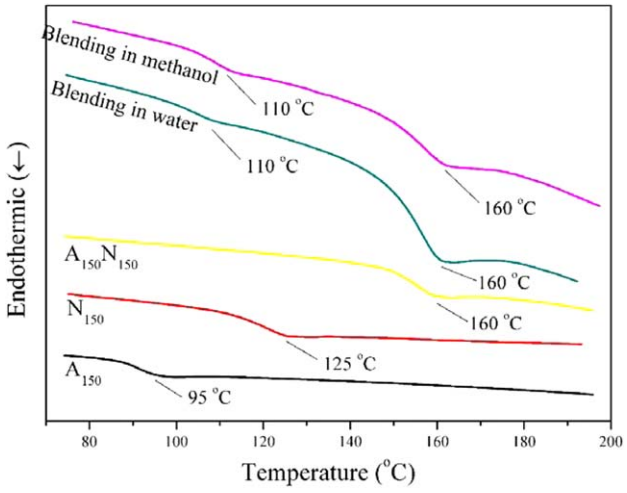

FIGURE 4 The determination of T_g for neat PAA (A_{150}), PNIPAAm (N_{150}), triblock copolymer of P(AA-*b*-NIPAm-*b*-AA) ($A_{150}N_{150}$), and the blends of A_{150} and N_{150} prepared by using cosolvent water or methanol. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 3 Self-Assembling Conditions and Morphologies of HOOC-PNIPAAm-COOH Homopolymer (N_{400}) and P(AA-*b*-NIPAAm-*b*-AA) Triblock Copolymers (A_xN_y)

	pH	Temperature (°C)	Size (nm)	Morphologies
N_{400}	10	60	300	Core-shell nanoparticles (Thermo-induced)
$A_{100}N_{150}$	4.5	60	150–250	Spherical nanogels (Thermo-induced)
$A_{150}N_{150}$	4.5	60	300–400	Spherical nanogels (Thermo-induced)
$A_{150}N_{75}$	7	60	450–550	Spherical nanogels (Thermo-induced)
$A_{150}N_{225}$	3.9	4	1,000–1,200	Spherical microgels (pH-induced)

LCST of the N_{400} was 35 °C indicated in Figure 5(a), slightly higher than the reported LCST of PNIPAAm at 32 °C. In addition, it was found that at pH 10.0 and 60 °C, this N_{400} could self-assemble into nearly monodispersed nanoparticles with a size of 300 nm as shown in Figure 5(b). This is because under this specific environmental condition, amphiphilic structure consisting of hydrophilic carboxylate ions at two ends and hydrophobic PNIPAAm main chain is obtained. As a result, the N_{400} could be organized into monodispersed nanoparticles with hydrophobic core (PNIPAAm) and hydrophilic shell-region (-COO^-).

Thermo-Induced Self-Assembling of $A_{100}N_{150}$ and $A_{150}N_{150}$

When the length ratio of PAA to PNIPAAm in triblock copolymers is high enough such as $A_{100}N_{150}$ and $A_{150}N_{150}$, they could not assemble into spherical nanoparticles at pH 10.0 and 60 °C as observed previously for the N_{400}. This is because they are too hydrophilic in the basic media due to the existence of PAA block with many carboxylate ions. By decreasing the pH into an acidic environment, the ionization extent of carboxylic acid groups could be greatly decreased, and so its hydrophilicity. When the pH is decreased to 4.5, both $A_{100}N_{150}$ and $A_{150}N_{150}$ copolymers are able to self-assemble into spherical nanoparticles at 60 °C. This temperature is apparently higher than their LCSTs which are determined from the optical transmittance at pH 4.5 as shown in Figure 6(a,b) for the $A_{100}N_{150}$ and $A_{150}N_{150}$, respectively. Different from the core-shell structure of N_{400} at pH 10 and 60 °C, Figure 6(c,d) show that the spherical particles of $A_{100}N_{150}$ and $A_{150}N_{150}$ are actually crosslinked nanogels. The size of $A_{100}N_{150}$ nanogels is in the range of 150 to 250 nm; however, it could be raised to 300 to 400 nm for the $A_{150}N_{150}$ due to its longer PAA block. The self-assembled nanoparticles under acid conditions are believed to be composed of hydrophobic PNIPAAm blocks in nano-sized coagulations and inter/intramolecular hydrogen-bonding of PAA blocks serving as physically crosslinking sites. Compared with the N_{400} shown in Figure 5(a), both $A_{100}N_{150}$ and $A_{150}N_{150}$ solutions have much higher transmittance at temperatures above their respective LCSTs, supporting the formation of swelling gel structure. Another evidence of the nanogel morphology is that the zeta potentials of $A_{100}N_{150}$ and $A_{150}N_{150}$ at pH 4.5 and 60 °C are relatively small at −2.67 and −1.67 mV, respectively. This indicates that the surface charge is not responsible for stabilizing these nanogel particles. In other words, the physical crosslinking of triblock copolymer chains through short-range hydrogen-bonding plays an important role for forming the nanogels.

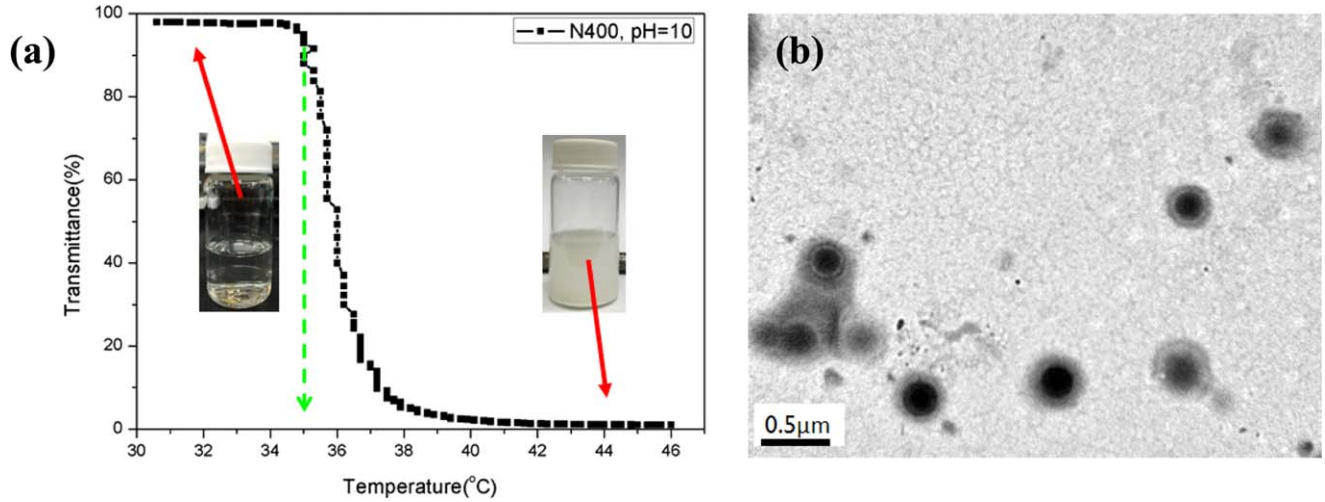

FIGURE 5 (a) The temperature dependence of optical transmittance at 480 nm of the aqueous solution of HOOC-PNIPAAm-COOH (N_{400}) at pH 10; (b) TEM image of the HOOC-PNIPAAm-COOH (N_{400}) at pH 10.0 and 60 °C[25]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

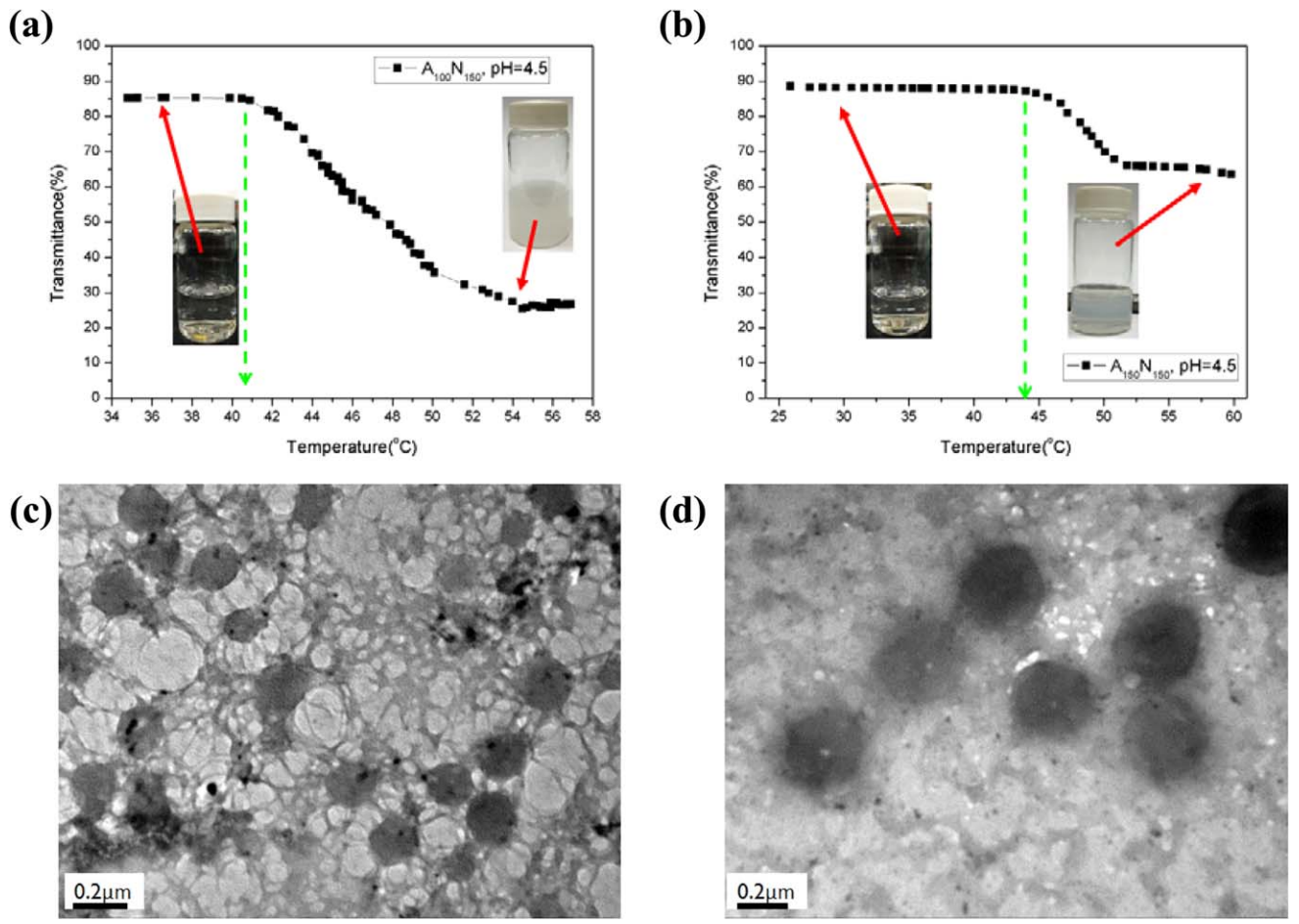

FIGURE 6 Temperature dependence of optical transmittance at 480 nm of the aqueous solutions of $A_{100}N_{150}$ (a) and $A_{150}N_{150}$ (b) both at pH 4.5. TEM images of the $A_{100}N_{150}$ (c) and $A_{150}N_{150}$ (d) both at pH 4.5 and 60 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Thermo-Induced Self-Assembling of $A_{150}N_{75}$

As the proportion of PAA block was over that of PNIPAAm in the triblock copolymer such as $A_{150}N_{75}$, the aforementioned nanogel structure collapsed and a massive coagulation was found at pH 4.5 and 60 °C. Therefore, the $A_{150}N_{75}$ was subjected to a higher pH value of 7.0 in order to provide a sufficient surface charge. Hopefully, spherical nanoparticles could be obtained. At pH 7.0, most carboxylic acid groups in the

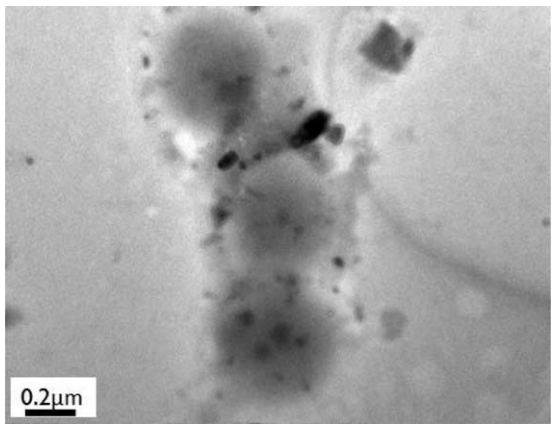

FIGURE 7 TEM image of the self-assembled $A_{150}N_{75}$ nanogels at pH 7.0 and 60 °C.

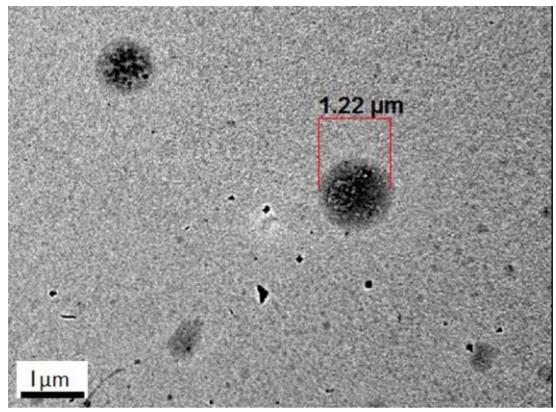

FIGURE 8 TEM image of the self-assembled $A_{150}N_{225}$ at pH 3.9 and 4 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

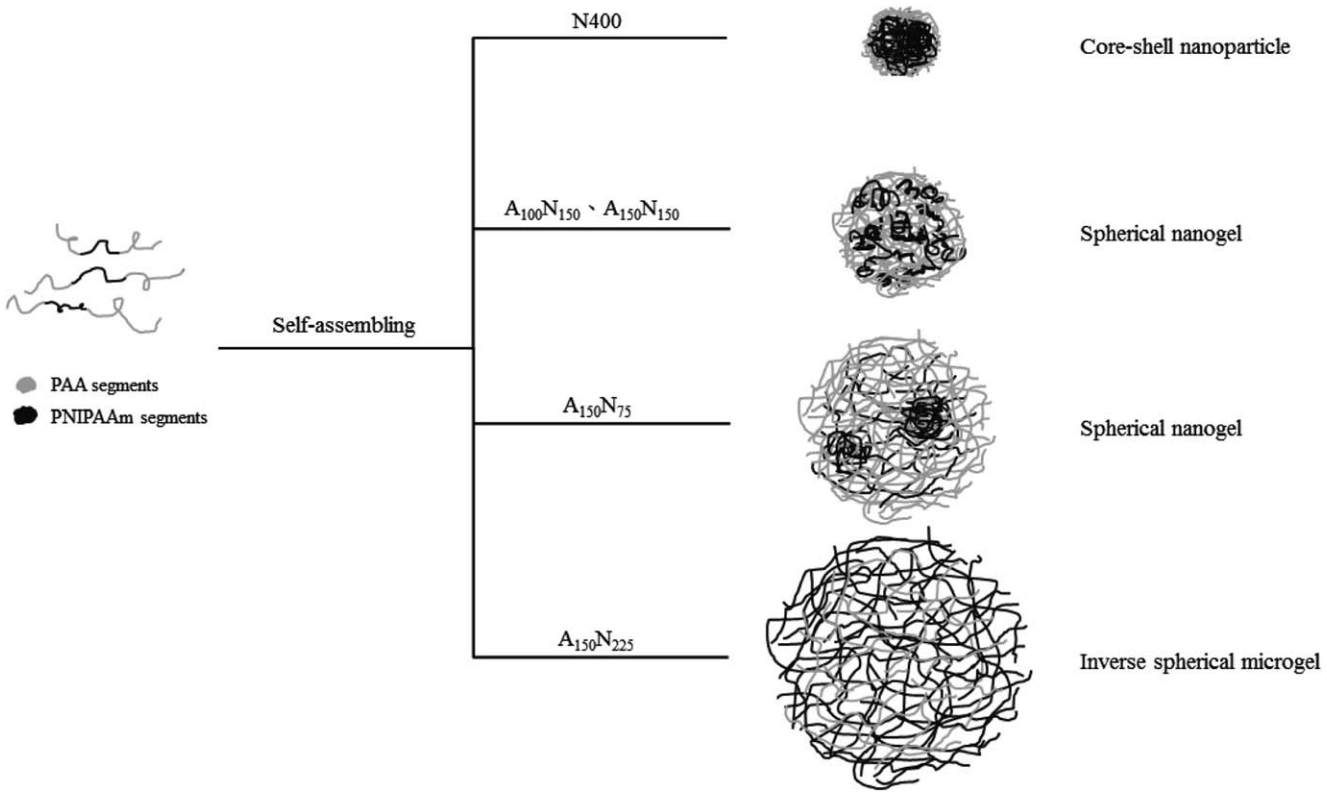

FIGURE 9 Different morphologies of self-assembled P(AA-*b*-NIPAAm-*b*-AA) with various compositions.

PAA block should be dissociated, providing sufficient negative charge to stabilize the nanogel particles in aqueous solution at a temperature higher than its LCST. Figure 7 shows that the $A_{150}N_{75}$ could self-assemble into nanogel particles with size ranging from 450 to 550 nm at pH 7.0 and 60 °C. The zeta potential of $A_{150}N_{75}$ at pH 7.0 and 60 °C is measured as −11.6 mV, supporting that the surface charge is increased with increasing the pH value which is beneficial for stabilizing the nanogels.

pH-Induced self-Assembling of $A_{150}N_{225}$

In the previous copolymer systems, the temperature is above their LCSTs and thus the PNIPAAm block is generally hydrophobic. We can decrease the temperature to below its LCST and render the PNIPAAm block to be hydrophilic. To provide the copolymer with amphiphilic properties, it is necessary to decrease the pH to a value lower than the pK_a of PAA block. The $A_{150}N_{225}$ triblock copolymer was thus dissolved in a solution of pH 3.9 and then cooled down to a temperature of 4 °C. The self-assembling behavior of the $A_{150}N_{225}$ was then monitored. Indeed, some microgel particles are observed as shown in Figure 8. This is because under the acidic environment at pH 3.9, most carboxylic acid groups in the PAA block would not dissociate and maintain their neutral form. Strong inter/intramolecular hydrogen bonding thus could be obtained. As a result, the $A_{150}N_{225}$ triblock copolymer would have a hydrophobic portion and form physical-crosslinking domains. And the hydrophilic nature of PNIPAAm block at 4 °C plays an important role to stabilize the microgels. As a result, a porous structure is observed under TEM observation.

In summary, well-defined, self-assembled nanoparticles of P(AA-*b*-NIPAAm-*b*-AA) could be obtained by manipulating the pH and temperature in order to change the hydrophilic/hydrophobic properties of PAA and PNIPAAm blocks. All the observed morphologies of the P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers with different compositions are depicted in Figure 9. For example, self-assembled nanoparticles of N_{400} and $A_{150}N_{75}$ at 60 °C could be obtained by means of raising the pH value, so that the dissociated carboxylic acid groups would be able to provide sufficient hydrophilicity or surface charge in order to stabilize the spherical structure of nanoparticles. On the other hand, physical-crosslinking via hydrogen bonding played a decisive role to the self-assembled nanogels of $A_{100}N_{150}$ and $A_{150}N_{150}$ triblock copolymers at pH 4.5 and 60 °C. Besides, a pH-induced self-assembling behavior was observed for the $A_{150}N_{225}$ solution by lowering the pH to 3.9 as well as the temperature to 4 °C, where a microgel structure was obtained.

CONCLUSIONS

A series of thermo- and pH-responsive ABA triblock copolymers, P(AA-*b*-NIPAAm-*b*-AA) with various compositions, were synthesized by RAFT copolymerization. GPC and ^{1}H NMR demonstrated the precise synthesis of well-defined copolymers. Their phase transitions, LCSTs and self-assembling behaviors were investigated under various pH

values and temperatures which could alter their hydrophilic/hydrophobic properties. TEM images revealed that the P(AA-*b*-NIPAAm-*b*-AA) copolymers could form nanoparticles in dilute aqueous solution only under some specific environmental conditions. Either physically crosslinked nanogels or core-shell nanoparticles were observed depending on the pH value, temperature and copolymer composition. In addition, various nanoparticles with their sizes ranging from hundreds nanometer to several micrometer could also be obtained. With these properties, it is believed that the P(AA-*b*-NIPAAm-*b*-AA) triblock copolymers have a great potential to be utilized as biomedical nanocapsules for drug encapsulation, smart delivery, triggered release, and targeting therapy.

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