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Direct Synthesis of Biotinylated Stimuli-Responsive Polymer and Diblock Copolymer by RAFT Polymerization Using Biotinylated Trithiocarbonate as RAFT Agent

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ABSTRACT: A new reversible addition−fragmentation chain transfer (RAFT) agent, biotinylated trithiocarbonate, was successfully synthesized. Temperature-responsive biotinylated polymer, poly(*N*-isopropylacrylamide) (PNIPAAm) and water-soluble poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA), have been prepared in one-step via RAFT polymerization using biotinylated trithiocarbonate as the RAFT agent. The results show that the polymerizations of *N*-isopropylacrylamide (NIPAAm) and *N*-(2-hydroxypropyl)methacrylamide (HPMA) are of living characters, and the corresponding polymers produced have ending biotin group. Biotin-ended diblock copolymer of PHPMA and PNIPAAm was also prepared by RAFT polymerization of NIPAAm using biotinylated PHPMA as macro chain transfer agent. The biotinylated PHPMA-*b*-PNIPAAm can form a new kind of core−shell nanostructure with biotin groups on the surface reversibly induced by temperature.

Introduction

Conjugates of biomolecules (antibodies, enzymes or proteins) with polymers combine the properties of the two components, and may lead to unique properties. They can be employed in numerous applications, such as affinity separations, immunoassays, medicine, enzyme recovery and bioengineering.[1−7] As a result, exploration of synthetic strategies to prepare polymers with special ending functionality through which polymers can conjugate to biomolecules has been an active research area for many years. Post-polymerization modification of polymer chain ends was undertaken to form the reactive end group, requiring numerous reaction and purification steps, and the degree of substitution still need to be improved.[8−10] Recently, the development of the controlled/living free radical polymerization techniques, such as stable free radical polymerization,[11−13] atom transfer radical polymerization (ATRP),[14,15] and reversible addition−fragmentation chain transfer (RAFT) polymerization,[16−20] has made it easy to directly synthesize polymers with designed ending functionality and low polydispersity. Controlled/living free radical polymerization has been employed to prepare polymer−protein conjugates. For example, Maynard's group described the preparation of pyridyl disulfide ended poly(2-hydroxyethyl methacrylate) by ATRP from an initiator with pyridyl disulfide group, and the conjugation of the polymers to proteins with thiol group.[21] Haddleton's group synthesized a series of α-functional poly[methoxy poly(ethylene glycol)] methacrylates and poly(glycerol) methacrylates by ATRP, and the conjugates of these polymers with proteins or peptides were successfully prepared.[22−24]

Conjugation of stimuli-responsive polymers to biomolecules yield "smart" bioconjugates that can respond to external stimuli, such as temperature, ionic strength, and pH, and these bioconjugates can be used for biosensors, enzyme recovery, triggered drug release, and affinity separations.[2,4,9] Poly(*N*-isopropylacrylamide) (PNIPAAm) is a thermosensitive polymer that exhibits a lower critical solution temperature (LCST) in water. PNIPAAm−protein conjugates have been synthesized and many applications in biosystem have been discovered.[4,6,25−27] Poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) is a hydrophilic and biocompatible polymer,[28] and the bioconjugates with PHPMA are often used in vehicles for site-specific gene and drug delivery.[29,30]

The affinity between biotin and proteins such as avidin and streptavidin is very strong;[31] therefore, polymer−protein conjugates can be easily formed from these proteins and polymers with a biotin moiety end. Biotinylated polymers can be synthesized by ATRP from biotinylated initiator as described by Wooley's[32] group and Maynard's group.[6,33] However, some water-soluble and stimuli-responsive monomers are not suitable for ATRP, and polymers obtained by ATRP often suffer from contamination of transition metal catalyst, need to be purified before conjugation. RAFT polymerization is arguably more versatile with respect to monomer choice than other living free radical polymerization techniques, can be applied to virtually most kinds of monomers without protection of functional groups, and produces polymers with designed reactive ends such as COOH, OH, and other functional ends.[19−20,34−35] Although Hoffman et al. synthesized biotinylated PNIPAAm by RAFT polymerization,[27] post-polymerization modification was still required to obtain thiol ended PNIPAAm, which was then biotinylated with functionalized biotin. Since thiol groups are easily oxidated, ethylenediaminetetra(acetic acid) was needed to prevent oxidation.

Here, we synthesized biotinylated trithiocarbonate as a RAFT agent, and we prepared biotinylated temperature-responsive PNIPAAm, biotinylated PHPMA, and biotinylated temperature-responsive PHPMA-*b*-PNIPAAm by RAFT polymerization without post-polymerization modification. Biotinylated PHPMA-*b*-PNIPAAm reversibly produced biotinylated core−shell nanoparticles with PNIPAAm as core, PHPMA as shell, and biotin groups on the surface by varying the temperature. These water-soluble polymer precursors should have potential applications in biosystems.

Experimental Section

Materials. Biotin (99%, Sigma-Aldrich), carbonyldiimidazole (reagent grade, Sigma-Aldrich), 2-(2′-aminoethoxy)ethanol (98%,

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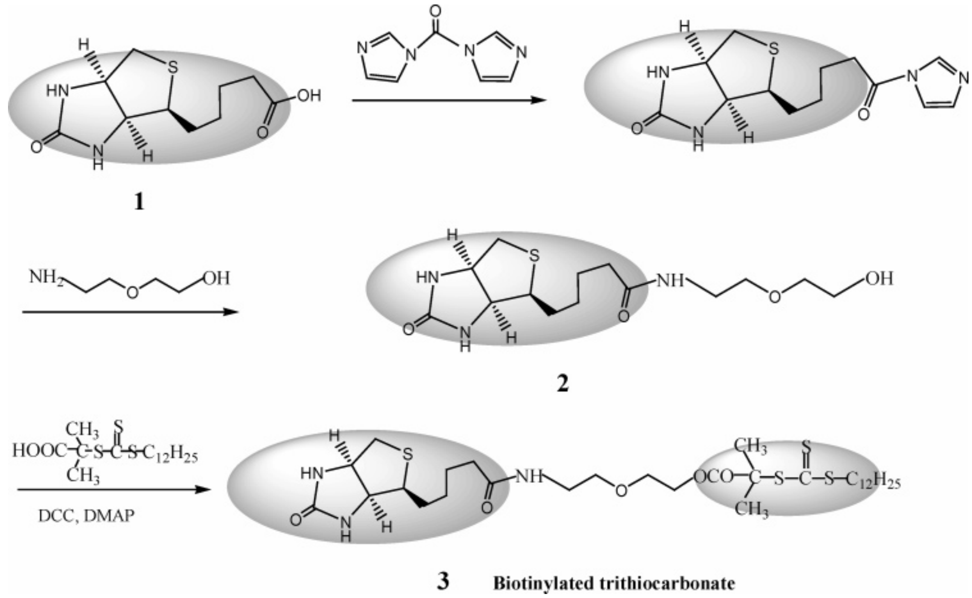
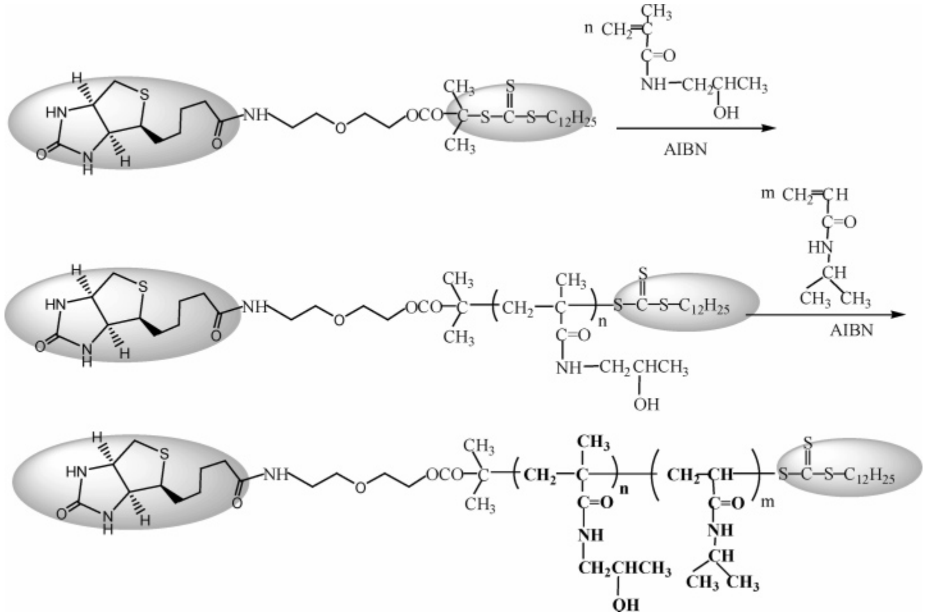
Scheme 1. Synthesis of Biotinylated Reversible Addition−Fragmentation Chain Transfer (RAFT) Agent[a]



[a] DCC = 1,3-dicyclohexylcarbodiimide; DMAP = 4-(*N*,*N*-dimethylamino)pyridine.

Scheme 2. Synthesis of Biotinylated Polymer and Diblock Copolymer[a]



[a] AIBN = 2,2′-azobis(isobutyronitrile).

Sigma-Aldrich), 1,3-dicyclohexylcarbodiimide (DCC, 99%, Sigma-Aldrich), 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 99%, Sigma-Aldrich), 1-dodecanethiol (97%, Fluka), acetone (99.5%, Sigma-Aldrich), tricaprylylmethylammonium chloride (Fluka, Aliquat-336), hexane ($\geq$95%, Aldrich), benzene ($\geq$99%, Aldrich), ethanol (>99.5, Aldrich), methanol ($\geq$99.8%, Aldrich), diethyl ether ($\geq$99.7%, Aldrich), *N*,*N*-dimethyformamide (DMF, $\geq$99.9%, Aldrich), and carbon disulfide ($\geq$99.9%, Sigma-Aldrich) were used as received. *N*-(2-Hydroxypropyl)methacrylamide (HPMA, reagent grade, Polysciences Inc) was recrystallized from methanol/ether (3/1, v/v) prior to use. 2,2′-Azobis(isobutyronitrile) (AIBN, 98%, Sigma-Aldrich) was recrystallized from ethanol and dried at room temperature under vacuum. *N*-Isopropylacrylamide (NIPAAm, 97%, Aldrich) was recrystallized from benzene/hexane (1/2, v/v). All other chemicals (reagent grade) were purchased from Aldrich.

Synthesis of *S*-1-Dodecyl-*S*′-(α,α′-dimethyl-α′′-acetic acid) Trithiocarbonate. 1-Dodecanethiol (20.2 g, 0.10 mol), acetone (58.0 g, 1.0 mol), and tricaprylylmethylammonium chloride (1.0 g, 0.0025 mol) were cooled to 0 °C under a nitrogen atmosphere, and aqueous sodium hydroxide (50%) (8.4 g, 0.105 mol) was added over 10 min. After the mixture was stirred for an additional 20 min, carbon disulfide (7.6 g, 0.10 mol) in acetone (10.0 g) was added over 30 min, and the color gradually turned red. After chloroform (17.8 g, 0.15 mol) was added, 50% aqueous sodium hydroxide (40 g) was added dropwise over 20 min. The mixture was stirred overnight. Water (200 mL) was added, followed by 80 mL of concentrated HCl to acidify the aqueous solution. After removing acetone, the solid was collected, and then stirred in 300 mL of 2-propanol. The undissolved solid was filtered off. The 2-propanol solution was concentrated, and the resulting solid was recrystallized from hexane to afford 12.0 g (yield: 32.9%) of yellow crystalline solid; mp 60−61 °C. ^{1}H NMR (in $CDCl_3$): δ 1.0 (t, 3H), 1.30−1.5 (m, 18H), 1.6−1.8 (m, 8H), 3.2 (t, 2H), 11.0 (s, 1H).

Synthesis of Biotinylated RAFT Agent. Biotin (0.50 g, 2.0 mmol) reacted with carbonyldiimidazole (0.64 g, 4.0 mmol) in *N*,*N*-dimethylformamide (DMF, 20 mL) at room temperature for 6 h. Then, 2-(2-aminoethoxy)ethanol (0.63 mL, 6.0 mmol) was added,

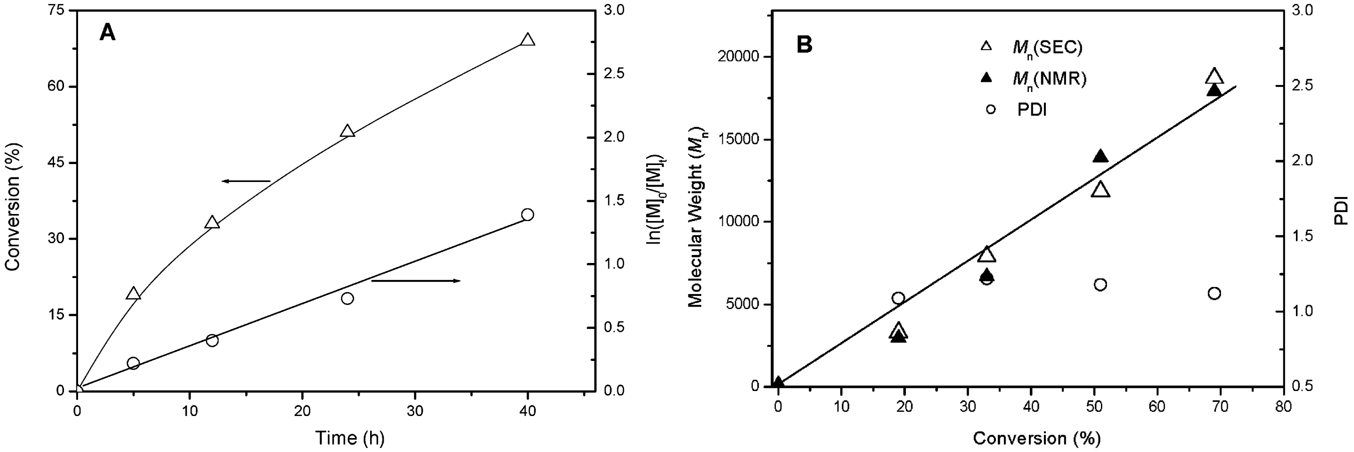


Figure 1. Variation of conversion and ln([M]$_0$/[M]$_t$) with polymerization time for reversible addition−fragmentation chain transfer (RAFT) polymerization of (*N*-isopropylacrylamide) (NIPAAm) (A) and the development of number-average molecular weight (M_n) and polydispersity (PDI) of biotinylated poly(*N*-isopropylacrylamide) (PNIPAAm)with monomer conversion (B).

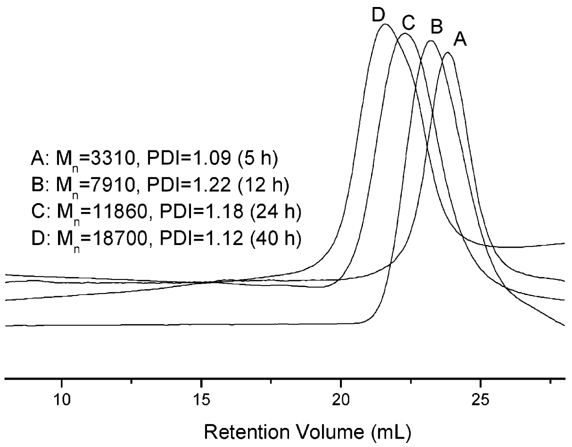


Figure 2. Size exclusion chromatogram (SEC) traces of biotinylated poly(*N*-isopropylacrylamide) (PNIPAAm) obtained at different time interval ([M]/[RAFT agent]/[AIBN] = 175:1:0.1).

and the mixture was stirred for an additional 18 h. After removing the solvent, the biotinylated alcohol was purified by chromatography on silica gel eluting with 1-butanol/acetic acid/water (80:10:10), yield: 58%. ^{1}H NMR (d_6-DMSO), *δ*: 4.2−4.5 ppm (2H, −C***H***−HNCONH−C***H***−), 3.5−3.7 ppm (4H, −C***H***$_2$OC***H***$_2$−), 2.7−2.8 ppm (2H, −CH−C***H***$_2$−S−CH), 2.2 ppm (2H, −C***H***$_2$−CO−), 1.4−1.8 ppm (6H, CHC***H***$_2$C***H***$_2$C***H***$_2$CH$_2$CO−).

Biotinylated alcohol (0.35 g, 1.0 mmol) was then dissolved in DMF (10 mL), and reacted with *S*-1-dodecyl-*S*′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (0.37 g, 1.0 mmol) in the presence of DCC (0.205 g, 1.0 mmol) and DMAP (0.015 g, 0.12 mmol) for 48 h at room temperature. The solid residue was filtered off, and the solvent was evaporated. The product was isolated after column chromatography (chloroform−methanol = 7:3), the yield was 48% (0.32 g, purity: >98%), ^{1}H NMR (d_6-DMSO), *δ*: 4.25−4.35 (2H, −C***H***−HNCONH−C***H***−), 4.10−4.20 (2H, −C***H***$_2$OCO−), 3.5−3.7 (4H, −C***H***$_2$OC***H***$_2$−), 3.0−3.1 (1H, −C***H***−S−), 2.9−3.0 (2H, C$_{11}$H$_{23}$C***H***$_2$−SC(S)−), 2.7−2.8 (2H, −CH−C***H***$_2$−S−CH), 2.2 (2H, −C***H***$_2$−CO−), 1.3−1.8 (32H, −CHC***H***$_2$C***H***$_2$C***H***$_2$CH$_2$CO-, 2-C(COOR)C***H***$_3$, −CH$_2$C$_{10}$***H***$_{20}$CH$_3$), 0.9 (3H, −CH$_2$C***H***$_3$), (−C***H***$_2$−NH is overlapped by water). ^{13}C NMR (d_6-DMSO, ppm): 221.8, 173.3, 167.6, 162.0, 70.2, 69.5, 68.8, 61.8, 60.2, 56.5, 55.1, 42.1, 40.5, 39.1, 36.2, 32.0, 28.2, 28.0, 25.8, 22.9, 14.6. Anal. ($C_{31}H_{55}O_5N_3S_4$) Calcd: C, 54.91; H, 8.18; N, 6.20; S, 18.92. Found: C, 54.25; H, 8.01; N, 6.27; S, 19.0.

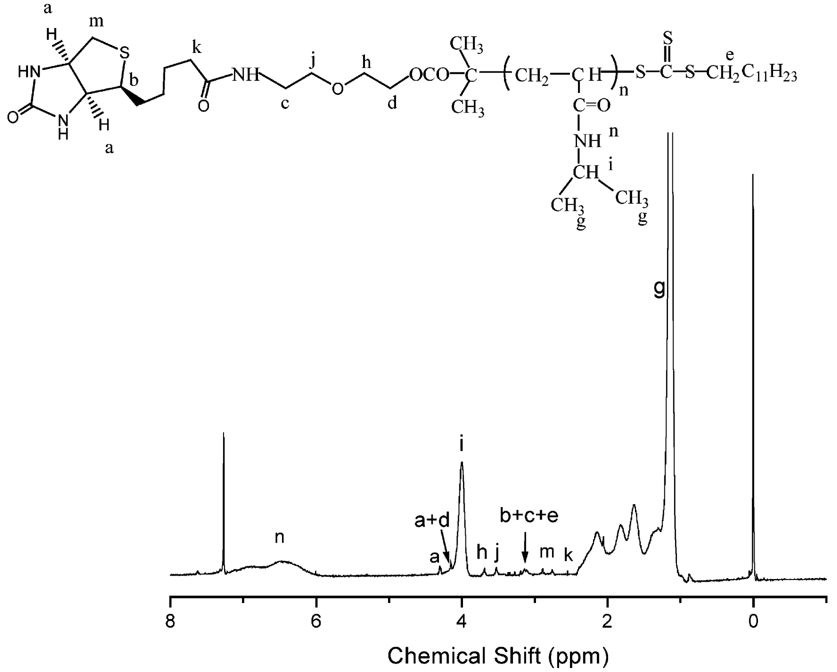


Figure 3. ^{1}H NMR spectrum of biotinylated poly(*N*-isopropylacrylamide) (PNIPAAm) with number-average molecular weight (M_n) of 11860.

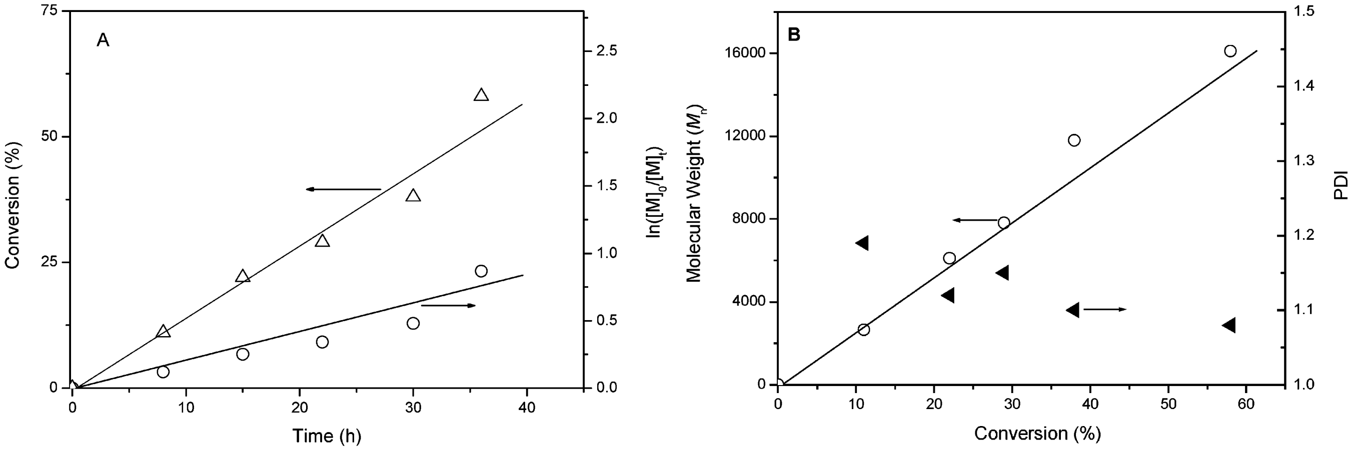


Figure 4. Variation of conversion and ln($[M]_0/[M]_t$) with polymerization time for the reversible addition−fragmentation chain transfer (RAFT) polymerization of (2-hydroxypropyl)methacrylamide (HPMA) (A) and the development of number-average molecular weight (M_n) and polydispersity (PDI) of biotinylated poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) with monomer conversion (B).

RAFT Polymerization of HPMA and NIPAAm with Biotinylated Trithiocarbonate. A solution of HPMA (10 g, 0.07 mol), biotinylated RAFT agent (283 mg, 0.40 mmol), and AIBN (6.7 mg, 0.04 mmol) in methanol (25 mL) was stirred for 10 min to ensure complete dissolution of these reagents. Aliquots were transferred to five different vials, which were then sealed with rubber septa. Each vial was purged with nitrogen for 10 min and immersed in a preheated water bath at 70 °C. Vials were removed at various time intervals, and polymerization was halted by immediate exposure to air and cooling with liquid nitrogen. The samples were analyzed using size exclusion chromatography (SEC) and NMR spectroscopy. The RAFT polymerization procedure of NIPAAm in DMF was similar to that of HPMA.

RAFT Block Copolymerization of NIPAAm with Biotinylated PHPMA as Macrochain Transfer Agent. A solution of biotinylated PHPMA (1.0 g, $M_n = 7800$, PDI = 1.15, 0.128 mmol), NIPAAm (3.0 g, 0.027 mol), and AIBN (2.0 mg, 0.012 mmol) in DMF (8 mL) was stirred for 10 min to ensure complete dissolution. Aliquots were transferred to three different vials, which were then sealed with rubber septa. Each vial was purged with nitrogen for 10 min and then immersed in a preheated water bath at 70 °C. Vials were removed at various time intervals, and the polymerization was halted by immediate exposure to air and cooling with liquid nitrogen.

Measurement of Hydrodynamic Sizes and Phase Transition Temperatures. The hydrodynamic diameters were determined using a Zetaplus particle size analyzer (Brookhaven Instruments) equipped with 35 mW vertically solid-state laser (658 nm). Biotinylated PHPMA-*b*-PNIPAAm (2.0 mg) was dissolved in distilled water (1.0 mL) and filtered through a 0.20 μm PVDF filter prior to measurement. To determine the phase transition temperature, the temperature was in the range of 25−40 °C, and the reading was taken after a 3 min equilibration at each temperature.

Measurements. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 instrument. Number-average molecular weight (M_n) and polydispersity indices (PDI = M_w/M_n) of polymers were determined by SEC in DMF at a flow rate of 1.0 mL·min^{-1} and 35 °C. The SEC system was comprised of a Waters 515 HPLC pump, 3 microStyragel columns (10^3, 10^4, and 10^5 Å), a Waters 2410 RI detector and a BIMwA detector with 30 mW vertically polarized solid state laser (660 nm) as a light source (Brookhaven Instruments, Inc.). SEC data were analyzed using PSS WinGPC Unity software from Polymer Standards. BIDNDC differential refractometer (Brookhaven Instruments, Inc.) was used to determine d*n*/d*c*. The transmittance of the solution was measured at a wavelength of 500 nm using a thermostatically controlled cuvette.

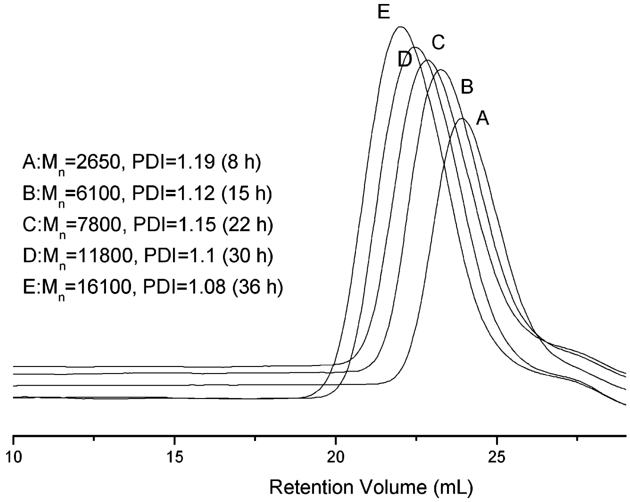


Figure 5. SEC traces of biotinylated poly(*N*-(2-hydroxypropyl)-methacrylamide) (PHPMA) obtained at different time interval ([M]/[RAFT agent]/[AIBN] = 175:1:0.1).

Results and Discussions

RAFT Polymerization of NIPAAm and HPMA Using Biotinylated Trithiocarbonate as RAFT Agent. Biotin has strong affinity with proteins such as avidin and streptavidin, and the biotinylated polymers can conjugate with proteins to form bioconjugates very easily. On the basis of the mechanism of RAFT polymerization,[16−20] the biotinylated polymer can be prepared directly by the RAFT polymerization using biotinylated chain transfer agent without post-polymerization modification. The synthesis of a RAFT agent, biotinylated trithiocarbonate, is shown in Scheme 1; the one-step procedure to synthesize PNIPAAm and PHPMA with a biotin end group and the preparation of the biotinylated diblock copolymer of PHPMA-*b*-PNIPAAm are shown in Scheme 2.

There were some irreversible terminations for propagating radicals in the ATRP of NIPAAm with biotinylated initiator.[33] In our experiment, the polymerization of NIPAAm was performed in DMF to produce biotinylated PNIPAAm using biotinylated trithiocarbonate as chain transfer agent. During the polymerization, the kinetic plot of ln($[M]_0/[M]_t$) vs polymerization time was linear (Figure 1A). The results for development of number-average molecular weight (M_n) and PDI of the biotinylated PNIPAAm are shown in Figure 1B. It is clear that M_n increased linearly with the increase of NIPAAm conversion and the PDIs were narrow, all M_ns obtained from SEC are close to the molecular weight values calculated from ^{1}H NMR based on the integral ratio of the peak at 4.0 ppm to the peak at 4.3 ppm, as listed in Figure 1B. The SEC results of the biotinylated

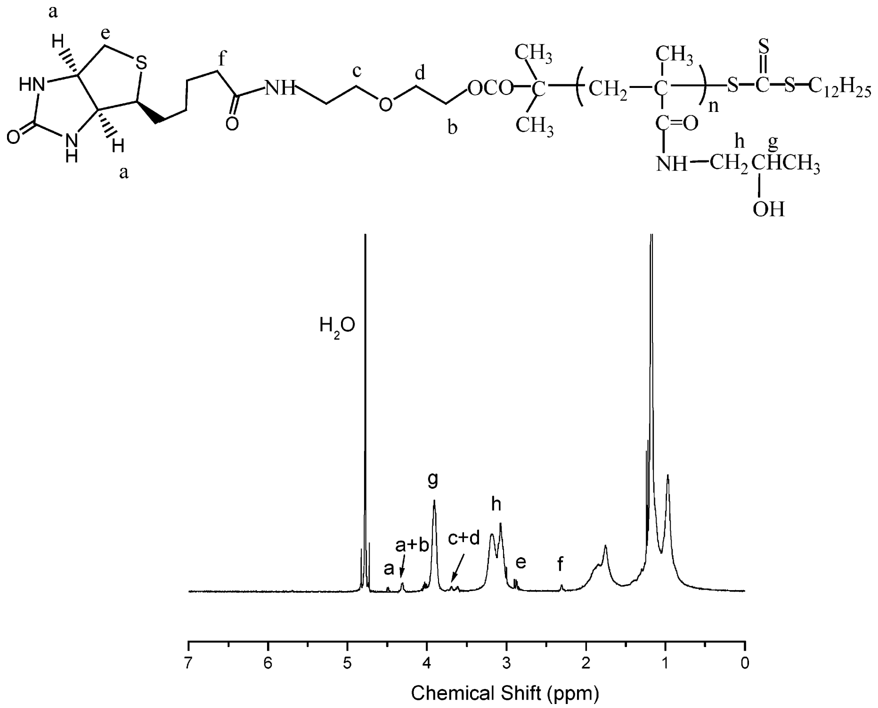


Figure 6. ^{1}H NMR spectrum (in D_2O) of biotinylated poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) with number-average molecular weight (M_n) of 7800 and polydispersity (PDI) of 1.15.

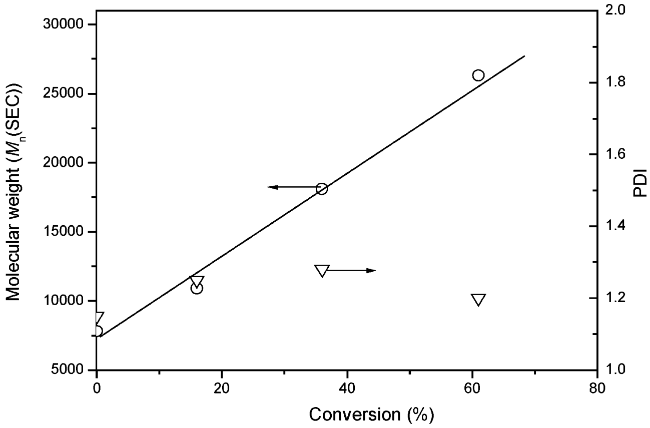


Figure 7. Relationship of number-average molecular weight and polydispersity (PDI) of biotinylated poly(*N*-(2-hydroxypropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) (PHPMA-*b*-PNIPAAm) with NIPAAm conversion.

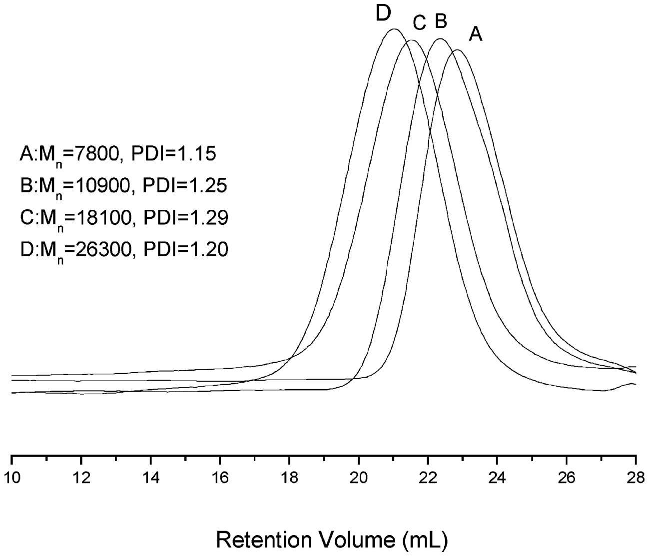


Figure 8. SEC traces of biotinylated poly(*N*-(2-hydroxypropyl)-methacrylamide)-*b*-poly(*N*-isopropylacrylamide) (PHPMA-*b*-PNIPAAm) obtained at different time interval ([M]/[RAFT agent]/[AIBN] = 210: 1:0.1).

PNIPAAm obtained at different intervals are shown in Figure 2. All of the SEC curves are symmetric, which indicates that there was almost no irreversible termination for the propagating radicals. Therefore, the RAFT polymerization of NIPAAm using biotinylated trithiocarbonate as chain transfer agent is of living character, and biotinylated trithiocarbonate is effective in the RAFT polymerization of NIPAAm.

The ^{1}H NMR spectrum of biotinylated PNIPAAm is shown in Figure 3. The characteristic resonances for PNIPAAm were very clear, such as signals at 4.0 and 1.1 ppm ascribed to methine α to NH and methyl protons, respectively. It is easy to identify some signals of the biotinylated RAFT agent, such as signals at 4.3 and 4.1 ppm for methine protons α to amide in the biotin unit and ester methylene protons of biotinylated trithiocarbonate; 3.7 and 3.5 ppm corresponding to methylene protons ($-C\boldsymbol{H}_2-O-C\boldsymbol{H}_2-$), 3.1 ppm for the methine proton α to sulfur in the biotin unit, the methylene protons α to the trithiocarbonate unit ($-S-C\boldsymbol{H}_2C_{11}H_{23}$), and methylene protons α to amide ($-C\boldsymbol{H}_2-NH-$), which indicate that PNIPAAm with ending biotin group has been successfully synthesized by RAFT polymerization using biotinylated trithiocarbonate as RAFT agent.

HPMA was polymerized in methanol using biotinylated trithiocarbonate as a RAFT agent to produce biotinylated PHPMA. The relationship of conversion and ln([M]$_0$/[M]$_t$) with polymerization time were plotted as shown in Figure 4A; ln([M]$_0$/[M]$_t$) increased linearly with polymerization time. The number-average molecular weights and PDIs for the produced biotinylated PHPMA were also obtained by SEC, and the results are shown in Figure 4B. Molecular weight increased linearly with the increase of HPMA conversion, and PDIs are narrow throughout the polymerization. The SEC curves of the produced

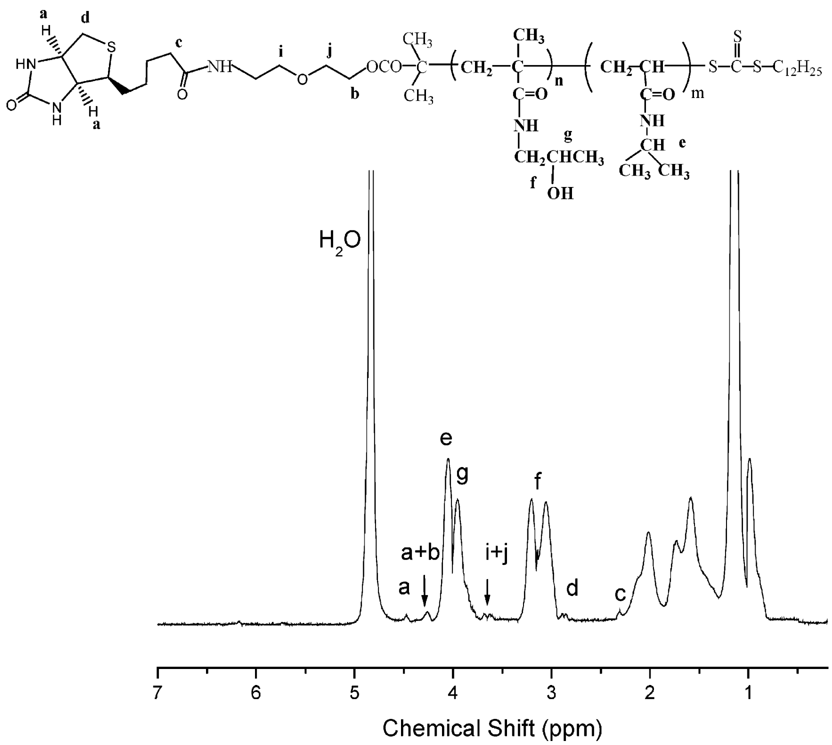
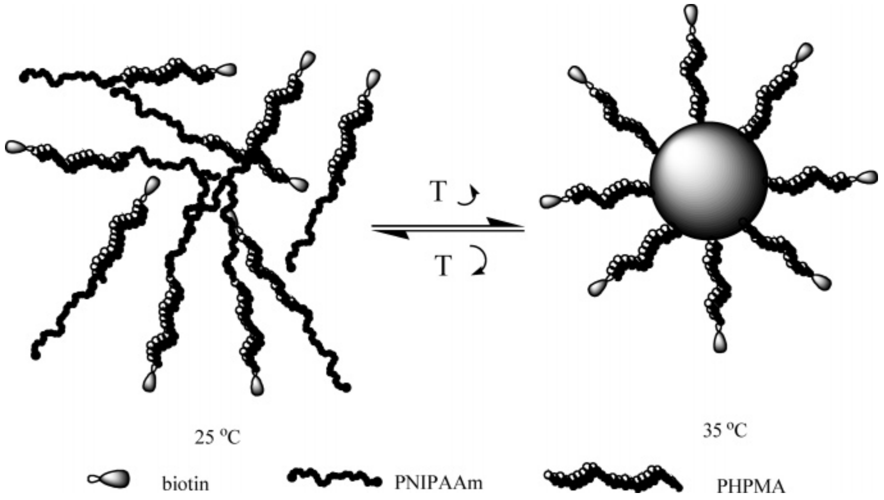


Figure 9. ^{1}H NMR spectrum (in D_2O) of biotinylated poly(*N*-(2-hydroxypropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) (PHPMA-*b*-PNIPAAm) with number-average molecular weight of 18100, polydispersity (PDI) of 1.29.

Scheme 3. Schematic of the Formation of Nano Core−Shell Structure with Biotin on the Surface Induced by Temperature



biotinylated PHPMA are shown in Figure 5. All of the curves are symmetric, which indicates that biotinylated trithiocarbonate is an effective RAFT agent for the RAFT polymerization of HPMA.

A typical ^{1}H NMR spectrum is shown in Figure 6, and the characteristic peaks for PHPMA were clearly found, such as signals at 3.9 ppm is ascribed to the methine proton ($-\mathrm{CH_2C}\boldsymbol{H}\mathrm{(OH)CH_3}$) of hydroxypropyl unit, signals at 3.0−3.4 ppm are for methylene protons ($-\mathrm{NH}-\mathrm{C}\mathbf{H}_2-$), signals at 0.7−2.2 ppm are assigned to methylene protons in the main chain of PHPMA, the protons of the side methyl unit, and the methyl protons of hydroxypropyl unit. The resonances due to the biotinylated RAFT agent appear at 4.5 and 4.3 ppm for methine protons α to NH in the biotin unit and ester methylene protons of biotinylated trithiocarbonate; 2.9 ppm is observed for methylene protons α to sulfur in the biotin unit and 2.3 ppm for the methylene protons α to acrylamide, which indicate that biotin-terminated PHPMA has been successfully synthesized by RAFT polymerization using biotinylated trithiocarbonate as RAFT agent.

Synthesis of Biotinylated Diblock Copolymer Using Biotinylated Macrochain Transfer Agent. A biotinylated diblock copolymer of PHPMA and PNIPAAm with well-defined structure could be prepared by the RAFT polymerization of NIPAAm using biotinylated PHPMA (M_n = 7800) as the macrochain transfer agent. The number-average molecular weight (M_n) development of the biotinylated diblock copolymer was traced by SEC. The result showed that the M_n of the block copolymer increased linearly with NIPAAm conversion, and the PDIs remained narrow throughout the polymerization as shown in Figure 7. The SEC curves of the block copolymers are shown in Figure 8; all of the peaks are symmetric, with no shoulders. Therefore, the RAFT polymerization of NIPAAm using biotinylated PHPMA as macrochain transfer agent can be controlled. A typical ^{1}H NMR spectrum of PHPMA-*b*-PNIPAAm is shown in Figure 9, and the characteristic

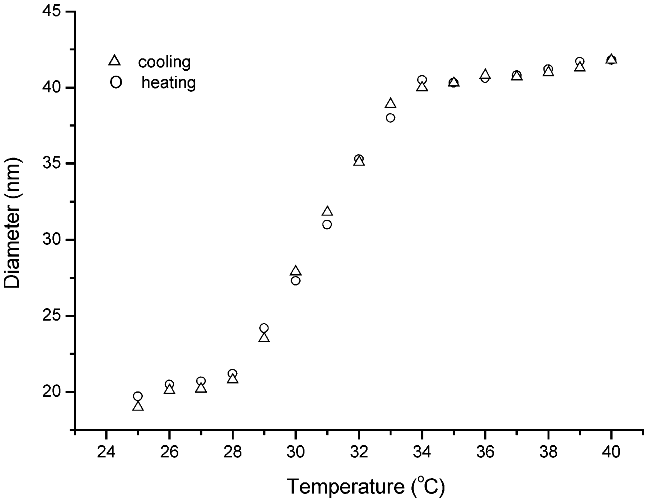


Figure 10. Change in diameter for biotinylated poly(*N*-(2-hydroxypropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) ($(HPMA)_{50}$-*b*-$(NIPAAm)_{90}$, number-average molecular weight (M_n) = 18 100, polydispersity (PDI) = 1.29) in distilled water with temperature.

resonances for PHPMA (such as signal at 3.9 ppm ascribed for the methine proton ($-CH_2C\boldsymbol{H}(OH)CH_3$) of hydroxypropyl unit) and PNIPAAm (such as the signal at 4.0 ppm ascribed to the methine proton of the isopropyl unit) were clearly found. Besides, some small peaks can be attributed to the biotinylated RAFT agent, such as signals at 4.5 and 4.2 ppm for methine protons and ester methylene proton, and 2.9 ppm for methylene protons α to sulfur in the biotin unit, which indicates that the produced diblock copolymer of PHPMA and PNIPAAm contains a biotin end group.

Reversible Formation of Biotinylated Nano Core−Shell Structure Induced by Temperature. Biotinylated nanoparticles and microparticles have high application potential and fundamental significance, and they can be utilized as model systems to study and mimic the multivalent interactions that occur in protein-cell recognition and cell−cell adhesion processes.[36−40] Recently, Wooley et al. reported the preparation of biotinylated shell cross-linked nanoparticles from poly(acrylic acid)-*b*-poly(methyl acrylate) with a biotin end group.[32]

PNIPAAm is a smart polymer whose conformation and solubility in water will change with temperature. At temperatures below the LCST, the intermolecular hydrogen bonding between the PNIPAAm chains and water molecules is predominant, the PNIPAAm chains are soluble in water; at temperatures above the LCST, intramolecular hydrogen bonding between C═O and N−H groups in PNIPAAm results in a compact and collapsed conformation of PNIPAAm chains, PNIPAAm is insoluble in water. Previous results showed that aggregation of PNIPAAm-*b*-PEO induced by temperature can form core−shell nanostructure with PNIPAAm core and PEO shell.[41] Biotinylated PHPMA-*b*-PNIPAAm is soluble in distilled water at 25 °C. However, PNIPAAm becomes insoluble in distilled water, and some PNIPAAm chains were collapsed together to form a nanoparticle with the increase of temperature above 32 °C, while the PHPMA chains were still soluble in water at this temperature and act as a shell to stabilize the nanoparticle (as shown in Scheme 3). Thus, core−shell structure with biotin on the surface can be formed from the biotinylated diblock copolymer of PHPMA and PNIPAAm.

We use the light scatter meter to trace the change of diameter of diblock copolymer with temperature and the formation of core−shell structure. Figures 10 and 11 shows that the diameter of PHPMA-*b*-PNIPAAm change from 19.7 to 42.0 nm when the temperature increased from 25 to 40 °C, and there is a sharp change in diameter at 32 °C, which corresponds to the phase transition of PNIPAAm from soluble to insoluble and PNIPAAm chains collapsed together to form a nanoparticle core. The diameter size increased from 19.7 to 42.0 nm, which results from that the solubility of PNIPAAm chains become poor with the increase of temperature, and some PNIPAAm chains entangled together to form a core, while the PHPMA chains are soluble to form shell at periphery of PNIPAAm core and stabilize the nanoparticle. Figure 11 shows the log−normal size distribution results of biotinylated PHPMA-*b*-PNIPAAm in distilled water at 25 °C (A) and biotinylated nanoparticles with PNIPAAm as core and biotinylated PHPMA as shell formed from biotinylated PHPMA-*b*-PNIPAAm in distilled water at 40 °C (B).

Poly(*N*-(2-hydroxypropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) (PHPMA-*b*-PNIPAAm) is sensitive to temperature, which also can be verified by the variation of transmittance at 500 nm for biotinylated PHPMA-*b*-PNIPAAm in distilled water with temperature. The transmittance at 500 nm

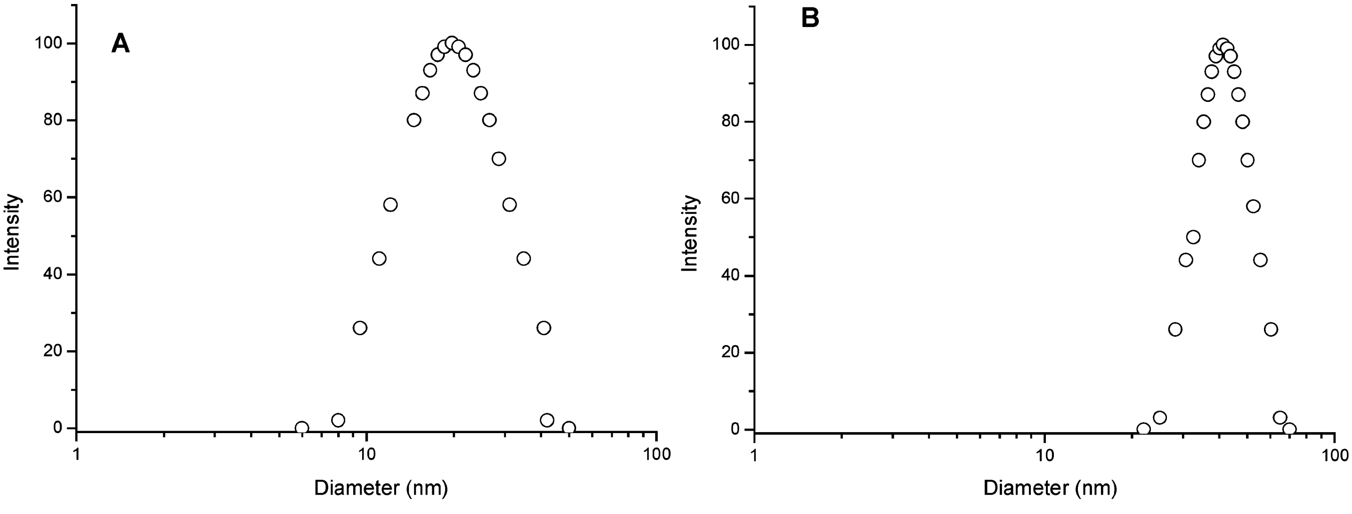


Figure 11. Log-normal size distribution results of biotinylated poly(*N*-(2-hydroxypropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) ($(HPMA)_{50}$-*b*-$(NIPAAm)_{90}$, number-average molecular weight (M_n) = 18 100, polydispersity (PDI) = 1.29) in distilled water at 25 °C (A) and biotinylated nanoparticles with PNIPAAm as core and biotinylated PHPMA as shell formed from biotinylated PHPMA-*b*-PNIPAAm in distilled water at 40 °C (B).

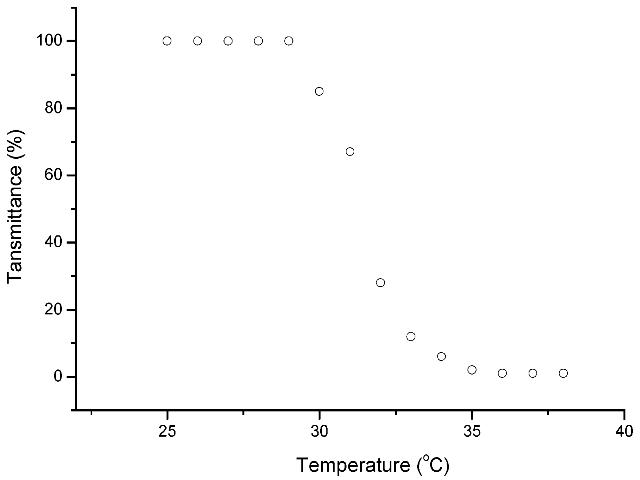


Figure 12. Variation of transmittance of poly(*N*-(2-hydroxypropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) ((HPMA)$_{50}$-*b*-(NIPAAm)$_{90}$, number-average molecular weight (M_n) = 18 100, polydispersity (PDI) = 1.29) in distilled water with temperature.

decreases with the increase of temperature as shown in Figure 12, which was resulted from that PNIPAAm chain become poorly soluble and collapsed together when temperature was increased, and there is a sharp transition at 32 °C, which corresponds to the phase transition temperature of PNIPAAm.

The conjugation of biotinylated smart PNIPAAm and PNIPAAm-*b*-PHPMA with protein, and potential applications of the formed biotinylated nanoparticles in controlled release and targeting delivery of drugs are in progress.

Summary

Biotinylated PNIPAAm, PHPMA, and PHPMA-*b*-PNIPAAm were successfully prepared by RAFT polymerization using biotinylated trithiocarbonate as chain transfer agent. The biotinylated trithiocarbonate is an effective RAFT agent for the polymerization of NIPAAm and HPMA. The biotinylated homopolymers and block polymers have controlled molecular weights and narrow PDIs. The biotinylated PHPMA-*b*-PNIPAAm has a temperature-responsive PNIPAAm segment, which can reversibly form a new core−shell nanostructure with biotin on the surface induced by temperature. The conjugations of these polymer precursors with proteins are in progress.

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