Aqueous RAFT Synthesis of pH-Responsive Triblock Copolymer mPEO−PAPMA−PDPAEMA and Formation of Shell Cross-Linked Micelles†

Xuewei Xu,‡ Adam E. Smith,‡ Stacey E. Kirkland,‡ and Charles L. McCormick*,‡,§

Department of Polymer Science and Department of Chemistry and Biochemistry, The University of Southern Mississippi, Hattiesburg, Mississippi 39406

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ABSTRACT: Herein we report the synthesis and characterization of polymeric cross-linking agents and novel shell cross-linked (SCL) micelles utilizing reversible addition−fragmentation chain transfer (RAFT) polymerization. A series of pH-responsive ABC triblock copolymers consisting of α-methoxypoly(ethylene oxide)-*b*-poly[*N*-(3-aminopropyl)methacrylamide]-*b*-poly[2-(diisopropylamino)ethyl methacrylate] (mPEO−PAPMA−PDPAEMA) have been synthesized via RAFT polymerization in aqueous media at 70 °C employing a PEO-based macro-chain transfer agent (macro-CTA). These triblock copolymers molecularly dissolve in aqueous solution at low pH (<5.0) due to protonation of primary amine residues on the PAPMA block and tertiary amine residues on the PDPAEMA block. Above pH 6.0, the polymers self-assemble into micelles consisting of PDPAEMA cores, PAPMA shells, and mPEO coronas. Hydrodynamic dimensions of the triblock copolymer micelles depend on both triblock copolymer composition and solution pH. Narrowly dispersed poly(*N*-isopropylacrylamide) was synthesized utilizing the difunctional CTA, 2-(1-carboxy-1-methylethylsulfanylthiocarbonylsulfanyl)-2-methyl-propionic acid (CMP). The chain ends of the PNIPAM were converted from carboxylic acids to *N*-hydroxysuccinimidyl esters (NHS) through dicyclohexylcarbodiimide (DCC) coupling, yielding an amine-reactive polymeric cross-linking agent, NHS−PNIPAM−NHS. SCL micelles were attained via reaction of PAPMA (shell) amine functionality with NHS-functionalized PNIPAM. These SCL micelles swell when the solution pH is lowered below the pK_a of the PDPAEMA block. The polymeric cross-linking agent NHS−PNIPAM−NHS synthesized in this work has inherent temperature-responsive segments and a cleavable trithiocarbonate unit which have future potential in mediating drug delivery from SCL micelles.

Introduction

In the past decade, structural design of shell cross-linked (SCL) micelles has received considerable attention since a combination of properties inherent to micelles, microgels, nanoparticles, and dendrimers can be achieved. Potential applications of SCL micelles include drug delivery, controlled release, emulsification, sequestration of metabolites, and entrapment of environmental pollutants.[1−18] The first examples of SCL micelles were reported by Wooley and co-workers,[19] who oligomerized pendant styrene groups using free radical chemistry. Numerous strategies have since been developed for the shell cross-linking of micelles including carbodiimide coupling,[20−23] 1,2-bis(2-iodoethoxy)ethane (BIEE),[24−26] divinyl sulfone (DVS),[27] glutaraldehyde,[28] activated esters,[29,30] polyelectrolyte complexation,[31,32] metal-catalyzed cross-linking,[33] and click chemistry.[34] Recently, our group developed a shell cross-linking method[29,30] which utilizes a facile reaction between activated esters and primary amines to prepare SCL micelles and reversible SCL micelles. For an excellent review on shell cross-linked micelles, the reader is referred to a recent publication by Read and Armes.[35]

Stimuli-responsive polymers, also called "smart polymers", can undergo phase transitions in response to external stimuli (temperature, pH, ionic strength, light, photochemical processes, etc.).[28,36−40] In particular, copolymers which exhibit a unimer-to-micelle transition in response to changes in temperature or pH have been extensively investigated due to potential biomedical applications.[30] In many cases, control of the solution pH is more convenient than manipulation of temperature or salt concentration.[41−45] Moreover, pH-induced micellization has been reported to yield micelle cores with greater hydrophobic character than those produced by temperature-induced micellization.[44]

Controlled/"living" radical polymerization techniques provide a powerful tool for synthesizing well-defined and complex architectures. Such techniques include nitroxide-mediated polymerization (NMP),[46] atom transfer radical polymerization (ATRP),[47] and reversible addition−fragmentation chain transfer (RAFT) polymerization.[48−51] Among these, RAFT is arguably the most versatile since it is compatible with a wide variety of functional monomers under conditions similar to those used in conventional free radical polymerization.[52,53] Our group has a long-standing interest in the aqueous RAFT (co)polymerization of anionic,[54] cationic,[55] zwitterionic,[56] and neutral[29,57] monomers to prepare water-soluble (co)polymers.

Herein, we report the successful aqueous RAFT polymerization of *N*-(3-aminopropyl)methacrylamide (APMA) using an α-methoxypoly(ethylene oxide) (mPEO)-based macro-chain transfer agent (macro-CTA) and subsequent chain extension with 2-(diisopropylamino)ethyl methacrylate (DPAEMA), generating mPEO−PAPMA−PDPAEMA triblock copolymers. In aqueous solution, this triblock copolymer self-assembles to form PDPAEMA−core micelles when the solution pH is increased above the pK_a of the PDPAEMA block. These micelles can be subsequently shell cross-linked using poly(*N*-isopropylacrylamide) (PNIPAM) end-functionalized with activated ester *N*-hydroxysuccinimide (NHS−PNIPAM−NHS). The polymeric cross-linking agent NHS−PNIPAM−NHS was prepared via RAFT polymerization of NIPAM using a difunctional CTA, 2-(1-carboxy-1-methylethylsulfanylthiocarbonylsulfanyl)-2-me-

* To whom correspondence should be addressed. E-mail: Charles.McCormick@usm.edu.

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‡ Department of Polymer Science.

§ Department of Chemistry & Biochemistry.

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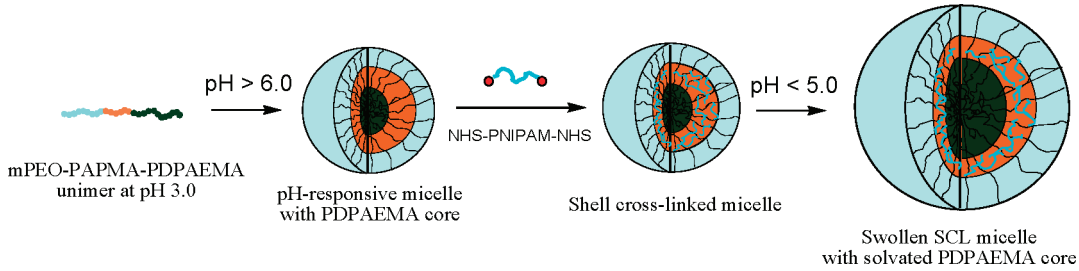
Scheme 1. pH-Responsive Micellization of mPEO−PAPMA−PDPAEMA Triblock Copolymer and Formation of Related SCL Micelles via Polymeric Cross-Linking Agent NHS−PNIPAM−NHS



thylpropionic acid (CMP). The PNIPAM cross-linking agent introduces potential temperature responsiveness while preventing the dissolution of micelles due to dilution effects. Additionally, the trithiocarbonate unit via RAFT polymerization of NIPAM presents a potentially cleavable site for micelle disassembly. The various morphologies accessible through manipulation of pH are represented in Scheme 1. Such pH-responsive SCL micelles have potential applications in drug delivery and controlled release.

Experimental Section

Materials. DPAEMA was purchased from Scientific Polymer Products Inc. and passed through a DHR-4 column to remove inhibitor prior to use. mPEO (M_n = 2000 g/mol, M_w/M_n = 1.10) (Sigma-Aldrich) and APMA hydrochloride (Polysciences Inc.) were used directly. NIPAM (Aldrich) was recrystallized twice from hexane. CMP was donated by Noveon and used as received. 4,4′-Azobis(4-cyanopentanoic acid) (V-501) was donated by Wako Chemicals. NHS (97%), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 99%), 4-(dimethylamino)pyridine (DMAP), ethyl ether, dioxane, and hexane were all purchased from Aldrich and used as received.

Characterization. The molecular weights and polydispersity indices (PDIs) of the copolymers were determined by aqueous size exclusion chromatography (ASEC) using an aqueous eluent of 1.0 wt % acetic acid/0.1 mol/L Na_2SO_4. A flow rate of 0.25 mL/min at 25 °C, Eprogen Inc. columns [CATSEC1000 (7 μm, 50 × 4.6), CATSEC100 (5 μm, 250 × 4.6), CATSEC1000 (7 μm, 250 × 4.6), and CATSEC300 (5 μm, 250 × 4.6)], a Polymer Laboratories LC 1200 UV/vis (λ = 275 nm), a Wyatt Optilab DSP interferometric refractometer (λ = 690 nm), and Wyatt DAWN DSP multiangle laser light scattering detectors (λ = 690 nm) were utilized, and Wyatt DNDC for Windows was used for d*n*/d*c* determinations. The molecular weights and PDIs of the polymeric cross-linking agent PNIPAM were determined by size exclusion chromatography (SEC)

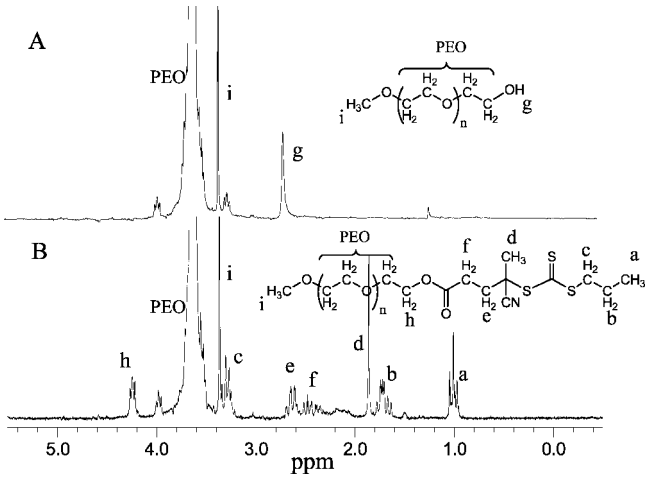


Figure 1. ^{1}H NMR spectra of mPEO (A) and macro-CTA mPEO−CPP (B) in $CDCl_3$.

Scheme 2. Synthetic Route for Preparation of pH-Responsive mPEO−PAPMA−PDPAEMA Triblock Copolymer via Aqueous RAFT Polymerization

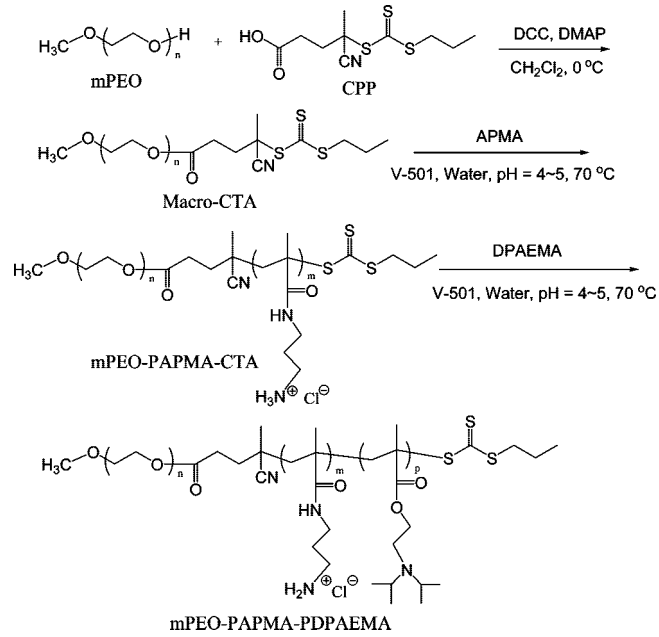


Table 1. Molecular Weight Data of mPEO−APMA Diblock Copolymers and mPEO−PAPMA−PDPAEMA Triblock Copolymers Prepared via Aqueous RAFT Polymerization

diblock/ triblock copolymers	M_n[a]	PDI[a]	M_n[b]	composition[b]
A	3 900	1.10	3 800	$mPEO_{46}$−$PAPMA_9$
B	4 000	1.12	4 100	$mPEO_{46}$−$PAPMA_{10}$
C	6 000	1.10	6 000	$mPEO_{46}$−$PAPMA_{21}$
D	10 300	1.08	9 200	$mPEO_{46}$−$PAPMA_{39}$
A1	14 200	1.19	11 000	$mPEO_{46}$−$PAPMA_9$−$PDPAEMA_{34}$
B1	20 300	1.26	18 400	$mPEO_{46}$−$PAPMA_{10}$−$PDPAEMA_{68}$
C1	16 800	1.18	13 700	$mPEO_{46}$−$PAPMA_{21}$−$PDPAEMA_{36}$
C2	31 800	1.23	27 600	$mPEO_{46}$−$PAPMA_{21}$−$PDPAEMA_{101}$

[a] As determined by ASEC, d*n*/d*c* = 0.164 (diblock copolymer) and d*n*/d*c* = 0.153 (triblock copolymer) were used to determine the molecular weights and PDIs. [b] Calculated from ^{1}H NMR data.

in a 0.02 M LiBr DMF eluent (1.0 mL/min, 60 °C). Viscotek I-Series Mixed Bed low-MW and mid-MW columns and Viscotek-TDA (632.8 nm RI, viscosity, 7 mW) 90° and 7° true low angle light scattering detectors (670 nm) were employed. The d*n*/d*c* of PNIPAM was determined to be 0.0731 at 632.8 nm in DMF at 60 °C with a Viscotek refractometer and Omnisec software. NMR spectra were recorded in D_2O or $CDCl_3$ with either a Varian Mercury 200 MHz or a Mercury Innova 500 MHz spectrometer. Dynamic light scattering (DLS) studies of the block copolymer at concentrations of 0.5 g/L in aqueous solution were conducted using a Malvern Instruments Zetasizer Nano ZS instrument equipped with

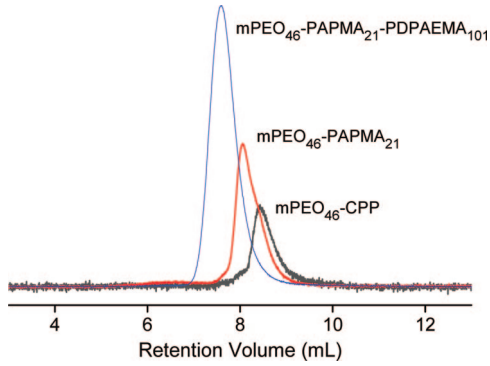


Figure 2. Typical ASEC traces of macro-CTA ($mPEO_{46}$−CPP), diblock copolymer ($mPEO_{46}$−$PAPMA_{21}$), and triblock copolymer ($mPEO_{46}$−$PAPMA_{21}$−$PDPAEMA_{101}$).

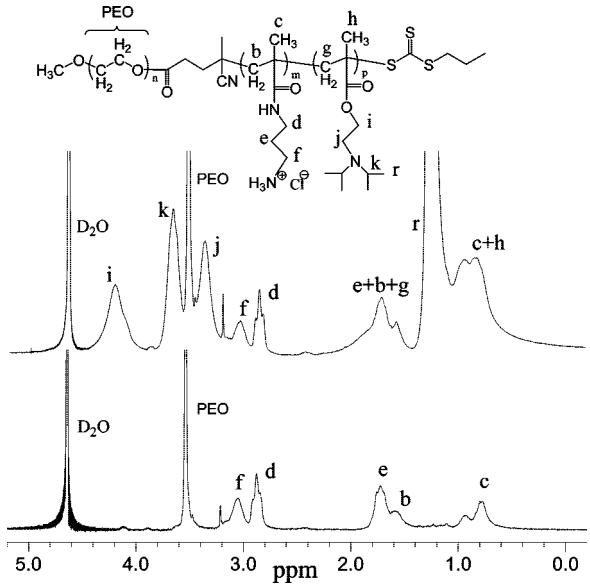


Figure 3. ^{1}H NMR spectra of diblock copolymer $mPEO_{46}$−$PAPMA_{21}$ (bottom) and triblock copolymer $mPEO_{46}$−$PAPMA_{21}$−$PDPAEMA_{101}$ (top) in D_2O.

a 4 mW He−Ne laser operating at $\lambda = 632.8$ nm, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple tau digital correlator electronics system. Atomic force microscopy (AFM) images were collected in tapping mode using standard RTESP silicon cantilever (length: 125 μm; spring constant: 20−80 N/m; resonance frequency: ∼300 kHz) (VEECO Probes, CA) in Dimension 3000 scanning probe microscope (Digital Instruments).

Synthesis of Monofunctional CTA 4-Cyano-4-(propylsulfanylthiocarbonyl)sulfanylpentanoic Acid (CPP). 1-Propanethiol (15.23 g, 0.20 mol) was added dropwise to a solution of potassium hydroxide (14.03 g, 0.25 mol) in 70 mL of water, followed by carbon disulfide (15.23 g, 0.20 mol) in one portion. The resulting solution was vigorously stirred at room temperature for 30 min and cooled to −5 °C. *p*-Tosyl chloride (19.05 g, 0.10 mol) in distilled acetone (100 mL) was added in portions over 10 min, and stirring was continued for 2 h. The acetone was then evaporated in an open vessel under stirring. Afterward, the red oil was extracted with dichloromethane; the organic layer was washed with water and dried over anhydrous $MgSO_4$ overnight. The $MgSO_4$ was filtered off, and dichloromethane was removed by rotary evaporation to yield solid bis(propylsulfanylthiocarbonyl) disulfide (27.80 g, yield: 92%) as a red solid. A solution of 4,4′-azobis(4-cyanopentanoic acid) (6.17 g, 0.022 mol) and bis(propylsulfanylthiocarbonyl) disulfide (6.04 g, 0.02 mol) in ethyl acetate (100 mL) was heated under reflux for 20 h. After removal of the volatiles in vacuo, the crude product was purified by column chromatography on silica gel with a mobile phase of diethyl ether/hexane (1/2, v/v). 9.42 g, yield: 85%. ^{1}H NMR ($CDCl_3$): δ 1.02 (t, 3H, **CH_3**−); 1.73 (m, 2H, CH_3−**CH_2**−CH_2−); 1.87 (s, 2H, **CH_3**−) 2.40−2.72 (m, 4H, −**CH_2**−**CH_2**−) 3.31 (t, 2H, −**CH_2**−S−).

Synthesis of PEO-Based Macro-CTA mPEO−CPP. In a 150 mL one-neck round-bottom flask equipped with a magnetic stirring bar, mPEO ($M_n = 2000$ g/mol, $M_w/M_n = 1.10$) (4.00 g, 2.0 mmol) was dissolved in 80 mL of toluene. After azeotropic distillation to remove traces of water, CPP (1.11 g, 4.0 mmol), DMAP (0.24 g, 2.0 mmol), and methylene chloride (50 mL) were added. After the solution was homogenized by stirring, the flask was placed in an ice bath. Then, DCC (0.82 g, 4.0 mmol) was added in portions. After 12 h of stirring at 0 °C, the reaction mixture was increased to room temperature and stirred for another 20 h. The precipitated dicyclohexylurea was filtered off. PEO-based macro-CTA mPEO−CPP was obtained as yellow solid by precipitation of the filtrate into excess diethyl ether three times and dried under vacuum at 35 °C for 24 h. The product (4.11 g, yield: 91%) was stored away from light at 4 °C in a sealed bottle.

Synthesis of mPEO−PAPMA Diblock Copolymers. APMA hydrochloride (2.00 g, 11.2 mmol) and mPEO−CPP (1.13 g, 0.5 mmol) were added along with deionized (DI) water (5.0 mL) to an ampule. V-501 (0.028 g, 0.1 mmol) dissolved in dioxane (1.0 mL) was then added. The solution was stirred until all the mPEO−CPP was dissolved. The ampule contents were sparged with nitrogen for ∼30 min, and then the ampule was placed in a preheated oil bath at 70 °C. The reaction was terminated after 8 h by cooling the reaction tube in an ice bath followed by exposure to air. The product was purified by dialysis against water (pH 4−5) and isolated by lyophilization (conversion: 95% (from ^{1}H NMR data), $M_n = 6000$ g/mol and PDI = 1.10, $mPEO_{46}$−$PAPMA_{21}$).

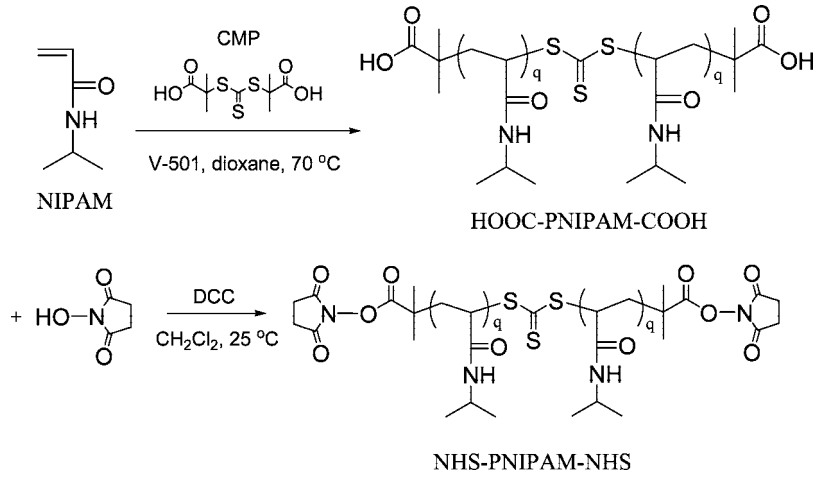
Chain Extension of mPEO−PAPMA. $mPEO_{46}$−$PAPMA_{21}$ (0.30 g, 0.05 mmol) and DPAEMA (1.50 g, 7.05 mmol) were dissolved in DI water (5.0 mL), and the solution pH was adjusted to 4.5. V-501 (0.0028 g, 0.01 mmol) dissolved in dioxane (1.0 mL) was then added. After sparging with nitrogen for 30 min, the polymerization was allowed to proceed at 70 °C for 6 h. The polymerization was quenched by cooling the reaction vessel in an ice bath and exposure to air. The product was purified by dialysis against DI water (pH 4−5) and isolated by lyophilization (conversion: 88% (from ^{1}H NMR data), $M_n = 31\,800$ g/mol, PDI = 1.23, $mPEO_{46}$−$PAPMA_{21}$−$PDPAEMA_{101}$).

Synthesis of NHS−PNIPAM−NHS. NIPAM (1.0 g, 8.84 mmol), CMP (0.056 g, 0.20 mmol), and V-501 (0.011 g, 0.04 mmol) were dissolved in 1,4-dioxane (5.0 mL) in an ampule. The ampule was sparged with nitrogen for ∼30 min and then placed in a preheated oil bath at 70 °C. The reaction was terminated after 10 h by cooling the reaction tube in an ice bath followed by exposure to air. The PNIPAM homopolymer was then precipitated in cold diethyl ether and dried under vacuum at 40 °C (conversion: 88%, $M_n = 4100$ g/mol, PDI =1.12). ^{1}H NMR (DMSO): δ 0.81−1.16 (b, −NH−CH(**CH_3**)**$_2$**); 1.16−1.69 (b, −(CH−**CH_2**)$_n$− and −S−(**CH_3**)$_2$C−COOH); 1.69−2.20 (b, −(**CH**−CH_2)$_n$−); 3.63−3.99 (b, −NH−**CH**(CH_3)$_2$); 11.87−12.20 (b, −**COOH**).

PNIPAM (1.23 g, 0.3 mmol) and NHS (0.104 g, 0.9 mmol) were added to a 100 mL flask and dissolved in 10 mL of CH_2Cl_2. After cooling to 0 °C, DCC (0.186 g, 0.9 mmol) was added. The solution was stirred for 24 h at room temperature. The crude product was then precipitated in cold diethyl ether twice and dried under vacuum at 30 °C (yield: 67%). ^{1}H NMR (DMSO): δ 0.81−1.11 (b, −NH−CH(**CH_3**)**$_2$**); 1.11−1.26 (−S−(**CH_3**)$_2$C−COONHS) 1.26−1.69 (b, −(CH−**CH_2**)$_n$−); 1.69−2.22 (b, −(**CH**−CH_2)$_n$−); 2.75−2.84 (b, −**CH_2**− of the NHS end groups); 3.66−4.00 (b, −NH−**CH**(CH_3)$_2$).

Preparation of Micelles and Shell Cross-Linked Micelles. The triblock copolymer $mPEO_{46}$−$PAPMA_{21}$−$PDPAEMA_{101}$ (10 mg) was molecularly dissolved in DI water (20 mL) at pH 3.0. The solution was filtered through a 0.2 μm filter. The solution pH was slowly increased to a final value of 10.0 in order to induce micelle formation. Shell cross-linking was achieved by adding the difunc-

Scheme 3. Preparation of Temperature-Responsive Polymeric Cross-Linking Agent NHS−PNIPAM−NHS via RAFT Polymerization in Dioxane at 70 °C, Using CMP as CTA and V-501 as the Initiator



tional polymeric cross-linking agent NHS−PNIPAM−NHS at pH 10.0 and stirring the solution for 2 h at room temperature.

Results and Discussion

PEO-Based Macro-CTA mPEO−CPP. PEO-based macroCTAs have been widely used in preparing diblock and triblock copolymers due to the steric stabilization, biocompatibility, and solubility PEO provides in aqueous media. For example, mPEO has been reacted with 4-cyano-4-[(thiobenzoyl)sulfanyl]pentanoic acid (CTP),[58] 3-benzylsulfanylthiocarbonylsufanylpropionic acid (BSPA),[59] and 2-dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid (DMP),[60] etc., to prepare related macroCTAs. In this research, a PEO-based macro-CTA was synthesized via the esterification of the terminal hydroxyl group of mPEO with the carboxyl group of CPP in the presence of DCC and DMAP. The substituted R group is expected to be a sufficiently good leaving group for methacrylate/methacrylamide monomers.[61,62] Figure 1 shows the ^{1}H NMR spectra of mPEO precursor (A) and mPEO−CPP (B) of which resonances of both mPEO and CPP were detected. The presence of the resonance at 4.2 ppm (h), attributed to the mPEO methylene protons (−CH_2−$\mathbf{CH_2}$−OCH(═O)−), confirms the formation of the esterified product. The capping of mPEO with CPP is calculated to be 95% efficient by comparing the peak area of the methyl protons of the CPP fragment at 1.0 ppm (a) and the methyl proton of mPEO at 3.4 ppm (i). This indicates that the macro-CTA mPEO−CPP can be successfully prepared at high yields.

mPEO−PAPMA Diblock and mPEO−PAPMA−PDPAEMA Triblock Copolymers. For the preparation of mPEO−PAPMA−PDPAEMA triblock copolymer, the PEO-based macro-CTA

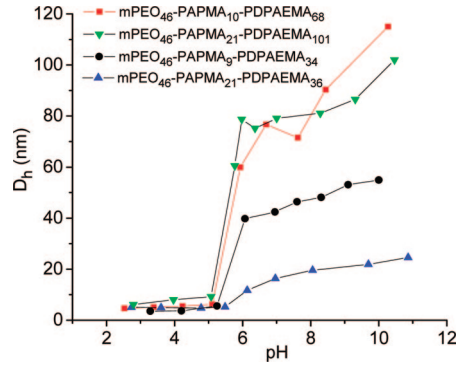
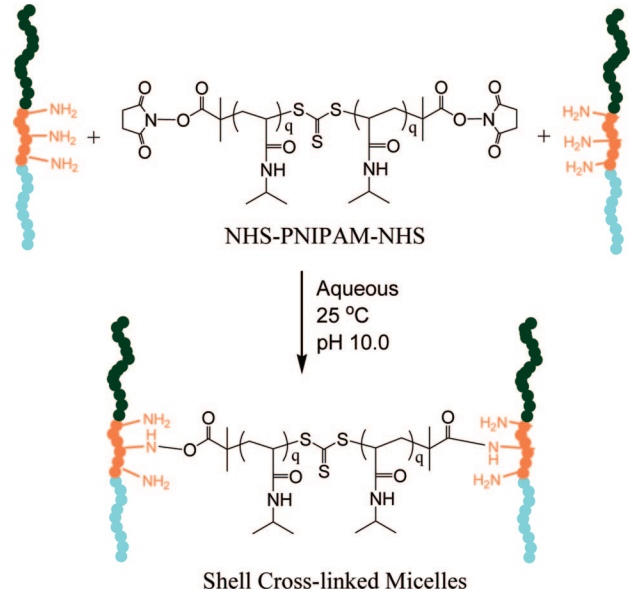


Figure 4. Variation of hydrodynamic diameter (D_h) with solution pH for triblock copolymers mPEO−PAPMA−PDPAEMA at 0.05 wt % in aqueous solution.

Scheme 4. Cross-Linking of the PAPMA Shell via Polymeric Cross-Linking Agent NHS−PNIPAM−NHS in Aqueous Solution at 25 °C



mPEO−CPP was employed to conduct the polymerization of APMA and subsequently DPAEMA in aqueous media at 70 °C, using V-501 as the initiator (Scheme 2). RAFT agents are generally not compatible with primary and secondary amine functionality due to the resulting aminolysis of the CTAs (dithioester or trithiocarbonate).[63] Nevertheless, our group[32] and Armes' group[64] have recently reported the successful RAFT polymerization of APMA in water and 2-aminoethyl methacrylate (AMA) in DMSO, respectively, by carefully controlling the polymerization conditions. In this work, the pH of the aqueous polymerization solution was maintained between 4 and 5 to avoid hydrolysis and/or aminolysis of the CTA.[65] Under these conditions, well-defined mPEO−PAPMA diblock copolymers and mPEO−PAPMA−PDPAEMA triblock copolymers were obtained (Table 1). ASEC chromatograms of the mPEO−CPP, mPEO−PAPMA, and mPEO−PAPMA−PDPAEMA are shown in Figure 2. All the traces are unimodal, and the PDIs are rather low (<1.30), indicating that the blocking efficiency is near-quantitative and the polymerization proceeds in a controlled fashion.

The typical ^{1}H NMR spectra for the mPEO−PAPMA diblock copolymer and the subsequent mPEO−PAPMA−PDPAEMA

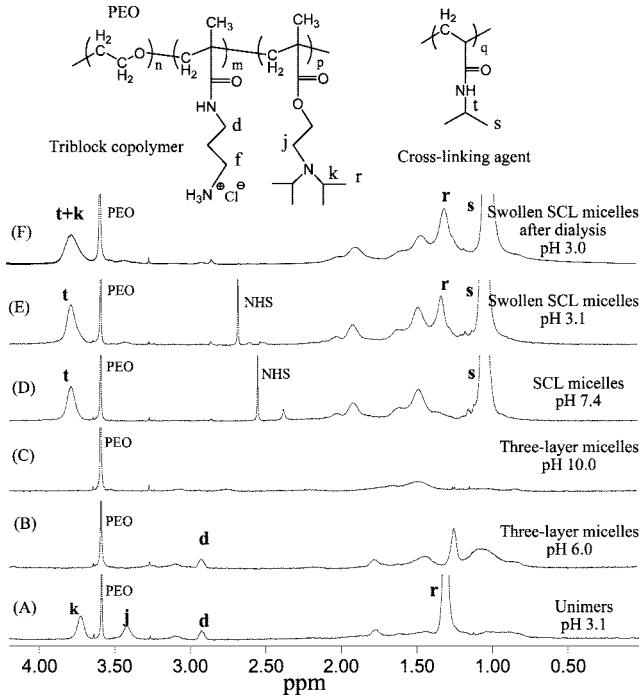


Figure 5. ^{1}H NMR spectra of the mPEO$_{46}$−PAPMA$_{21}$−PDPAEMA$_{101}$ triblock copolymer and the SCL micelles in D_2O (concentration: 0.05 wt %) at selected pH values at 25 °C.

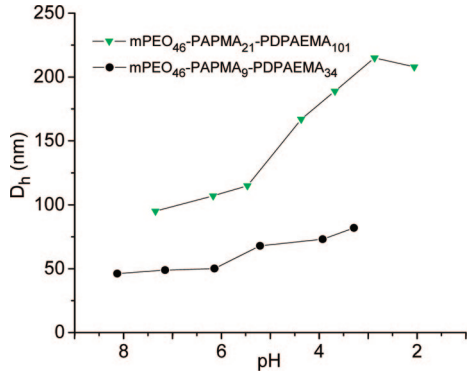


Figure 6. Variation of micelle D_h with solution pH after shell cross-linking.

triblock copolymer are shown in Figure 3. Block copolymer compositions were determined by comparing resonances of the mPEO block (∼3.6 ppm) to those associated with the PAPMA block (∼2.9 ppm) and PDPAEMA block (∼4.2 ppm). The molecular weight information on the diblock and triblock copolymers is summarized in Table 1.

Synthesis of Polymeric Cross-Linking Agent NHS−PNIPAM−NHS. A difunctional, symmetrical CTA, CMP, was utilized to generate PNIPAM containing carboxylic acid end groups. The PNIPAM chain ends were converted to activated esters through the esterification of the carboxylic acid end groups with NHS in the presence of DCC (Scheme 3). To ensure all termini of the PNIPAM consisted of carboxylic acid groups, V-501, a diazo initiator containing two carboxyl groups, was chosen to initiate the RAFT polymerization.

PNIPAM is a well-known, temperature-responsive polymer which undergoes a sharp coil-to-globule transition in water, leading to phase separation above the lower critical solution temperature (LCST).[66−68] The LCST of a NIPAM homopolymer is significantly dependent on both the molecular weight of the polymer and the hydrophilicity of the end group.[69,70] It should be noted that the trithiocarbonate group, built into the polymeric cross-linking agent, NHS−PNIPAM−NHS, potentially provides a site for cleavage in reducing environments.

Self-Assembly Behavior of mPEO−PAPMA−PDPAEMA Triblock Copolymers. The DPAEMA homopolymer is a weak polybase with a pK_a of about 6.0.[71] At room temperature, poly(DPAEMA) is water-soluble below pH 5.0 as a weak polycation due to protonation of the tertiary amine groups. The PAPMA is also a polycation containing primary amine groups, and the mPEO block is permanently water-soluble. Thus, the triblock copolymer is expected to self-assemble into a three-layer, "core−shell−corona" micelle in aqueous solution at pH values above the pK_a of the PDPAEMA block.

As observed in Figure 4, at pH values less than 5, all mPEO−PAPMA−PDPAEMA triblock copolymers exist as unimers with hydrodynamic diameters less than 10 nm. By increasing the solution pH value above 6.0, micellization occurs as the PDPAEMA blocks are deprotonated, resulting in increased hydrophobicity. It is clearly shown that different size micelles are formed by the different triblock copolymers. At pH 7.0, mPEO$_{46}$−PAPMA$_{21}$−PDPAEMA$_{36}$ forms small micelles with D_h of about 20 nm; mPEO$_{46}$−PAPMA$_9$−PDPAEMA$_{34}$ forms micelles with D_h of about 40 nm, while mPEO$_{46}$−PAPMA$_{21}$−PDPAEMA$_{101}$ and mPEO$_{46}$−PAPMA$_{10}$−PDPAEMA$_{68}$ form the largest micelles with D_h above 80 nm. This indicates that the size of the micelles is strongly dependent on the composition of the triblock copolymers. With similar mPEO−PAPMA block lengths, the triblock copolymer with the longest PDPAEMA block forms larger micelles. With similar PDPAEMA block lengths, the triblock copolymer with the shortest mPEO−PAPMA block formed smaller micelles. This is consistent with previous results reported by Eisenberg and co-workers[72−74] which showed that increasing the hydrophobic length or decreasing the hydrophilic length leads to larger aggregates, sometimes even transitioning from micelles to vesicles. Furthermore, for each triblock copolymer, larger micelles are formed with increasing solution pH. This can be explained by the increased hydrophobicity of the triblock copolymer at pH values higher than the pK_a of the PAPMA block (∼8.7, see Supporting Information, Figure 1S).

The successful micellization was also confirmed by ^{1}H NMR. Figure 5A−C shows the ^{1}H NMR spectra recorded for mPEO$_{46}$−PAPMA$_{21}$−PDPAEMA$_{101}$ at different pH values. At pH 3.1, the representative signals for each block are present indicating the triblock copolymers are fully solvated. At pH 6.0, the signals due to the PDPAEMA block at 3.7 ppm (k) and 3.4 ppm (j) are significantly broadened, indicating the formation of micelles comprised of DPAEMA cores. In more alkaline media (pH 10.0), the signal attributed to the PAPMA block at 2.9 ppm (d) is suppressed due to the decreased hydrophilicity of the PAPMA block above its pK_a. This is indicative of partial desolvation of PAPMA chains in the shell at pH values above the pK_a of PAPMA.

Shell Cross-Linking of Micelles. SCL micelles can be prepared by the addition of a cross-linking agent containing two or more functional groups which readily react with primary amines. The reaction of an activated ester and a primary amine is facile under mild conditions (ambient temperature, aqueous solution).[29] In this work, NHS−PNIPAM−NHS serves as a narrowly dispersed polymeric cross-linking agent. The capping efficiency of the NHS end groups is nearly quantitative as indicated by ^{1}H NMR spectrum (not shown). The cross-linking reaction was performed at pH 10.0 in order to ensure the majority of amine groups on the PAPMA block were deprotonated (pK_a ∼ 8.7). In order to limit the aminolysis of the trithiocarbonate group embedded in the cross-linking agent NHS−PNIPAM−NHS, 10% mole excess of cross-linking agent

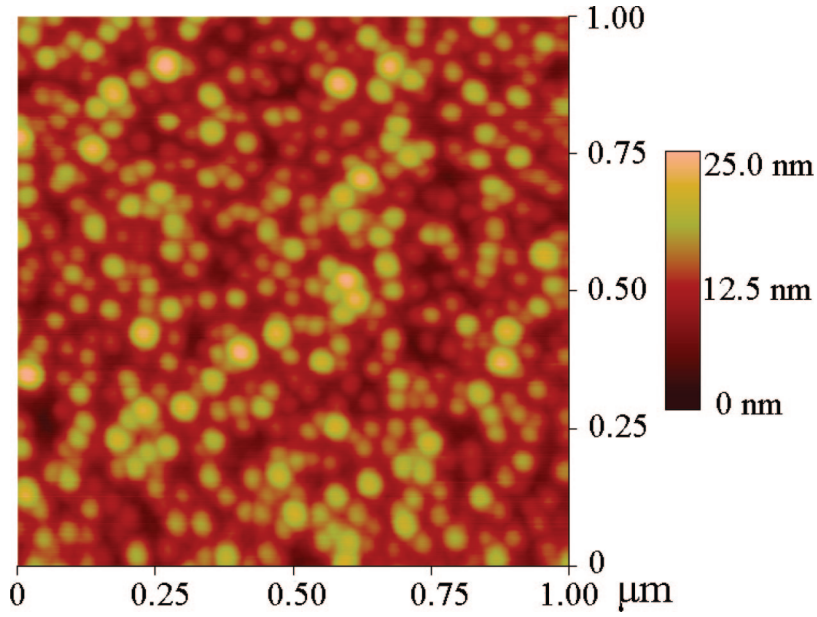


Figure 7. AFM images of SCL micelles formed by $mPEO_{46}-PAPMA_9-PDPAEMA_{34}$ triblock copolymer. Samples were prepared by drop deposition (5 μL; concentration: 0.05 wt %; pH: 8.1) onto freshly cleaved mica and allowed to dry in air.

was added. The reaction reaches 100% conversion after 2 h according to a previous report.[29] After shell cross-linking, the solution pH was lowered from 10.0 to 7.4 (for $mPEO_{46}-PAPMA_{21}-PDPAEMA_{101}$) and 8.1 (for $mPEO_{46}-PAPMA_9-PDPAEMA_{34}$). DLS shows a slight decrease in size of the SCL micelles compared to the non-cross-linked micelles. For example, the diameter of $mPEO_{46}-PAPMA_9-PDPAEMA_{34}$ micelles at pH 10.0 is 55 nm. After cross-linking (pH 8.1), the diameter decreases to 46 nm.

The formed SCL micelles are quite stable. For $mPEO_{46}-PAPMA_9-PDPAEMA_{34}$ triblock copolymer micelles, DLS indicates D_h of 45 nm after 10 days and 48 nm after a month. Obviously, the cross-linking agents are intact and are still capable of "locking" the micellar structures.

After cross-linking the micelles, the pH of the resulting solution was adjusted to less than 5.0. DLS experiments demonstrated that the micelle size increased with a decrease in solution pH (Figure 6). If shell cross-linking were unsuccessful, micelles would dissociate into unimers upon this change in pH. The degree of swelling is highly dependent on the length of PDPAEMA block. For $mPEO_{46}-PAPMA_{21}-PDPAEMA_{101}$, the micelles swell from 95 nm (pH 7.4) to 215 nm (pH 2.9) while the $mPEO_{46}-PAPMA_9-PDPAEMA_{34}$ triblock copolymer SLC micelles swell from 46 nm (pH 8.1) to 82 nm (pH 3.3). Under acidic conditions, the swollen SCL micelles are still stable. For the $mPEO_{46}-PAPMA_9-PDPAEMA_{34}$ triblock SCL micelles, the size does not change significantly (from 82 to 89 nm) after 2 months at pH 3.3.

Further evidence of successful shell cross-linking is provided by ^{1}H NMR. As shown in Figure 5F, the signals related to NHS completely disappeared after dialyzing against DI water (pH ~4.0) using 12 000 Da molecular weight cutoff dialysis tubing. The peaks attributed to PNIPAM block remain unchanged, indicating the micelles are successfully cross-linked by PNIPAM.

Figure 7 shows an AFM image of the SCL micelles prepared from the $mPEO_{46}-PAPMA_9-PDPAEMA_{34}$ triblock copolymer. It can be seen that relatively uniform spherical micelles were formed. The sizes range from 40 nm to 50 nm, similar to the value derived from DLS.

Conclusions

A series of mPEO−PAPMA−PDPAEMA triblock copolymers have been successfully synthesized via aqueous RAFT polymerization. The pH-responsive PDPAEMA comprises the core of the micelles at high pH (pH < 6.0), and the permanently hydrophilic mPEO block forms the corona. Micelle sizes are shown to be dependent on the solution pH and compositions of the triblock copolymers. The amine-containing middle block, PAPMA, is easily cross-linked by the difunctional polymer NHS−PNIPAM−NHS. After cross-linking, the size of the SCL micelle increases with decreasing solution pH due to the swelling of the PDPAEMA block. ^{1}H NMR measurements confirm the formation of SCL micelles.

Traditionally, difunctional small molecules have been utilized for cross-linking reactions. In this work, a polymeric cross-linking agent with potential temperature-responsive and cleavable properties was synthesized via RAFT polymerization and utilized to form SCL micelles. Preliminary work has indicated temperature-induced response of these SCL micelles and successful cleavage of the cross-linking agent, leading to the dissociation of SCL micelles into unimers. A detailed study of this reversible, multimode, stimuli-responsive behavior is underway and will be the subject of a future paper. It is anticipated that the drug loading and release properties of SCL micelles cross-linked by a temperature-responsive polymer can likely be tailored by controlling the pore size of the cross-linked shell.

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Supporting Information Available: Acid titration curve for PAPMA homopolymer. This material is available free of charge via the Internet at http://pubs.acs.org.

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