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Reversible Imine Shell Cross-Linked Micelles from Aqueous RAFT-Synthesized Thermoresponsive Triblock Copolymers as Potential Nanocarriers for "pH-Triggered" Drug Release†

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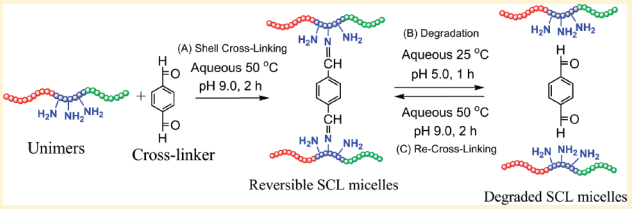
Supporting Information

ABSTRACT: A temperature-responsive triblock copolymer, α-methoxypoly(ethylene oxide)-*b*-poly(*N*-(3-aminopropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) (mPEO−PAPMA−PNIPAM), was synthesized via aqueous RAFT (*a*RAFT) polymerization. At room temperature, the polymer is hydrophilic and exists as unimers in aqueous solution. Increasing the solution temperature above the lower critical solution temperature (LCST) of the PNIPAM block leads to self-assembly into micelles with PNIPAM cores, PAPMA shells, and mPEO coronas with hydrodynamic diameter (D_h) values of ca. 52 nm. The PAPMA shell was cross-linked with terephthaldicarboxaldehyde (TDA) at pH 9.0 to generate shell cross-linked (SCL) micelles with cleavable imine linkages. The reversible pH- and temperature-dependent formation and cleavage of the (SCL) micelles was followed by dynamic light scattering and NMR spectroscopy. As well, the release of the model hydrophobic drug prednisolone 21-acetate (PA) from loaded SCL micelles was studied at specific pH and temperature conditions. PA was released at pH < 6.0 as hydrolytic cleavage of the imine cross-links within the swollen SCL micelles occurred. Such "pH-triggered" release behavior conceptually demonstrates that the reversible SCL micelles prepared by this simple procedure from temperature-responsive triblock copolymers have promise as therapeutic nanocarriers in biomedicine.



INTRODUCTION

Amphiphilic block copolymers are capable of self-assembling into a variety of structures (i.e., micelles[1,2] and vesicles[3−5]) depending on the hydrophilic and hydrophobic block lengths. These assemblies have been studied due to their promise as an effective means of delivering lipophilic drugs via a loaded hydrophobic core. Equally important for drug delivery applications, the hydrophilic corona imparts aqueous stability in the biological environment.[6−8] It is well-accepted that the micellar carriers can be tailored to allow delivery of drugs to specific sites while minimizing unwanted side effects.[9] To date, various copolymer micelles have been developed as potential delivery vehicles for model drugs with inherently low water solubility.[10−15]

However, certain limitations of self-assembled nanostructures preclude their use in practical applications. One major limitation is the dissociation of the amphiphilic micelles into unimers after administration into physiological media due to dilution effects.[16] When the copolymer concentration falls below the critical micelle concentration (CMC), as it does when administered to a patient, the nanostructure dissociates, resulting in an undesirable "burst" release of the active compound.[17] To address such stability issues, shell cross-linking (SCL), pioneered by Wooley et al.[18,19] and Armes et al.,[20,21] has been widely used. Since then, various methods have been developed for the formation of SCL micelles, including carbodiimide coupling,[22,23] polyelectrolyte complexation,[24,25] click chemistry,[26] and other facile methods.[21]

To date, other than a few notable exceptions discussed below, most SCL micelles are prepared from polymers synthesized by tedious, multistep procedures requiring organic solvents and protecting group chemistry. Micelle formation is often induced in mixed solvents, and shell cross-linking involves producing nondegradable covalent linkages between reactive functionalities in the shell. Such permanent cross-linking of micelles is generally not desirable for drug delivery. Alternatively, the hydrolytic decomposition of properly designed nanocarriers under specific conditions could lead to drug release at specific sites and aid in the clearance of the polymer. This can be achieved, in principle, by designing nanoparticles with cross-links that can be cleaved/re-formed under specific biological conditions. Such reversible SCL micelles have potential advantages in drug delivery since the release rate could be dictated by inherent physiological conditions. Recently, our group prepared such a reversible SCL micelle system utilizing a disulfide-containing cross-linker, cystamine, and investigated the controlled release behavior of the model drug, dipyridamole.[27] Subsequently, we reported shell cross-linking via another thiol-sensitive cross-linker, dimethyl 3,3′-dithiobispropionimidate (DTBP).[28] Beside these, acid cleavable acetal-type cross-linkers, such as 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane[29]

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and di(2-acryloyloxyethoxy)-4-hydroxyphenylmethane[30] have been employed by the CAMD group to obtain cross-linked micelles that are stable at high pH values but quickly degrade into free block copolymers at low pH values as the acetal cross-linking sites are transformed into an aldehyde and an alcohol group. Such acetal linkers[31−33] and hydrazone,[34−36] which are both cleavable at a pH of ∼5.5, have been extensively studied for the controlled release of antitumor drugs. Another acid-sensitive linkage formed from the reaction of a benzaldehyde and a primary amine was recently employed to prepare reversible, "stealth" polycationic micelles which were stable at physiological pH (7.4) while cleaved at endosomal pH (6.0−5.0).[37] The acid-labile property of imine cross-links was further investigated by Ding et al.,[38] who reported the "burst" release of doxorubicin (DOX) at endosomal pH.

The self-assembly of amphiphilic block copolymers usually requires that copolymers have well-defined structures and narrow polydispersities. Remarkable advancements in controlled radical polymerization (CRP) techniques including reversible addition−fragmentation chain transfer (RAFT)[39−46] polymerization now allow the precise construction of sophisticated architectures appropriate for use as delivery vehicles for diagnostics and therapeutic agents in the rapidly expanding area of nanomedicine.[47] In this work, we describe to our knowledge the first SCL micelles with pH-reversible imine cross-links. The temperature-responsive ABC triblock copolymer, α-methoxypoly(ethylene oxide)-*b*-poly(*N*-(3-aminopropyl)methacrylamide)-*b*-poly(*N*-isopropylacrylamide) (mPEO−PAPMA−PNIPAM), synthesized by *a*RAFT polymerization, undergoes thermo-induced micellization in water at increased temperature. The micelles are then cross-linked by adding a dialdehyde cross-linker, terephthaldicarboxaldehyde (TDA). The resultant SCL micelles can be cleaved and re-formed by adjusting the solution pH. ^{1}H NMR spectroscopy, dynamic light scattering (DLS), and transmission electron microscopy (TEM) are utilized to characterize the reversible SCL micelles. To demonstrate the potential of this system, the model hydrophobic drug, prednisolone 21-acetate (PA), is loaded into the reversible SCL micelle core and subsequently released under specific conditions.

EXPERIMENTAL SECTION

Materials. mPEO-NH_2 (M_n=5000 g/mol, M_w/M_n = 1.13) (Sigma-Aldrich) and *N*-(3-aminopropyl)methacrylamide (APMA) hydrochloride (Polysciences Inc.) were used as received. *N*-Isopropylacrylamide (NIPAM) (Aldrich) was recrystallized twice from hexane. 4-Cyano-4-(ethylsulfanylthiocarbonyl)sulfanylpentanoic acid (CEP) was synthesized following the previously reported procedure[48] (^{1}H NMR (300 MHz acetone-d_6) δ: 1.35 (t, −S−CH_2−CH_3); δ 1.85 (s, −C(CN)−CH_3); δ 2.4−2.67 (m, −CH_2−CH_2−); δ 3.42 (q, −S−CH_2−CH_3)). 4,4′-Azobis(4-cyanopentanoic acid) (V-501) and 2,2′-azobis(2-(2-imidazolin-2-yl)propane) (VA-044) were donated by Wako Chemicals. Terephthaldicarboxaldehyde (TDA) (99%), prednisolone 21-acetate (PA) (97%), *N*-hydroxysuccinimidyl ester (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 size exclusion chromatography (SEC) using a DMF eluent (0.02 M LiBr) at a flow rate of 1.0 mL/min in combination with Viscotek I-Series Mixed Bed low-MW and mid-MW columns and a Viscotek-TDA 302 (RI, viscosity, 7 mW 90° and 7° light scattering detectors (670 nm)) at 35 °C. The d*n*/d*c* of each (co)polymer was determined in DMF at 35 °C using a Viscotek refractometer and Omnisec software. NMR spectra were recorded in D_2O or $CDCl_3$ with either a Varian Mercury 300 MHz or a Mercury Innova 500 MHz spectrometer. 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 a 4 mW He−Ne laser operating at λ = 632.8 nm, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple tau digital correlator electronics system. TEM was conducted using a JEOL JEM-2100 electron microscope at an acceleration voltage of 200 kV. The samples were prepared by placing 5.0 μL of the micelle solution on a Formvar/carbon-coated, 200 mesh copper grid followed by water evaporation. A Varian Cary 500 UV−vis spectrophotometer was used to measure the absorbance of PA at λ_{max} = 243 nm.

Synthesis of PEO-Based Macro-CTA mPEO−CEP. In a 50 mL one-neck round-bottom flask equipped with a magnetic stirring bar, CEP (0.53 g, 2.0 mmol) and NHS (0.28 g, 2.4 mmol) were dissolved in 20 mL of chloroform. After the solution was homogenized by stirring, the flask was placed in an ice bath. Then, DCC (0.54 g, 2.6 mmol) was added in portions. After 4 h of stirring at 0 °C, the reaction mixture was allowed to warm to room temperature and stirred overnight. The precipitated dicyclohexylurea was removed by filtration to yield clear CEP activated ester solution.

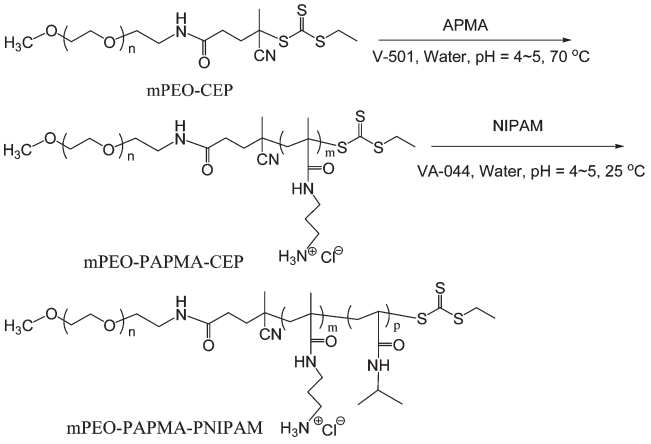
In another 100 mL round-bottom flask, mPEO-NH_2 (M_n = 5000 g/mol, 2.0 g, 0.4 mmol) was dissolved in 20 mL of chloroform and mixed with TEA (0.09 g, 0.8 mmol). Then, the mPEO-NH_2 solution was added dropwise into the previously prepared CEP activated ester solution within 30 min with stirring. The solution was allowed to stir overnight at room temperature. PEO-based macro-CTA, mPEO−CEP, was obtained as yellow solid by multiple precipitations (3) of the concentrated solution into excess diethyl ether followed by drying under vacuum at 35 °C for 24 h. The product (1.77 g, yield 88%) was stored in the dark at 4 °C in a sealed bottle.

Synthesis of mPEO−PAPMA Diblock Copolymer. APMA hydrochloride (0.50 g, 2.8 mmol) and mPEO−CEP (1.0 g, 0.2 mmol) were added along with deionized (DI) water (5.0 mL) to an ampule. V-501 (11.2 mg, 0.04 mmol) dissolved in methanol (0.5 mL) was then added. The solution was stirred until all the mPEO−CEP was dissolved. The ampule contents were purged with nitrogen for ∼30 min, and then the ampule was placed in a preheated oil bath at 70 °C. The reaction was terminated after 4 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: 89% (from ^{1}H NMR data), M_n = 7300 g/mol and PDI = 1.09, $mPEO_{113}$−$PAPMA_{12}$−CEP).

Chain Extension of $mPEO_{113}$−$PAPMA_{12}$−CEP. In a 25 mL ampule, $mPEO_{113}$−$PAPMA_{12}$−CEP (0.40 g, 0.056 mmol) and NIPAM (0.80 g, 7.07 mmol) were dissolved in DI water (5.0 mL), and the solution pH was adjusted to 4.5. VA-044 (18.1 mg, 0.053 mmol) dissolved in DI water (1.0 mL) was then added. The ampule was placed in ice bath. After purging with nitrogen for 30 min at 0 °C, the polymerization was allowed to proceed at 25 °C for 4 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: 87% (from ^{1}H NMR data), M_n = 20 100 g/mol, PDI = 1.21, $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$).

Preparation of Shell Cross-Linked Micelles. The triblock copolymer $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ (10 mg) was molecularly dissolved in DI water (20 mL) at pH 5.0. The solution was filtered through a 0.2 μm filter. The solution pH was then adjusted to a value of 9.0. After purging with N_2 for 15 min, the solution temperature was slowly increased from room temperature to 50 at 0.5 °C/min in order to induce micelle formation. Shell cross-linking was achieved by adding the dialdehyde cross-linker TDA (preadjusted pH to 9.0, TDA/APMA molar ratio = 1:2) into the micelle solution using a syringe. The resulting solution was stirred for 1 h prior to cooling to room temperature. DLS and NMR were utilized to confirm the formation of SCL micelles.

Scheme 1. Synthetic Route for Preparation of Temperature-Responsive mPEO−PAPMA−PNIPAM Triblock Copolymer via Aqueous RAFT Polymerization



Drug Release from SCL Micelles. $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ (100 mg) and the model drug PA (40 mg) were dissolved/dispersed in 100 mL of HPLC water. The solution pH was increased to 9.0 using 0.1 M NaOH, and the temperature was increased to 50 at 0.5 °C/min heating rate. The micelle solution was kept at 50 °C for 1 h. The appropriate amount of TDA cross-linker (TDA/amine molar ratio = 1:2) was then added. After stirring for 1 h, the drug-loaded SCL micelle solution was filtered through a 0.45 um filter to remove the PA precipitate and divided into portions. One portion (12.5 mL) was transferred to a dialysis tube (molecular weight cutoff (MWCO) = 6000−8000 Da) which was immersed in 450 mL of phosphate buffer solution at selected pH values and temperatures. Aliquots were taken at predetermined times, and the rate of drug release was monitored by UV−vis spectroscopy.

RESULTS AND DISCUSSION

Synthesis and Sequential Chain Extension of mPEO−CEP Macro-CTA. In this work, a PEO-based macro-CTA was designed and synthesized for preparation of the temperature-responsive triblock copolymer mPEO−PAPMA−PNIPAM which is expected to form micelles in aqueous media above the LCST of the PNIPAM block. The designed micelles consist of PNIPAM cores, PAPMA shells, and mPEO coronas. The non-immunogenic, permanently hydrophilic mPEO block stabilizes the micelles. The PAPMA block, containing primary amine groups, provides a reactive site which is convenient for a variety of shell cross-linking reactions. PEO-based macro-CTAs have been widely used in preparing diblock and/or triblock copolymers.[49,50] Most are synthesized via an esterification reaction between mPEO−OH and a carboxylic-functionalized CTA which generates an ester linkage. However, the ester bond is readily attacked by primary amines under basic conditions[51] which may lead to the loss of the mPEO stabilizing corona. Thus, a more chemically stable linkage, such as an amide, is desirable. This synthesis was achieved by the reaction of mPEO-NH_2 with an activated ester CTA via a modified literature procedure.[52] A stable amide bond was generated without competitive degradation of the trithiocarbonate group. The capping of mPEO with CEP was calculated to be ~94% efficient from the ^{1}H NMR data (Figure 1A) by comparing the peak area of the methyl protons of the CEP fragment at 1.88 ppm (c) with that of the methyl protons of mPEO at 3.37 ppm (g).

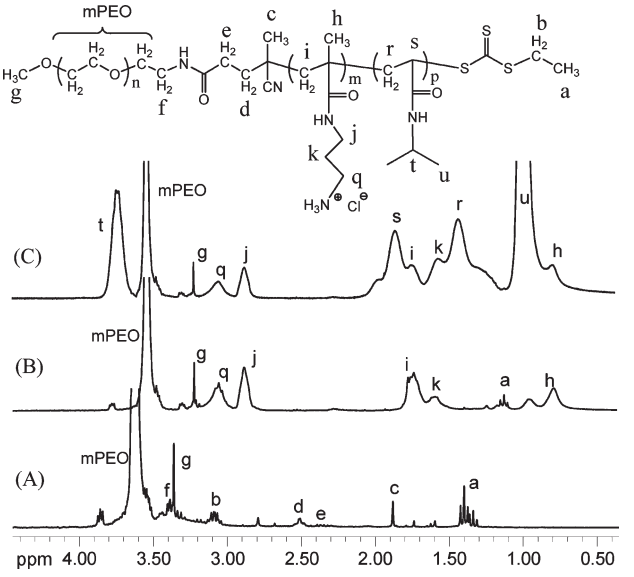


Figure 1. ^{1}H NMR spectra of (A) macro-CTA $mPEO_{113}$−CEP in $CDCl_3$, (B) diblock copolymer $mPEO_{113}$−$PAPMA_{12}$−CEP in D_2O (pH 5.0), and (C) triblock copolymer $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ in D_2O (pH 5.0).

Table 1. Molecular Weight Data of Macro-CTA mPEO−CEP, Diblock Copolymer mPEO−PAPMA−CEP, and Triblock Copolymer mPEO−PAPMA−PDPAEMA Prepared via Aqueous RAFT Polymerization

(co)polymer[a]	M_n[b]	PDI[b]	M_n[a]	M_n(theo)
$mPEO_{113}$−CEP	5100	1.13		5200
$mPEO_{113}$−$PAPMA_{12}$−CEP	7300	1.09	7100	7500
$mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$	20100	1.21	23500	19900

[a] Determined by ^{1}H NMR spectroscopy. [b] Determined by DMF SEC (d*n*/d*c* values: diblock copolymer (0.042), triblock copolymer (0.032)).

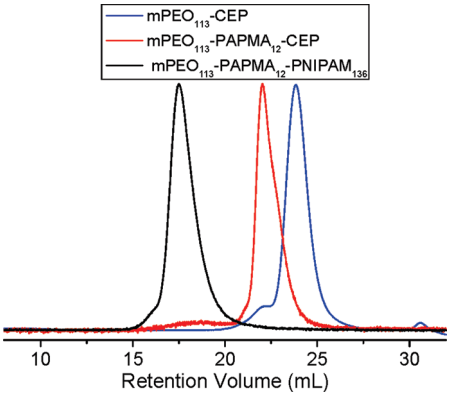


Figure 2. ASEC traces of macro-CTA ($mPEO_{113}$−CEP), diblock copolymer ($mPEO_{113}$−$PAPMA_{12}$−CEP), and triblock copolymer ($mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$).

The resulting macro-CTA, $mPEO_{113}$−CEP, was then employed in RAFT polymerization of the primary amine-containing monomer, APMA, in aqueous media at 70 °C, using V-501 as the free radical initiator (Scheme 1). As we previously reported,[53] by carefully controlling the polymerization conditions, in our case, maintaining the pH of the aqueous polymerization solution

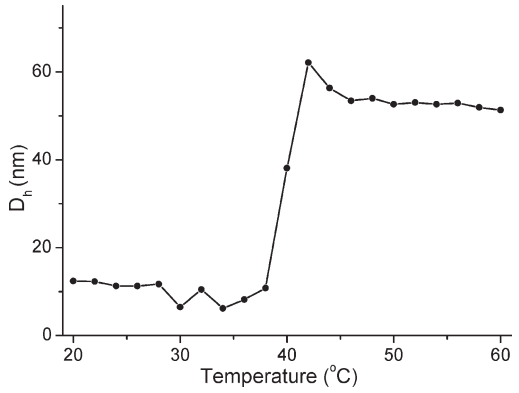


Figure 3. Variation of hydrodynamic diameter (D_h) with solution temperature for triblock copolymers $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ at pH 9.0 in aqueous solution (concentration: 0.05 wt %).

between 4 and 5, hydrolysis and/or aminolysis of the CTA is prevented.[54] The generated $mPEO_{113}$−$PAPMA_{12}$−CEP diblock macro-CTA was then utilized for triblock formation by chain extension with NIPAM in water. To avoid the precipitation of the PNIPAM block at high temperature, the (*a*RAFT) polymerization was conducted at room temperature using VA-044 as the free radical source.[55] Under conditions described in the Experimental Section, the well-defined diblock copolymer $mPEO_{113}$−$PAPMA_{12}$−CEP and triblock copolymer $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ were obtained (Table 1). SEC chromatograms of the $mPEO_{113}$−CEP, $mPEO_{113}$−$PAPMA_{12}$−CEP, and $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ are shown in Figure 2. A small shoulder was observed from GPC trace of $mPEO_{113}$−CEP, which is likely attributed to small amount of high molecular weight mPEO impuritiy. The diblock and triblock copolymer traces are unimodal and the PDIs are rather low (≤1.21), indicating that the blocking efficiency is high and the polymerization proceeds in a controlled fashion.

The ^{1}H NMR spectra for the macro-CTA mPEO−CEP, diblock copolymer mPEO−PAPMA, and the subsequent triblock copolymer mPEO−PAPMA−PDPAEMA are shown in Figure 1. 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 PNIPAM block (~3.8 ppm). The composition of the triblock copolymer determined from ^{1}H NMR data is $mPEO_{113}$−$PAPMA_{12}$−$PDPAEMA_{136}$. All SEC and NMR data for the (co)polymers are summarized in Table 1.

Thermo-Induced Micellization of the Triblock Copolymer. It is well established that PNIPAM becomes dehydrated as a result of an entropy gain resulting from the release of water molecules upon association of the isopropyl groups above its LCST.[56−60] The dehydration of the NIPAM block causes the block copolymers to undergo a transition from molecularly dissolved unimers to aggregates. The temperature-induced micellization can be followed with dynamic light scattering. As shown in Figure 3, at 40 °C, a large increase in the hydrodynamic diameter of the $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ triblock copolymer occurs, followed by a slight decrease in size as the solution temperature is increased further, which is the typical micellization behavior of PNIPAM-containing copolymers.[61,62] The copolymer exists as unimers with average hydrodynamic diameters (D_h) of 11.2 nm below the LCST. As the temperature is increased above the LCST (in this case, 40 °C), the unimers associate to form

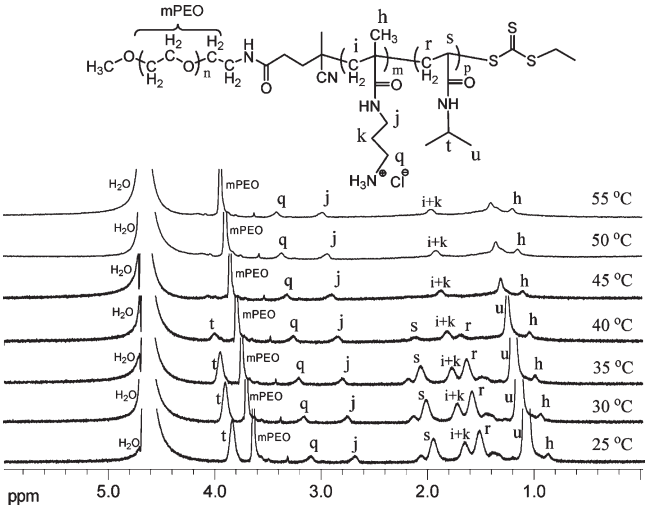


Figure 4. ^{1}H NMR spectra of the $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ triblock copolymer and the thermo-induced micelles in D_2O at increasing temperature (concentration: 0.05 wt %; pH: 9.0).

aggregates of 62.1 nm before reaching an equilibrium size of 52.6 nm at 50 °C.

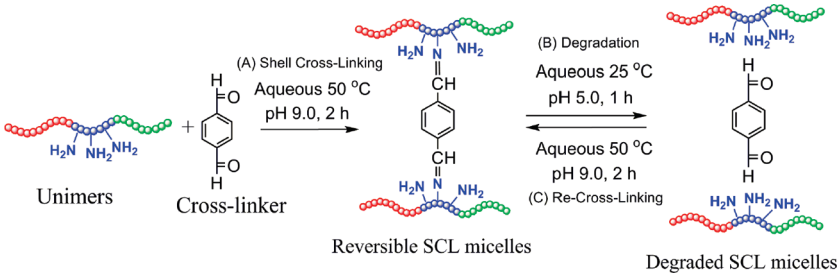
Figure 4 shows the ^{1}H NMR spectra recorded for the triblock copolymer $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ at increasing temperatures. At 25 °C, the copolymer chains are fully solvated, and the signals associated with each block are visible. At the LCST of 40 °C, where DLS shows that thermo-induced micellization begins, the signals associated with the NIPAM block (t and u) have been attenuated. Increasing the solution temperature above 40 °C causes the NIPAM signal to disappear since the PNIPAM block becomes hydrophobic and forms the core of micelles. It is worth noting that both of the DLS and NMR measurements were performed in aqueous solution at pH 9.0 since the subsequent shell cross-linking reaction occurs under basic conditions.

Formation of TDA Shell Cross-Linked Micelles. Once the thermo-induced micelles are formed, SCL micelles can be prepared by adding a bifunctional or multifunctional compound which can react with the pendent primary amine functionalities on the PAPMA middle block of $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$. In this present work, a dialdehyde cross-linker, TDA, was utilized to cross-link the PAPMA shell (Scheme 2).

Once the triblock polymer forms "core−shell−corona" three-layer micelles at 50 °C, an appropriate amount of cross-linker TDA (TDA/APMA molar ratio = 1:2) was added. The cross-linking reaction was allowed to proceed at pH 9.0 for 2 h. Subsequently, the resulting solution was allowed to cool to room temperature. The absence of unimer peaks upon cooling indicated the shell cross-linking was successful. DLS experiments show that micelle size increases from 53.2 nm at 50 °C to ~92.0 nm at room temperature (pH 9.0) (data are shown in Figure 8). When the solution temperature is lowered below the LCST of the NIPAM block, the NIPAM core becomes hydrophilic, resulting in the anticipated swelling of the SCL micelles.

TEM (Figure 5) was also utilized to characterize the SCL micelles. Grids were allowed to dry at room temperature; it should be noted that micelle structures remain intact (although below NIPAM LCST) due to cross-links. Micrographs of the SCL micelles at pH 9.0 and room temperature show micelle structures ranging in size from 80 to 95 nm, in agreement with the DLS results (~92.0 nm), signifying successful shell cross-linking. SCL micelles are quite

Scheme 2. Cross-Linking, Cleavage, and Re-Cross-Linking of $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ Triblock Copolymer Micelles in Aqueous Solution Triggered by Changing the Solution pH



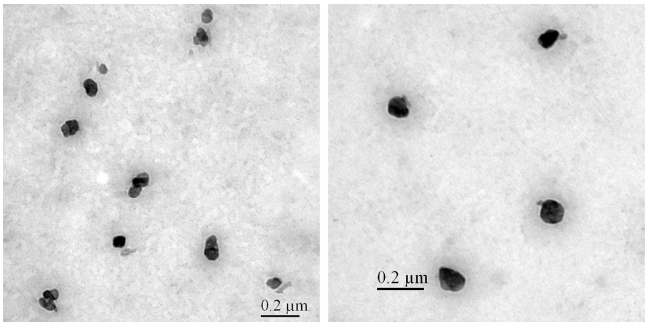


Figure 5. TEM images of the swollen SCL micelles at pH 9.0 at room temperature (copolymer concentration: 0.05 wt %).

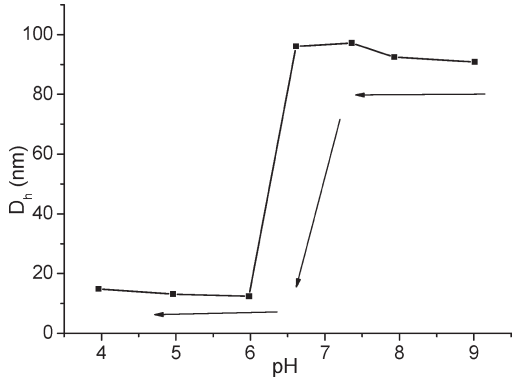


Figure 6. Variation of hydrodynamic diameter (D_h) with solution pH for TDA cross-linked $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ micelles at 25 °C in aqueous solution (concentration: 0.05 wt %).

stable at pH 9.0 at room temperature. DLS studies of solutions aged for more than 1 month under these conditions indicate that the micelles maintain constant size.

Reversible Cleavage of SCL Micelles. It is well-known that the imine linkage is not stable under acidic conditions (below pH 6.8).[63] The imine cross-links are expected to be hydrolyzed at low pH, consequently causing the dissociation of the SCL micelles. As shown in Figure 6, gradually decreasing solution pH leads to the change of D_h from ~92.0 nm (swollen SCL micelles) at pH 9.0 to ~12.0 nm (unimers) at pH 5.0 at room temperature. The D_h of SCL micelles dramatically decreases at pH 6.0−6.5, which indicates the cleavage of imine linkages. Thus, the TDA SCL micelles are demonstrated to be stable at basic condition (pH > 7.0), while dissociated into unimers at acidic condition (pH < 6.5). Imine linkages have also been

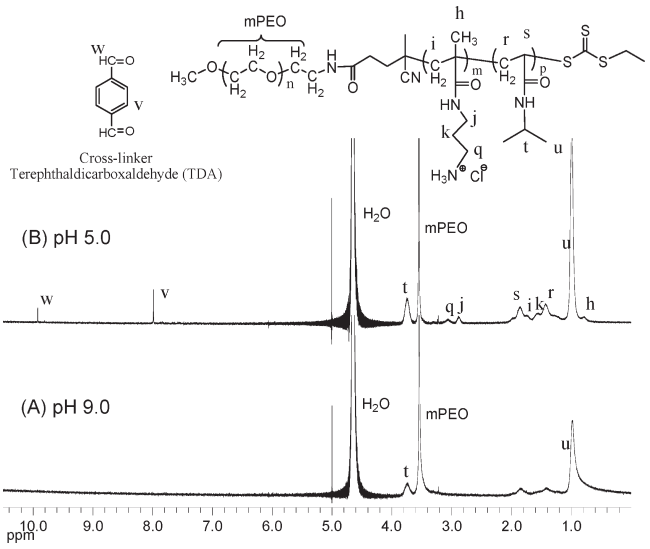


Figure 7. ^1H NMR spectra of TDA cross-linked $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ micelles in D_2O at pH 9.0 (A) and pH 5.0 (B) at room temperature.

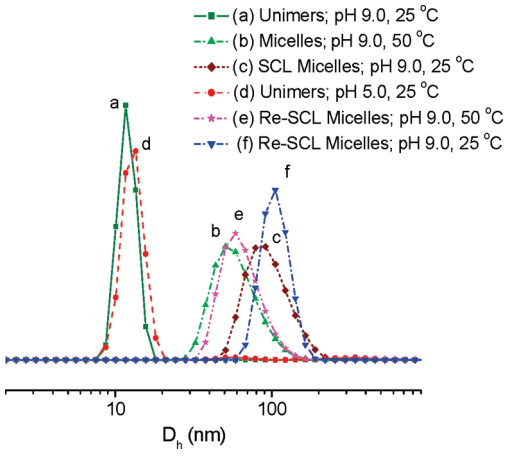


Figure 8. Dynamic light scattering size distribution of the triblock copolymer $mPEO_{113}$−$PAPMA_{12}$−$PNIPAM_{136}$ under various conditions (copolymer concentration: 0.05 wt %).

utilized by Gu and co-workers[37] for grafting cleavable PEG chains onto an amphiphilic block copolymer, in which the critical pH for cleavage of the imine was determined to be 6.8.

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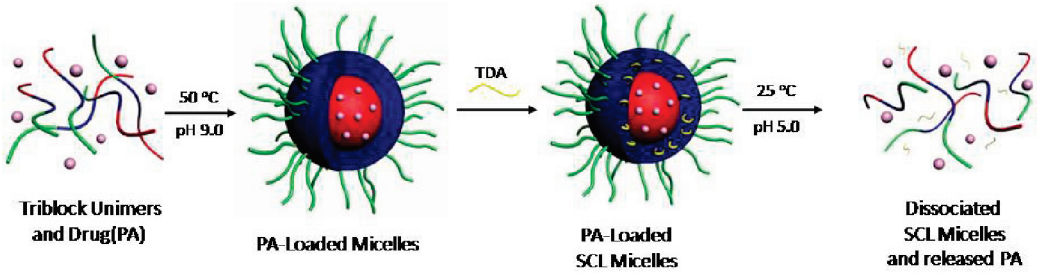
Scheme 3. Schematic Illustration of the Formation of Reversible Shell Cross-Linked (SCL) Micelles from the Triblock Copolymer, $mPEO_{113}-PAPMA_{12}-PNIPAM_{136}$



To further prove the success of TDA cross-linking, the solution containing the SCL micelles was purified by dialysis against DI water at pH 9.0 to remove unreacted cross-linker followed by lyophilization. ^{1}H NMR analyses of the purified TDA SCL micelle sample were performed at pH 9.0 and pH 5.0, respectively (Figure 7). At pH 9.0, attenuation of the PNIPAM peaks and disappearance of the TDA peaks were observed which can be attributed to the limited motion available to these blocks when in a core−shell−corona structure. In contrast, at pH 5.0, the peaks assigned to PEO, PAPMA, and PNIPAM blocks are all visible. Also, the peaks at 8.00 and 9.97 ppm related to TDA cross-linker appear. The NMR data give further evidence that the imine cross-links are hydrolyzed to TDA and primary amine at pH 5.0, resulting in dissociation of TDA SCL micelles back into unimers. By comparing the integration of the peaks v and peak j, the degree of cross-linking is calculated to be ~85% (although this is an overestimate since intramolecular cross-linking cannot be avoided). The ^{1}H NMR spectrum of SCL micelles in $CDCl_3$ was also obtained (Figure S1) in which the peak at 8.06 ppm assigned to TDA cross-linker is visible, although attenuated. Thus, the ^{1}H NMR study clearly demonstrates the successful formation of SCL micelles and the acid-labile behavior of imine linkages.

After dissociation of the SCL micelle solution at pH 5.0, the micelles are reformed upon increasing the temperature to above 40 °C. The reassembled micelles can also be re-cross-linked by simply adding an appropriate amount of NaOH solution (0.1 M) to increase the solution pH back to 9.0. DLS results demonstrate the re-cross-linked micelles exhibit swelling behavior similar to that of the original SCL micelles. Thus, the reversible SCL micelles were successfully prepared via the reaction of TDA with the primary amine groups of the triblock copolymer. The cleavage and regeneration of imine linkages are strongly dependent on solution pH. The unimodal distribution observed in the DLS measurements indicates that there was no intermicellar cross-linking (Figure 8). Advantages of the imine cross-linking approach include the ability to carry out the reaction under mild conditions and the lack of small molecule organic byproducts which may be toxic to cells.

Temperature-Responsive and "pH-Triggered" Release of PA. To date, various classes of SCL micelles have been reported.[21] However, only a few studies concerning the drug release behavior have appeared in the literature.[15,27,64] As described above, the acid-labile imine linkage can be used in the reversible formation of SCL micelles and is, therefore, expected to trigger the release of a model hydrophobic drug, prednisolone acetate (PA) (Scheme 3). PA is widely used anti-inflammatory/anti-proliferative drug and has very low solubility in water.[65,66] It has a maximum UV absorption at 243 nm[67] with a molar absorption coefficient (ε) value of 1.358 $\times$ 10^4 L mol^{-1} cm^{-1} in methanol. Additionally, PA is a good candidate for model drug experiments due to its consistent reported value of water solubility in the range (5.0−9.0) of interest. The PA loading capacity (LC) of reversible SCL micelles is calculated by using the following equation[68]

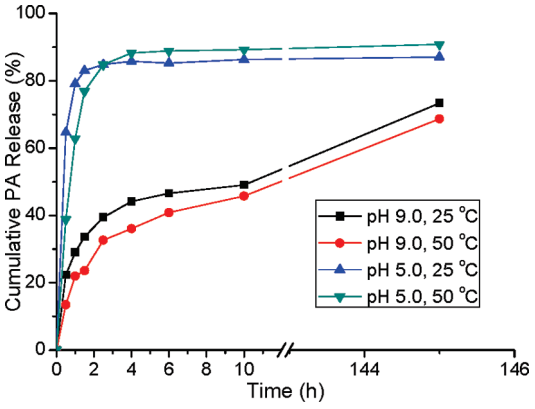


Figure 9. Cumulative PA release from TDA cross-linked temperature-responsive micelles formed by the triblock copolymer $mPEO_{113}-PAPMA_{12}-PNIPAM_{136}$.

$$LC = \frac{W_{drug}}{W_{micelle}} \times 100\%$$

in which W_{drug} is the total amount of PA loaded in reversible SCL micelles, this value being determined by measuring the UV−vis absorption of drug-loaded SCL micelles in methanol. $W_{micelle}$ is the weight of SCL micelles. The LC, in this case, is calculated to be 21.6 wt %, which is relatively high compared to other systems.[10,69] The high LC might be attributed to the fairly long PNIPAM block of the triblock copolymer leading to the formation of micelles with large cores.

The drug release profile from the reversible SCL micelles was evaluated at pH 5 and 9 at both room temperature and 50 °C (Figure 9). The rate of drug release is slightly faster at 25 °C than at 50 °C regardless of solution pH. This can be attributed to the difference in hydrophobicity of the PNIPAM core below and above its LCST. In contrast to temperature-responsive behavior, the release of PA from TDA cross-linked $mPEO_{113}-PAPMA_{12}-PNIPAM_{136}$ micelles can be triggered by lowering the solution pH below 6.5 since SCL micelle dissociation occurs rapidly under such conditions. Also as shown in Figure 9, ~80% of PA loaded into the SCL micelles was released within 2 h at pH 5.0. However, only 40−50% of PA was released at pH 9.0. These results indicate that

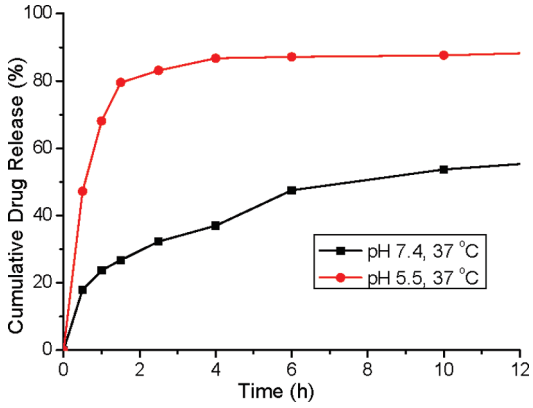


Figure 10. Cumulative PA release from reversible SCL micelles under simulated physiological and endosomal conditions (pH 7.4, 37 °C and pH 5.5, 37 °C, respectively).

the solution pH, which affects the imine linkages, plays an important role in the controlled release of the model drug.

To better understand the PA controlled release behavior from our reversible SCL micelles, studies were performed at pH and temperature values that simulate that of the human body. As shown in Figure 10, about of 50% of PA was released after10 h under simulated physiological conditions (i.e., pH 7.4, 37.0 °C). In contrast, a burst release (more than 80% of PA was released within 2 h) was observed at a lower pH (i.e., pH 5.5, 37 °C, endosomes have pH values from 5.0−6.5). At both pH values, swelling of the SCL micelles below the LCST of the core is responsible for facilitating the release of a portion of PA; however, burst release of PA at lower pH is attributed to hydrolysis of the imine linkages and micelle disintegration.

CONCLUSIONS

In summary, triblock copolymer micelles were prepared from a thermoresponsive ABC triblock copolymer synthesized using (*a*RAFT) polymerization. SCL micelles were readily obtained using dialdehyde as a cross-linker. These SCL micelles can be reversibly cleaved by simply adjusting the solution pH. At high pH (>7.0), the SCL micelles are stable. In contrast, at low pH (<6.5), where the imine cross-links are cleaved, the SCL micelles dissociate into unimers. The hydrophobic drug, PA, was utilized as model drug to investigate the release behavior at conditions simulating those of the living systems. The "pH-triggered" burst release of PA was clearly observed at endosomal conditions (pH 5.5, 37 °C), as compared to slow, sustained release under normal physiological conditions (pH 7.4, 37 °C). Therefore, the imine cross-linked micelles appear to provide an attractive platform for developing SCL nanostructures for controlled drug delivery.

ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectrum (in $CDCl_3$) of TDA cross-linked micelles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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