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ARTICLE

Temperature and pH Double Responsive Hybrid Cross-Linked Micelles Based on P(NIPAAm-*co*-MPMA)-*b*-P(DEA): RAFT Synthesis and "Schizophrenic" Micellization

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ABSTRACT: Poly(*N*-isopropylacrylamide-*co*-3-(trimethoxysilyl)propyl methacrylate)-*b*-poly(2-(diethylamino)ethyl methacrylate) (P(NIPAAm-*co*-MPMA)-*b*-P(DEA)) copolymer was synthesized by reversible addition−fragmentation chain transfer (RAFT) polymerization. The temperature and pH responsive schizophrenic micellization behaviors of P(NIPAAm-*co*-MPMA)-*b*-P(DEA) and the cross-linking of P(NIPAAm-*co*-MPMA) blocks using inorganic silica-based cross-linking strategy were investigated in detail. Transmission electron microscopy (TEM) showed that the resultant core cross-linked (CCL) and shell cross-linked (SCL) micelles displayed regular spherical shapes with different sizes in *N*,*N*′-dimethylformamide (DMF) and aqueous media. The structure changes of CCL and SCL micelles at different pHs and temperatures were characterized by ^{1}H NMR. Optical absorption measurements showed that the lower critical solution temperatures (LCSTs) of the CCL and SCL micelles were 37.5 and 40.5 °C, respectively. In vitro drug release study showed that the drug-loaded CCL and SCL micelles displayed thermoresponsive and pH double responsive release behaviors.

Introduction

In recent years, the stimulus responsive polymeric micelles have attracted a lot of attention for their unique core−shell structures and intelligent properties, including pH, thermo, ionic strength responsibilities, and so on.[1−4] Taking advantage of these distinctive virtues, many of them have been developed as drug carriers for controlled release so far. For example, thermally responsive amphiphilic copolymer poly(*N*-isopropylacrylamide) (PNIPAAm)-*graft*-polyphosphazene (PNIPAAm-*g*-PPP) was synthesized for controlled release of diflunisal (DIF).[5] Analogously, a series of amphiphilic pH-sensitive cholesteryl-bearing carboxymethylcellulose derivatives (CCMCs) were prepared and used in controlled release of indomethacin (IND).[6]

As compared with traditional intelligent micelles, a novel class of micelles, the building blocks of which are both stimulus responsive, were defined as "schizophrenic micelles".[7,8] Lowe et al. prepared poly(*N*-isopropylacrylamide-*block*-4-vinylbenzoic acid) (PNIPAAm-*b*-PVBZ) and reported its schizophrenic micellization behavior in aqueous solutions.[9] Their study showed that the normal and inverse micelles could be simply obtained by tuning the pH and temperature of the aqueous solution because the PNIPAAm block is thermosensitive and the PVBZ block is pH-sensitive. However, in the practical applications of traditional intelligent micelles and schizophrenic micelles, the stability of the micelles is an important issue since the micellar structure can hardly keep stable after being diluted by a large amount of gastric fluid, blood, or other body fluids in the process of drug release.[10]

To address this problem, core cross-linked (CCL) and shell cross-linked (SCL) micelles have been developed for maintaining the micellar structural integrity.[11,12] Cross-linking agents for the preparation of CCL or SCL micelles mainly include three types: (i) organic small molecules, such as diamine, dialdehyde, and diyne;[13−15] (ii) inorganic molecules, like 3-(triethoxysilyl) propyl methacrylate (TEPM), 4-(1-methylsilacyclobutyl)-styrene (SBS), and calcium chloride;[16−18] (iii) macromolecules, such as poly(*N*-isopropylacrylamide) (PNIPAAm);[19] polyelectrolyte, poly([ar-vinylbenzyl]trimethylammonium chloride) (PVBTAC), and poly(sodium 2-acrylamido-2-methylpropanesulfonate) (PAMPS).[20,21] Among them, 3-(trimethoxysilyl)propyl methacrylate (MPMA) is an inorganic cross-linking agent used in the "silica-based" cross-linking strategy. By copolymerization of organic monomers with MPMA, organic/inorganic hybrid cross-linked micelles could be prepared facilely. Furthermore, the resultant hybrid micelles would bear characteristics of polymers as well as inorganic structures, such as rigid structure, low cost, low toxicity, and ease in purification.[22]

It is well-known that PNIPAAm homopolymer is thermoresponsive with a thermosensitive phase transition temperature around 33 °C,[23] and poly(2-(diethylamino)ethyl methacrylate) (P(DEA)) is pH-sensitive, which dissolves in acidic solutions as a weak cationic polyelectrolyte and becomes insoluble in neutral and alkaline solutions due to the deprotonation of tertiary amine groups.[24] Herein, we synthesized a diblock copolymer (P(NIPAAm-*co*-MPMA)-*b*-P(DEA)) by RAFT polymerization and investigated its thermoresponsive and pH-responsive schizophrenic micellization behaviors. Using the copolymer, organic/inorganic hybrid CCL and SCL micelles were further prepared using silica-based cross-linking strategy (Figure 1). Moreover, in vitro drug release behaviors of the resulting CCL and SCL micelles at

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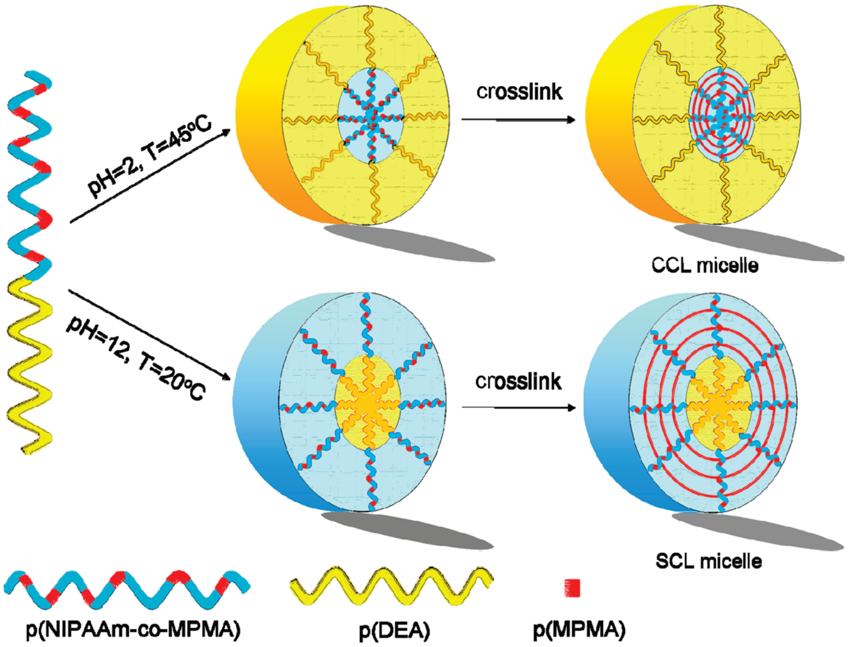


Figure 1. Schematic illustration of schizophrenic micellization behavior of P(NIPAAm$_{235}$-*co*-MPMA$_5$)-*b*-P(DEA$_{362}$) and cross-linking of the micellar core or shell in aqueous media.

different pH values and temperatures were further investigated to evaluate their potential as smart drug carriers.

Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAAm) (Acros) and 3-(trimethoxysilyl)propyl methacrylate (MPMA) (Wuhan University Chemical Plant, Wuhan, China) were used as received. 2-(Diethylamino)ethyl methacrylate (DEA) was purchased from Sigma-Aldrich. *N,N*′-Dimethylformamide (DMF) and tetrahydrofuran (THF) were obtained from Shanghai Chemical Reagent Co. and used after distillation. 2-(2-Carboxyethylsulfanylthiocarbonylsulfanyl)propionic acid (CPA) was prepared according to previous article.[25] *N,N*′-Azobis(isobutyronitrile) (AIBN) was purchased from Shanghai Chemical Reagent Co. and used after recrystallization. All other reagents and solvents were used without further purification.

Synthesis of P(NIPAAm-*co*-MPMA) (Macro-CTA) by RAFT Polymerization. *N*-Isopropylacrylamide (NIPAAm) (2.28 g, 20 mmol), MPMA (95.3 μL, 0.4 mmol), CPA (25.4 mg, 0.1 mmol), and AIBN (1.64 mg, 0.01 mmol) were dissolved in anhydrous DMF (8 mL, freshly distilled) in a dry glass flask equipped with a magnetic stir bar. The reaction mixture was degassed and then sealed. The flask was immersed in the preheated oil bath at 70 °C. After polymerization for 70 min, the reaction flask was removed from oil bath. Then the reaction mixture was poured into diethyl ether to precipitate the product. The product was collected by filtration and purified twice by redissolution/reprecipitation with THF/diethyl ether and finally dried in vacuum overnight to obtain macro-CTA.

Synthesis of P(NIPAAm-*co*-MPMA)-*b*-P(DEA) by RAFT Polymerization. 2-(Diethylamino)ethyl methacrylate (DEA) (1000 mg, 5.4 mmol), macro-CTA (350 mg, 0.013 mmol), and AIBN (1 mg, 0.006 mmol) were dissolved in anhydrous THF (8 mL, freshly distilled) in a dry glass flask equipped with a magnetic stir bar. The reaction mixture was degassed and then sealed. Then the flask was immersed in the preheated oil bath at 70 °C. After polymerization for 25 h, the flask was removed from oil bath, and the reaction mixture was poured into cold *n*-hexane (−70 °C) to precipitate the product. The copolymer was collected by filtration, purified twice by redissolution/reprecipitation with THF/*n*-hexane, and finally dried in vacuum overnight.

^{1}H NMR Characterization. ^{1}H NMR spectra were recorded on a Varian Unity 300 MHz spectrometer using $CDCl_3$ and D_2O as the solvents. A trace amount of NaOH or DCl was added to adjust pH value of D_2O. The testing temperature was 20 °C unless otherwise specified.

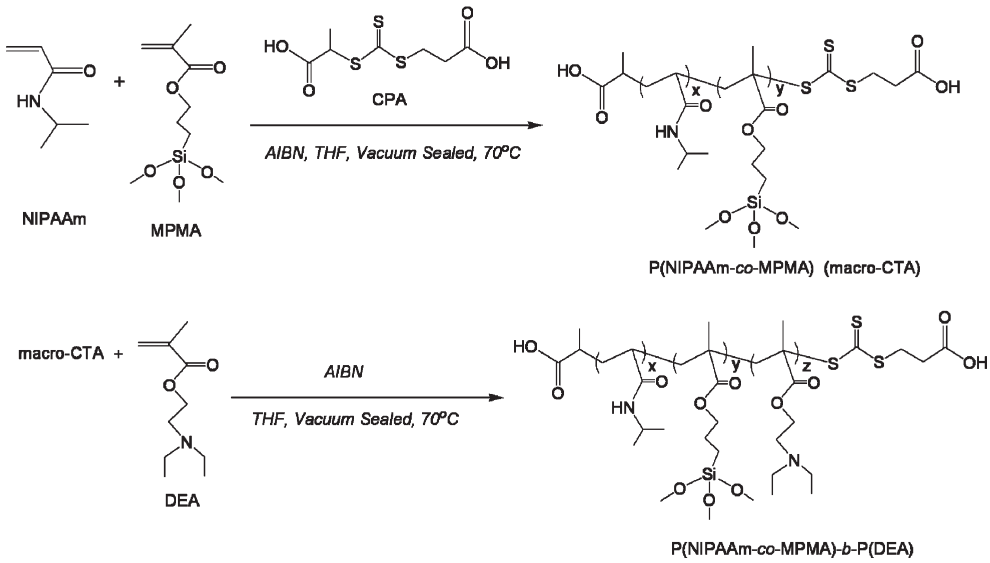
SEC-MALLS Measurements. The size-exclusion chromatography and multiangle laser light scattering were used to determine the molecular weights of polymers. A dual-detector system consisting of a MALLS device (DAWN EOS, Wyatt Technology) and an interferometric refractometer (Optilab DSP, Wyatt Technology) was used. The columns used were styragel HR1 and HR4 (Waters). The concentration of the copolymer was kept constant at 10 mg/mL, and THF was used as the eluent at a flow rate of 0.3 mL/min. The MALLS detector was operated at a laser wavelength of 690 nm.

Micelle Formation. 5 mg of P(NIPAAm-*co*-MPMA)-*b*-P(DEA) copolymer was dissolved in 0.2 mL of THF, which was then added into 15 mL of aqueous solution (pH 2, 45 °C) dropwise under vigorous stirring to form core cross-linked micelles (CCL) or added into 15 mL of aqueous solution (pH 12, 20 °C) to form shell cross-linked micelles (SCL). The ionic strength of all the aqueous solutions mentioned above was adjusted to 0.1 M by adding NaCl in order to avoid the effect resulting from ion strength variation.[26] After the acid- or base-catalyzed sol−gel process (15 h), THF and byproduct methanol from the hydrolysis of the silyl ethers were removed by dialysis against distilled water for 2 days (dialysis tube was purchased from Shanghai Chemical Reagent and molecular weight cutoff was 8000−12 000 g/mol). The final product was freeze-dried.

Transmission Electron Microscopy (TEM) Observation. A drop of micelle solution was placed on a copper grid with carbon film and stained by a 0.2% (w/v) solution of phosphotungstic acid before measurement by JEM-100CX II transmission electron microscope (TEM) at an acceleration voltage of 100 keV. In order to visualize the micelle at 45 °C by TEM, a drop of micelle solution preheated to 45 °C was placed onto a copper grid with carbon film, which was then placed in an oven at 45 °C for quick drying.

Size Distribution Measurement. Zetasizer Nano ZS (Malvern Instruments) was used to determine the size and size distribution of cross-linked micelles (laser wavelength was 632.8 nm). The micelle solutions (micelle concentration = 330 mg/L, I = 0.1 M)

Scheme 1. Synthesis of Diblock P(NIPAAm$_{235}$-*co*-MPMA$_5$)-*b*-P(DEA$_{362}$) Copolymer



were passed through a 0.45 μm pore size filter (Shanghai Chemical Reagent Co.) prior to measurements.

LCST Behaviors. The optical absorbance of P(NIPAAm-*co*-MPMA)-*b*-P(DEA) polymer solution and micelle solutions (500 mg/L) at various temperatures was measured at 500 nm using a Lambda Bio40 UV−vis spectrometer (Perkin-Elmer). Sample cells were thermostated in a circulator bath at different temperatures from 25 to 53 °C prior to the measurements, and the heating rate was set at 0.1 °C min^{-1}. The LCST was defined as the temperature producing a half increase of the total increase in optical absorbance.

Drug Loading and in Vitro Drug Release. The P(NIPAAm-*co*-MPMA)-*b*-P(DEA) (40 mg) and model drug prednisone acetate (5 mg) were dissolved in 4 mL of THF, which was then added into 50 mL of aqueous solution (pH 2, 45 °C) dropwise under vigorous stirring for 15 h to form drug-loaded CCL micelles. After the sol−gel process, the solution was put into a dialysis tube (molecular weight cutoff: 8000−12 000 g/mol) and subjected to dialysis against 500 mL of dialyzate (pH 2, 45 °C) for 24 h, which was replaced every 8 h to remove the unloaded free drug, THF, and byproduct methanol. Subsequently, the CCL solution in dialysis tube was divided into four equipartitions, which were placed into dialysis tubes, and then put into aqueous media (10 mL) with different pH values, i.e., pH 2, 20 °C (labeled as A-1); pH 2, 45 °C (labeled as A-2); pH 12, 20 °C (labeled as A-3); and pH 12, 45 °C (labeled as A-4), for drug release.

Drug-loaded SCL micelles were prepared in aqueous medium with pH 12 at 20 °C, and other procedures were the same as the preparation of drug-loaded CCL micelles aforementioned, and the four samples for drug release in different media were labeled as B-1, B-2, B-3, and B-4 according to conditions mentioned above.

The ionic strength of all aqueous solutions mentioned above was kept constant at 0.1 M. The molecular weight of prednisone acetate was 400, which was much smaller than the MWCO (8000−12 000) of the dialysis tube. Therefore, prednisone acetate released from the micelles can diffuse freely through the dialysis tube into the outside aqueous medium, while the cross-linked micelles could not penetrate through the dialysis tube into the outside aqueous medium for their molecular weights are much higher than the MWCO of the dialysis tube. The aqueous medium outside the dialysis tube was withdrawn periodically to determine the drug concentration and held constant by adding fresh medium with the same volume after each sampling. Since the characteristic absorption peak of prednisone acetate in aqueous solutions was at 242 nm,[27−29] the amount of prednisone acetate released from micelles was measured using UV absorbance at 242 nm. The total amount of prednisone acetate loaded in the micelles was the sum of the drug released in the aqueous medium and the drug remaining in the cross-linked micelles after drug release. After drug release, the solution in the dialysis tube was lyophilized, and then the dried micelles were dissolved in DMF and the drug concentration was measured by UV absorbance at 270 nm to determine the amount of prednisone acetate remained in the micelles. The entrapment efficiency (EE) and drug loading (DL) are defined as follows:

$$\mathrm{EE} = (\text{mass of drug loaded in micelles/mass of drug fed initially}) \times 100\%$$

$$\mathrm{DL} = (\text{mass of drug loaded in micelles/mass of drug-loaded micelles}) \times 100\%$$

Results and Discussion

Synthesis of P(NIPAAm-*co*-MPMA)-*b*-P(DEA) Copolymer. The synthesis of the P(NIPAAm-*co*-MPMA)-*b*-P(DEA) diblock copolymer consists of two consecutive steps, i.e., the synthesis of P(NIPAAm-*co*-MPMA) by the RAFT polymerization using CPA as a chain transfer agent and the synthesis of P(NIPAAm-*co*-MPMA)-*b*-P(DEA) diblock copolymer through RAFT polymerization using P(NIPAAm-*co*-MPMA) as a macro-CTA. The detailed synthesis route is illustrated in Scheme 1.

^{1}H NMR was employed to characterize the structure of the diblock copolymers. Figure 2A shows the characteristic peaks of P(NIPAAm-*co*-MPMA). ^{1}H NMR (CDCl$_3$, ppm, TMS): 5.9−7.0 (a, $-\mathrm{N}H-$ in NIPAAm units), 4.0 ppm (b, $-\mathrm{C}H(\mathrm{CH_3})_2$ in NIPAAm units), 3.6 ppm (e, $-(\mathrm{OC}H_3)_3$ in MPMA units), 1.0 ppm (c, $-\mathrm{CH}(\mathrm{C}H_3)_2$ in NIPAAm units), and 0.9 ppm (d, $-\mathrm{C}H_3$ in $-\mathrm{CH_2C}(\mathrm{C}H_3)\mathrm{CO}-$ in MPMA units). From the integration ratio of b:e = 5.27:1, the ratio of two types of units in the copolymer could be determined as NIPAAm:MPMA = 47:1. Figure 2B shows the ^{1}H NMR spectrum of P(NIPAAm-*co*-MPMA)-*b*-P(DEA). Both signals ascribed to P(NIPAAm-*co*-MPMA) and P(DEA) blocks could be observed in Figure 2B, i.e., 4.0 ppm (b + g, $-\mathrm{C}H(\mathrm{CH_3})_2$ in NIPAAm units and $-\mathrm{OC}H_2-$ in DEA units), 2.7 ppm (h, $-\mathrm{OCHH_2C}H_2-$ in DEA units), 2.5 ppm (i, $-\mathrm{N}(\mathrm{C}H_2\mathrm{CH_3})_2$ in DEA units), 1.1 ppm (j, $-\mathrm{N}(\mathrm{CH_2C}H_3)_2$

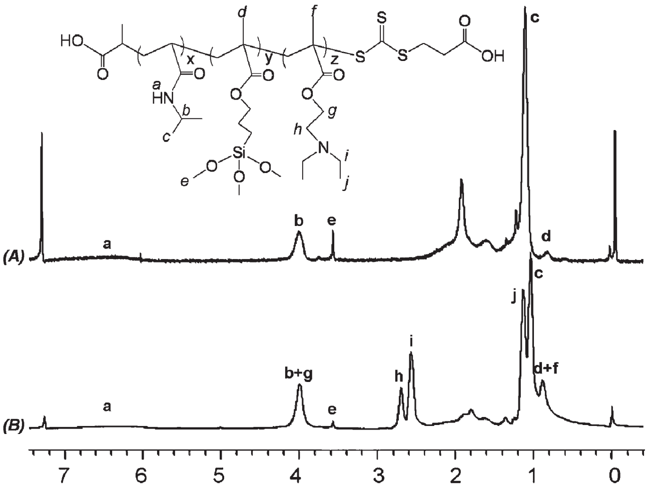


Figure 2. ^{1}H NMR spectra of P(NIPAAm$_{235}$-*co*-MPMA$_5$) (A) and P(NIPAAm$_{235}$-*co*-MPMA$_5$)-*b*-P(DEA$_{362}$) (B) in CDCl$_3$.

Table 1. Molecular Weights of Copolymers Measured by SEC-MALLS

copolymer	M_n	M_w	M_w/M_n (PDI)
P(NIPAAm$_{235}$-*co*-MPMA$_5$)	27 000	34 000	1.26
P(NIPAAm$_{235}$-*co*-MPMA$_5$)-*b*-P(DEA$_{362}$)	94 000	130 000	1.38

in DEA units), and 0.9 ppm (d + f, $-CH_3$ in $-CH_2C(CH_3)$-CO$-$ in MPMA units and $-CH_2C(CH_3)CO-$ in DEA units).

SEC-MALLS was utilized to determine the molecular weights of the polymers. It can be seen from Table 1 that M_n and polydispersity (M_w/M_n) of P(NIPAAm-*co*-MPMA)-*b*-P(DEA) (94 000 and 1.38) are both larger than that of P(NIPAAm-*co*-MPMA) (27 000 and 1.27). In addition, both polymers have unimodal molecular distributions from the SEC traces (data not shown). The data of molecular weights also suggest that the reactivity of macro-CTA (P(NIPAAm-*co*-MPMA)) is high; as a result, DEA monomer can be inserted into the macro-CTA. Similar RAFT polymerization to insert the second or third block was also reported in the literature.[30,31] Based on the molar ratio (47:1) of NIPAAm units to MPMA units aforementioned and the M_n (27 000) of P(NIPAAm-*co*-MPMA), the numbers of MPMA units and NIPAAm units are calculated to be 5 and 235, respectively. Then the number of DEA units is further calculated to be 362 from the M_n (94 000) of P(NIPAAm-*co*-MPMA)-*b*-P(DEA). Therefore, the copolymer is labeled as P(NIPAAm$_{235}$-*co*-MPMA$_5$)-*b*-P(DEA$_{362}$).

Cross-Linked Micelle Formation. A schematic illustration of formation of CCL and SCL micelles in aqueous media is presented in Figure 1. The lyophilized CCL and SCL micelles could not be dissolved in DMF, indicating the success of cross-linking. The morphology of cross-linked micelles was observed by TEM. Two typical TEM images and size distributions of micelles are presented in Figure 3. As shown in Figures 3A$_1$ and 3B$_1$, both of the CCL and SCL micelles display well-defined spherical shapes with diameters ranged from 40 to 90 nm and 45 to 85 nm, respectively. Since the copolymer can be completely dissolved in DMF, the observed nanospheres in DMF indicate the successful cross-linking of core or shell. From the dynamic light scattering (DLS) data measured by a Zetasizer, the average sizes of CCL and SCL micelles are 267 and 316 nm, respectively (Figures 3A$_2$ and 3B$_2$). The difference between the sizes measured by DLS and determined by TEM is due to the following reason. The sizes measured by the DLS are based on the swollen micelles in aqueous solutions, and the sizes observed by TEM are for the dried micelles. According to previous literature, the average diameter measured by DLS is about 2$-$4 times larger than that measured by TEM.[32-34] Our results are in agreement with previous studies.

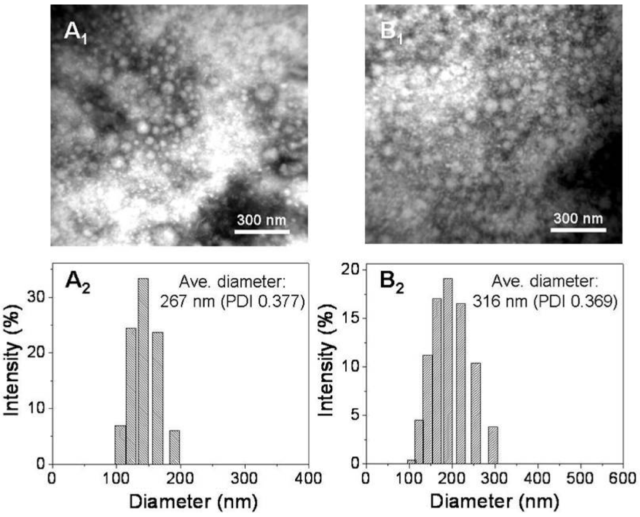


Figure 3. TEM images and sized distributions measured by a Zetasizer of CCL micelles (A) and SCL micelles (B) in DMF.

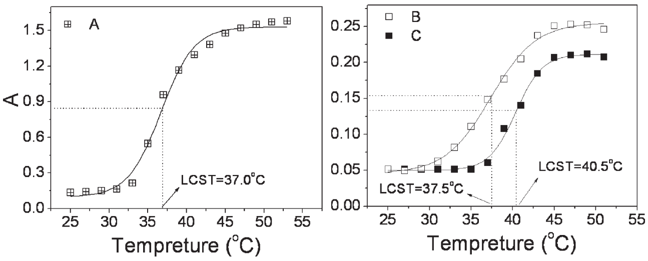


Figure 4. Thermosensitive behaviors of (A) P(NIPAAm$_{235}$-*co*-MPMA$_5$)-*b*-P(DEA$_{362}$) polymer dissolved in distilled water directly, (B) CCL micelles in the aqueous medium with pH 2, and (C) SCL micelles in the aqueous medium with pH 12.

Thermoresponsive Properties of Cross-Linked Micelles. In order to determine whether the P(NIPAAm$_{235}$-*co*-MPMA$_5$)-*b*-P(DEA$_{362}$) micelles exhibit a thermal response, we measured the optical absorbances of copolymer in distilled water (A), CCL micelles in aqueous medium with pH 2 (B), and SCL micelles in aqueous medium with pH 12 (C). As presented in Figure 4, the LCSTs of (A), (B), and (C) are 37.0, 37.5, and 40.5 °C, respectively, which are higher than that of pure PNIPAAm homopolymer. According to previous studies, the introduction of a small proportion of hydrophobic MPMA as the cross-linking agent into PNIPAAm chains slightly changes the LCST behavior (both response rate and LCST value).[22] In addition, the existence of terminal carboxylic acid groups affects the LCST and the effect is non-negligible.[35] Thus, it seems that the elevated LCST is attributed to the end-group effect. Additionally, it should be noted that although the LCST values of (A), (B), and (C) are similar, the extent of turbidity variation of (A) is much greater than that of (B) and (C), which is probably attributed to the cross-linking of MPMA units, namely, the existence of silica cross-linked core or shell limits the aggregation of PNIPAAm chains above the LCST.[22]

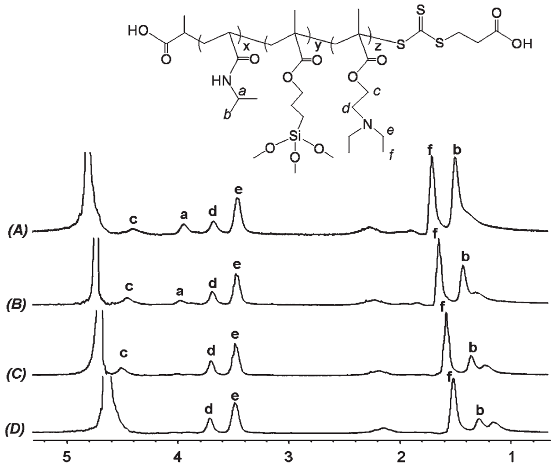


Figure 5. ^{1}H NMR spectra of CCL micelles in D_2O (pH 2) at different temperatures (A) 20, (B) 30, (C) 40, and (D) 50 °C.

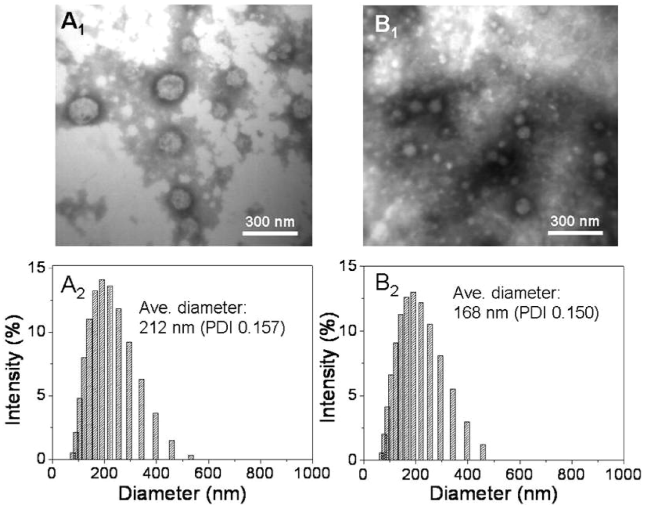


Figure 6. TEM images and sized distributions measured by a Zetasizer of CCL micelles in aqueous media: (A) pH 2, 20 °C; (B) pH 2, 45 °C.

We further characterized the thermal response of the CCL micelles by ^{1}H NMR in D_2O (pH 2) at different temperatures of 20, 30, 40, and 50 °C (Figure 5). It is found that the characteristic signals of NIPAAm units (δ = 3.9 and 1.3 ppm, i.e., peaks a and b) and DEA units (δ = 4.4, 3.7, 3.5, and 1.5 ppm, i.e., peaks c, d, e, and f) are distinct at 20 °C. After heating to 30 °C, the peaks assigned to NIPAAm units are suppressed significantly, and the corresponding signals become weaker and weaker with the increase of temperature to 40 and 50 °C. The peak a at δ = 3.9 ppm completely disappears at high temperatures, indicating the phase transition of PNIPAAm chains from the hydrophilic to the hydrophobic state. In addition, the peak c at δ = 4.4 ppm from DEA units could not be observed at 50 °C, which is possibly attributed to the fusion of peak c with the signal from D_2O into a broad one.

Thermal sensitivity of the cross-linked micelles is further visualized by TEM. As shown in Figures 6A$_1$ and 6B$_1$, the lyophilized CCL micelles redispersed in aqueous media with pH 2 have the sizes ranged from 75 to 165 nm at 20 °C and 40 to 80 nm at 45 °C, respectively. Obviously, the average size of micelles decreases when temperature increases to 45 °C. The reason is that the P(NIPAAm-*co*-MPMA) block contracts at temperature above the LCST, leading to the shrinkage of micelles and the decrease in the micellar size. From Figures 6A$_2$ and 6B$_2$, the average sizes of CCL micelles determined by the Zetasizer are 212 nm at 20 °C and 168 nm at 45 °C. As mentioned before, the difference between the sizes measured by DLS and determined by TEM is due to the different states of micelles in aqueous media and after drying.

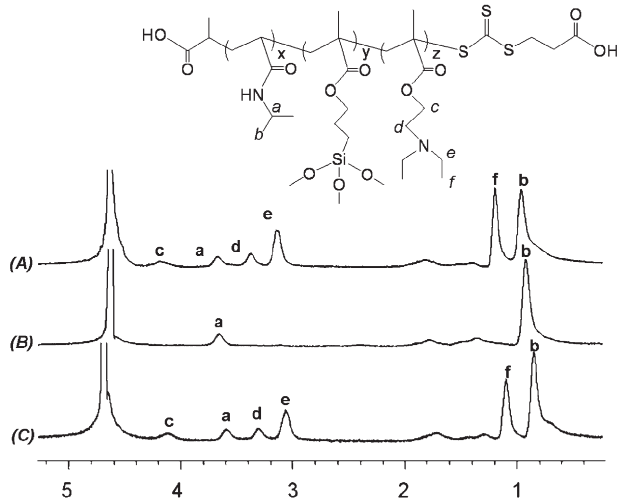


Figure 7. ^{1}H NMR spectra of SCL micelles in D_2O: (A) pH 2 by adding DCl; (B) pH 12 by adding NaOH in (A); (C) pH 2 by adding DCl in (B).

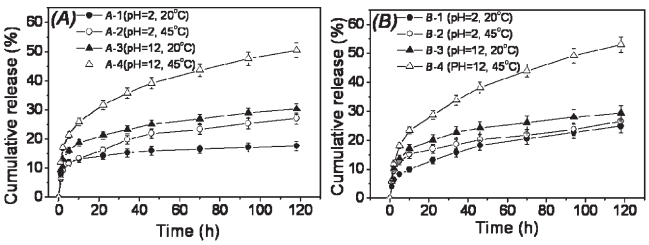


Figure 8. TEM images and sized distributions measured by a Zetasizer of SCL micelles in aqueous media: (A) pH 2, 20 °C; (B) pH 12 by adding NaOH in (A), 20 °C.

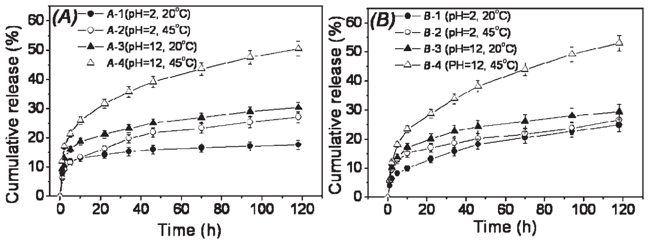


Figure 9. Cumulative drug releases from (A) CCL micelles prepared at pH 2 and 45 °C and (B) SCL micelles prepared at pH 12 and 20 °C in the aqueous media with different pH values at different temperatures.

pH Responsive Properties of Cross-Linked Micelles. ^{1}H NMR and TEM were both employed to verify the pH sensitivity of the cross-linked micelles. As shown in Figure 7A, in the ^{1}H NMR spectrum of SCL micelles in D_2O at pH 2, all the characteristic peaks of NIPAAm units (δ = 3.6 and 0.8 ppm, i.e., peaks a and b) and DEA units (δ = 4.1, 3.3, 3.1, and 1.1 ppm, i.e., peaks c, d, e, and f) are distinct because both blocks are soluble. At pH 12 (Figure 7B), the characteristic peaks of DEA units (δ = 4.1, 3.3, 3.1, and 1.1 ppm, i.e., peaks c, d, e, and f) disappear, which is due to the fact that P(DEA) is a weak polybase and therefore could turn from a hydrophilic to hydrophobic state when subjected to the pH change from acidic to alkaline due to the deprotonation of tertiary amine groups. However, as

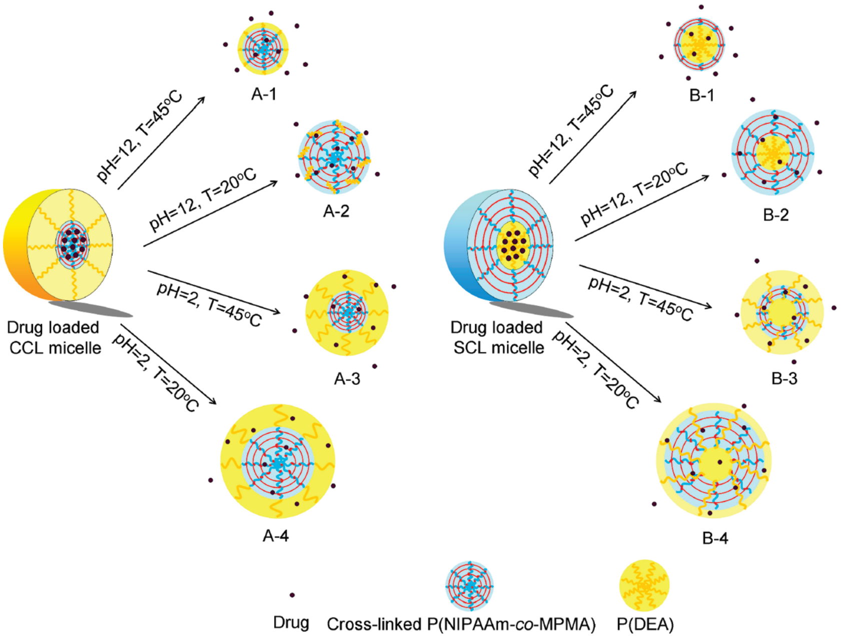


Figure 10. Schematic illustration of temperature and pH controlled drug release from CCL and SCL micelles.

shown in Figure 7C, the peaks of DEA units re-emerge when pH value of the aqueous medium is adjusted back to 2 again, confirming the transition of P(DEA) from hydrophobic to hydrophilic state in the acid medium.

Figures 8A_1 and 8B_1 are TEM images of lyophilized SCL micelles after redispersed in aqueous media with pH 2 and pH 12, respectively, at 20 °C. As shown in TEM images, the sizes of micelles range from 120 to 215 nm at pH 2 and 55 to 100 nm at pH 12. Apparently, the size of micelles at pH 2 is bigger than that of pH 12. The reason is that the SCL micelles were fully swollen since both two blocks were soluble under pH 2 at 20 °C. However, P(DEA) block would become hydrophobic at pH 12, leading to the contraction of P(DEA) chains and consequently the shrinkage of micelles. The sizes measured by the Zetasizer at different pH values (Figures 8A_2 and 8B_2) show a similar trend.

Thermoresponsive and pH Responsive Drug Release. In order to study the temperature and pH double controlled drug release properties of CCL and SCL micelles, an anti-inflammation drug, prednisone acetate, was chosen as a model drug. The drug-loaded CCL micelles were prepared at pH 2 and 45 °C (A), and the drug-loaded SCL micelles were prepared at pH 12 and 20 °C (B). The EE and DL values were 28.1% and 3.4% for CCL micelles and 31.5% and 3.8% for SCL micelles, respectively.

As shown in Figure 9, both release profiles at pH 12, 45 °C (A-4 and B-4) exhibit a fastest cumulative release behavior. The reason is that CCL and SCL micelles will both turn completely hydrophobic at pH 12, 45 °C; as a result of the shrinkage of the micellar structure, the drug is squeezed out more quickly from the micelles. Similar results were reported in previous publications.[22,36−39] On the basis of the experiments, the cumulative drug release within 118 h for A-4 and B-4 is 50.6% and 53.0%, respectively. On the contrary, when drug release is performed in aqueous media of pH 2, 20 °C, the total amount of drug released within 118 h is the lowest. The data for A-1 and B-1 are only 17.7% and 24.9%, respectively. Under this circumstance, both core and shell of the cross-linked micelles change to be hydrophilic. According to previous studies on non-cross-linked micelles, the drug would diffuse out quickly due to the deformation of micellar structure under this condition.[40] However, herein the cross-linking would keep the micellar structure although the core and shell turn to be hydrophilic simultaneously and thus effectively sustain the diffusion of the drug from the micelle to the aqueous medium. At pH 12, 20 °C for A-2 and B-2, the P(NIPAAm-*co*-MPMA) block of the micelles is hydrophilic and the P(DEA) block is hydrophobic. On the contrary, at pH 2, 45 °C for A-3 and B-3, the P(DEA) block turns to be hydrophilic and the P(NIPAAm-*co*-MPMA) block becomes hydrophobic. In brief, one of the two blocks keeps hydrophilic and the other keeps hydrophobic. As mentioned above, the hydrophobic blocks cause the shrinkage of the partial micellar structure, leading to the accelerated drug release. As a result, the drug release rates are lower than that from the micelles with two hydrophobic blocks and higher than that from the micelles with two hydrophilic blocks. A schematic illustration of the thermo- and pH-induced drug release from CCL and SCL micelles in aqueous media is presented in Figure 10. To the best of our knowledge, there is no literature reporting the drug release study on double hydrophobic or hydrophilic cross-linked micelles. The mechanism of drug release remains to be investigated further and may be our next subject of research.

Conclusions

In summary, pH and thermo double responsive hybrid CCL and SCL micelles based on P(NIPAAm-*co*-MPMA)-*b*-P(DEA) were prepared by the cross-linking of P(NIPAAm-*co*-MPMA) blocks using inorganic silica-based cross-linking strategy. The pH and thermo double sensitivities of the CCL and SCL micelles were studied by ^{1}H NMR and TEM. Taking advantage of their LCSTs being slightly above 37 °C as well as double responsive properties, the as-prepared CCL and SCL micelles would have great potential as novel polymeric carriers for both temperature and pH controlled drug release.

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