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Sulfobetaine-Based Homo- and Copolymers by RAFT: Cross-Linked Micelles and Aqueous Solution Properties

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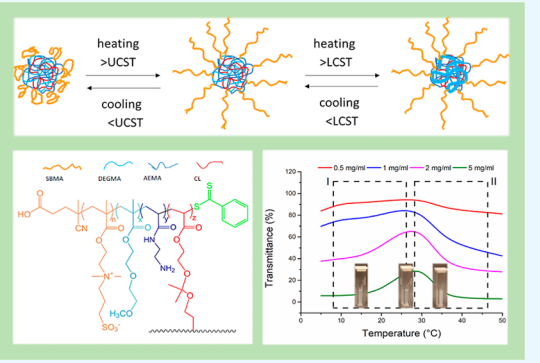
ABSTRACT: In this study, we describe the synthesis and aqueous solution behavior of temperature-sensitive *N*-(3-sulfopropyl)-*N*-methacroyloxyethyl-*N*,*N*-dimethylammonium betaine (SBMA) homopolymers and core cross-linked micelles (CCMs) with an SBMA shell. Reversible addition−fragmentation chain transfer polymerization has been utilized to synthesize sulfobetaine homopolymers, followed by CCM formation during copolymerization in the presence of an acid-degradable cross-linker. First, SBMA homopolymers of varying chain lengths were synthesized, and it has been demonstrated that an increase in the chain length and concentration of the homopolymer resulted in an increase in the upper critical solution temperature (UCST). Besides, micelles showed concentration-dependent dual temperature-sensitive behavior with UCST and LCST transitions. Also, homopolymers and CCMs were characterized by FTIR, ^{1}H-NMR, GPC, and TEM. Micelle formation and temperature sensitivity were also investigated by DLS. As a result, stabilized micelles were successfully prepared with the motivation of preventing premature drug release and achieving a pH- and temperature-controlled system. Due to their dual-responsive characteristics, the CCMs show promising potential to be used as smart drug carriers for controlled delivery.



KEYWORDS: *zwitterionic, cross-linked micelle, UCST, LCST, controlled drug delivery, thermoresponsive polymers*

1. INTRODUCTION

Temperature-sensitive polymers are a class of "smart" materials exhibiting conformational changes in response to a temperature change. Owing to thermoresponsive properties, their applications have gained high popularity in many fields including drug release, in which a facile control of stimuli responsiveness is required. Temperature sensitivity is attributed to the hydrophilic and hydrophobic components' balance and interaction of free functional groups in a solvent.[1] Based on the interactions with the aqueous medium, these polymers are classified into two main types: polymers showing a lower critical solution temperature (LCST)- and an upper critical solution temperature (UCST)-type behavior. The LCST is the point below which the polymer is soluble in water. On the other hand, the polymer becomes monobasic or soluble above a specific transition temperature called the UCST. Poly(*N*-isopropyl acrylamide) (poly(NIPA) is the most popular polymer having LCST transition in an aqueous solution.[2] Also, using diethylene glycol methacrylate (DEGMA) in a copolymer structure allows obtaining temperature-sensitive systems that can be used for drug delivery applications. According to Seuring and Agrawal, UCST polymers have problems with the stable UCST behavior, and therefore much effort has been focused on designing optimum polymers considering effective parameters such as pH, molecular weight, ionic strength, concentration, and electrolytes in solution.[3]

Sulfobetaines, as zwitterionic polymers, show UCST-type transition by electrostatic interactions in the aqueous medium, and the UCST depends on ionic strength due to the presence of cationic ammonium and anionic sulfonate groups in the chemical structure. These polymers are usually synthesized by radical/reversible addition−fragmentation chain transfer (RAFT) polymerization of sulfobetaine monomers.[4,5] Taking advantage of such characteristics, micellar structures can be prepared, in which the shell part is composed of betaine polymer chains. In response to a stimulus, micelles can be expanded at physiological conditions, and their responsiveness depends on the polymer chain length, concentration, and ionic strength.[6] In addition, sulfobetaines have recently attracted a high interest due to their excellent biocompatibility. Although it is widely accepted that polyethylene glycol (PEG) is nonantigenic and nonimmunogenic, recent clinical studies

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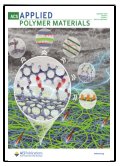

Scheme 1. Synthesis of the Homopolymer by RAFT Polymerization

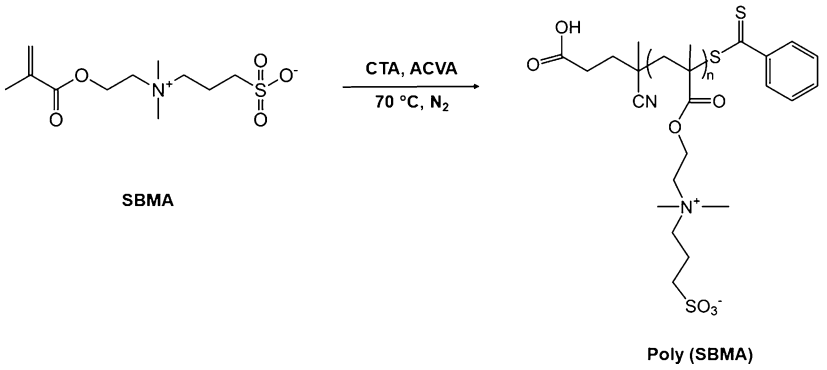

Table 1. Characterization of Poly(SBMA) Homopolymers: Molecular Weight, PDI, and Cloud Point Values[a]

					cloud point (°C)	
sample	monomer/CTA/initiator	M_n (kDa)	M_w (kDa)	PDI (M_w/M_n)	10 (mg/mL)	5 (mg/mL)
M1	40/1/0.2	10	10.5	1.045		
M2	60/1/0.2	18.6	19.0	1.023		
M3	65/1/0.2	19.2	20.0	1.044		
M4	100/1/0.2	31.5	32.7	1.040	12	11
M5	120/1/0.2	69.4	72.7	1.047	24	19
M6	180/1/0.2	75.7	79.2	1.046	26	22

[a] $\mathbf{M}_n$ and $\mathbf{M}_w$ were estimated by GPC. No cloud points were observed for M1, M2, and M3.

show that anti-PEG antibodies were detected in not only patients treated with PEGylated therapeutics but also patients treated with PEGylated drugs treatment-naive with a prevalence from <1 to 72%.[7] Therefore, it is of great importance to deeply examine the use of sulfobetaines as an alternative of PEG for the shell part in the micellar structure.

Cross-linking in the micelle structure has been proposed in the last decade as a way to prevent early micelle dissociation and premature drug release.[8,9] They can be designed as environmentally degradable or hydrolyzable systems at low pH or reductive conditions, which can lead to time- or site-controlled release of bioactive agents.[10] Owing to the versatility of RAFT polymerization, it is possible to obtain cross-linked micelles during copolymerization and improve the functionality for further modifications.[8,9] The main concept to obtain a stable and multifunctional micelle-based nanocarriers relies on using a hydrophilic polymer in the shell part, while other components are used for the core part together with a specific cross-linker. Also, using polymers with the LCST or UCST behavior reveals temperature sensitivity that can be utilized for controlled delivery of the drugs. Besides, there have been many reports about the synthesis and potential applications of dual-responsive ("schizophrenic") micelles exhibiting the LCST and UCST behavior in one polymeric system. According to several studies, dual-responsive schizophrenic micelles have the potential to be used in varying "smart" applications such as controlled uptake, transport, and release, and also as smart emulsifiers. Besides, Papadakis et al. speculated that nanocarriers with an adjustable structure and payload can be achieved upon small temperature changes.[11] Vishnevetskaya et al. reported that the tunable thermoresponsive properties of schizophrenic micelles make them promising candidates for a variety of applications, such as the development of switchable surfaces and thermo-optical devices.[12] Sun et al. prepared a hydrogel consisting of DMAEMA and *N*-(3-sulfopropyl)-*N*-methacroyloxyethyl-*N*,*N*-dimethylammonium betaine (SBMA), and exhibiting an LCST/UCST/pH-sensitive swelling behavior. Moreover, they stated that deswelled hydrogels below the UCST and LCST could lead to an "off" effect on long-term drug release.[13] According to Morimoto and Yamamoto's report, an LCST−UCST-like thermoresponsive zwitterionic copolymer can be used to stabilize or control the activity of proteins.[14] Rajan and Matsumura[12] suggested that tunable dual-thermoresponsive core−shell nanogels can be used in different applications such as drug delivery, biosensing, and development of thermoresponsive scaffolds. Kim et al. reported that dual-thermoresponsive micelles can be applied to drug delivery systems.[6] According to these studies, by means of controlling and altering the LCST/UCST transition of the polymers, micelles with dual-thermoresponsive characteristics are promising to reach tunable copolymeric systems, especially for drug loading and release studies.

Herein, we report the synthesis and aqueous solution behavior of temperature-sensitive SBMA containing homopolymers and acid-degradable core cross-linked micelles (CCM). We investigated the chain length and concentration dependency of homopolymers' cloud point temperature. Also, the temperature sensitivity, phase transition behavior, and size change of CCMs formed by SBMA on the shell and DEGMA in the core were analyzed. Besides, the contribution of SBMA and DEGMA to the temperature sensitivity was discussed. As a result, a stable cross-linked micellar system exhibiting LCST and UCST transitions was obtained as a potential drug nanocarrier.

2. MATERIALS AND METHODS

2.1. Materials. 4-Cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPAD as CTA), 4,4′-azobis(4-cyanovaleric acid) (ACVA), diethylene glycol methyl ether methacrylate (DEGMA), 2,2-dimethoxypropane, 2-hydroxyethyl methacrylate (HEMA), *p*-toluenesulfonic acid monohydrate (*p*-TSA·H_2O), triethylamine, NaCl were

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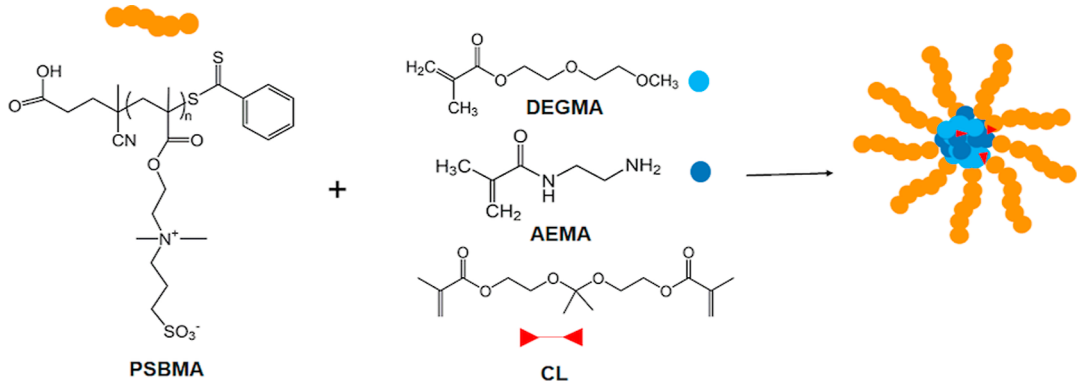
Scheme 2. CCMs Synthesis by RAFT Polymerization



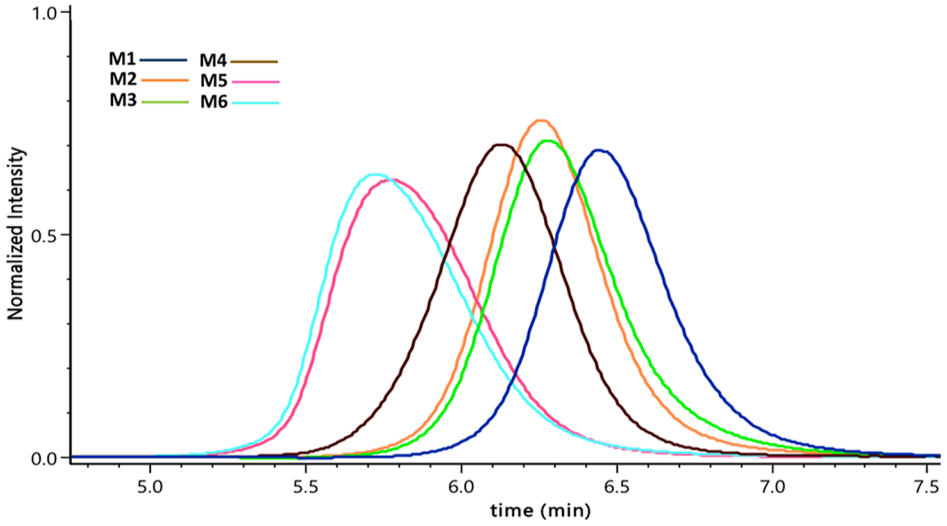


Figure 1. Normalized GPC traces of the homopolymers (M1−M6).

purchased from Aldrich. SBMA (sulfobetaine, SBMA), 1,4 dioxane, 2-propanol, hexane, ethyl acetate, sodium nitrate, and acetonitrile for liquid chromatography were purchased from Merck. *N*-(2-Aminoethyl) methacrylamide hydrochloride (AEMA) was purchased from PolyScience and sodium azide from SERVA.

2.2. Methods. *2.2.1. Synthesis and Characterization of Poly(SBMA) Homopolymers as MacroCTAs by RAFT Polymerization.* In order to synthesize poly(SBMA) homopolymer as macroCTA (Scheme 1), the SBMA monomer with varying concentrations (Table 1) (2−9 mmol, 40−180 equiv), the chain transfer agent (CPAD) (0.054 mmol, 1 equiv), and the initiator (ACVA) (0.011, 0.2 equiv, in dioxane) were dissolved in 0.5 M aqueous NaCl at pH 7−7.5, and then the solution sealed with a septum was purged with N_2 for 30 min. The polymerizations were conducted at 70 °C for 30 min, and the polymer was purified by dialysis against water (MWCO 3500) for 3−4 h.

Following this step, poly(SBMA) was precipitated from ethanol three times. Then, polymers were recovered by vacuum drying, and pale pink-colored polymers were obtained. ^{1}H-NMR and Fourier transform infrared (FTIR) spectroscopy were used to identify and characterize homopolymers [macroCTA(s)]. Gel permeation chromatography (GPC) was used for molecular weight determination.

2.2.2. Cross-Linked Micelle Synthesis by RAFT. In order to synthesize CCMs, the acid-sensitive cross-linker (CL, 2,2-dimethacroyloxy-1-ethoxypropane) was first synthesized according to the literature.[15,16] MacroCTA (0.0033 mmol, 2 equiv), AEMA (0.26 mmol, 162 equiv), DEGMA (0.75 mmol, 468 equiv), and an acid-sensitive cross-linker (5% of macroCTA) were dissolved in distilled water, and then ACVA (0.0016 mmol, 1 equiv) in isopropyl alcohol as an initiator was added to the solution (Scheme 2). The solution was degassed by purging with N_2 for 30 min and placed in a preheated oil bath at 70 °C. The reaction was allowed to stir overnight. Following the reaction, the micelle solution was dialyzed against distilled water to remove the unreacted substance using a dialysis membrane with MWCO 3500, and then the product was dried by lyophilization, resulting in a white solid. In order to compare the SBMA length on the UCST−LCST behavior of core cross-linked micelles, CCM3 and CCM6 were synthesized from M3 and M6 homopolymers, respectively. The chemical structures of purified CCMs were characterized by FTIR and ^{1}H-NMR spectroscopy, while the size and size distribution of the samples were determined by dynamic light scattering (DLS). Besides, CCMs' morphology and size were analyzed by transmission electron microscopy (TEM).

2.2.3. Characterization. ^{1}H-NMR spectra of the samples were acquired using a Bruker Ultrashield 300 MHz liquid NMR spectrometer using related deuterated solvents. FTIR spectra of samples were acquired using a Thermo Scientific Nicolet 6700 FTIR spectrometer (Madison, USA).

GPC was used to determine the molecular weight and molecular weight distribution of macroCTA(s). The chromatogram was taken on a TOSOH EcoSEC HLC-8320 GPC/SEC system with an RI detector and a Wyatt miniDAWN Treos-II MALS (multiangle light scattering) detector equipped with a PSS SUPREMA analytical 100 Å column (8 × 300 mm, 10 μm, Polymer Standards Service, USA Inc) at room temperature. In the mobile phase, a solvent mixture containing 80% aqueous 0.1 M ammonium sulfate-20% acetonitrile with 0.0125% sodium azide was used. The mobile phase and the polymer solutions were filtered by PTFE membrane filters with a 0.2 μm pore. The flow rate of the mobile phase was set to 2.0 mL/min.

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The injection volume of the filtered poly(SBMA) solutions was adjusted to 50 μL.

The cloud points of macroCTAs and CCMs with varying concentrations (1, 2, 5, 10 mg/mL and 0.5, 1, 2, 5 mg/mL for macroCTAs and CCMs, respectively) in the aqueous solutions were determined by turbidity measurement using a UV/Vis spectrophotometer (at 400 nm) (Thermo Scientific, Genesys 10 S equipped with a Peltier temperature controller). The cloud point temperatures were determined as the point at which the solution transmittance was 50%.[5]

The hydrodynamic diameters of CCMs in water were determined using a Malvern Zetasizer NanoZS DLS. DLS measurements were performed at various concentrations of micelles (0.5, 1, and 2 mg/mL) and temperatures (20−35 °C). All solutions were prepared with 1 M NaCl to modulate the interactions among zwitterionic poly(SBMA) chains. The measurement was carried out for 3 min after the temperature changed, and each measurement was repeated in triplicate. Besides, TEM was used for the size and morphology analysis of CCMs (CCM3).

3. RESULTS AND DISCUSSION

3.1. Characterization of Homopolymers and Micelles.

In this study, RAFT polymerization has been utilized due to the fact that it allows the formation of well-defined polymers with good control over the molecular weight (M_n), dispersity (M_w/M_n), and direct cross-linking in aqueous solutions. The syntheses of macroCTA and CCMs by RAFT polymerization were depicted in Schemes 1 and 2. The polymerization was conducted at neutral pH in order to provide that CTA and initiator were soluble in the medium.

Initially, macroCTA of SBMA was synthesized with varying monomer concentrations ($[M]_0$) at constant $[CTA]_0/[I]_0$ ratios (1/0.2) to observe the effect of the SBMA chain length on physicochemical characteristics. Molecular weight analysis was performed by GPC with an eluent of 80% 0.1 M ammonium sulfate and 20% acetonitrile. According to the GPC analysis, the homopolymers in relatively low polydispersities were obtained, as expected from the RAFT method. Moreover, increasing the SBMA monomer ratio results in an increase in the molecular weight of homopolymers, as given in Table 1. A clear shift in the molecular weight is demonstrated by GPC, as the overlaid molecular weight distributions shown in Figure 1. In Donovan et al.'s study, SBMA was polymerized by RAFT (CTA/I: 5/1 and 0.706 M monomer), and a similar molecular weight regime was obtained.[4]

Following the synthesis of poly(SBMA) homopolymers, we proceeded to the CCM synthesis and characterization. As mentioned in the Materials and Method part, DEGMA and AEMA were used as comonomers because this combination allows for determining the effect of hydrophilicity and temperature sensitivity of the core part. Thus, the resulting polymer will have a potential use for further drug delivery applications with controlled release at different temperatures.

For the structural analysis of the poly(SBMA) homopolymer and CCM, FTIR and ^{1}H-NMR spectroscopies were used. The FTIR analysis of the homopolymer and CCM were given in Figure 2A,B. According to the FTIR spectrum of homopolymer, the absorption band at 3450 cm^{-1} was assigned to the O−H bending, the absorption band at 1720 cm^{-1} was assigned to the vibrations from the carboxylic acid groups (C═O stretching), and the absorption band at 2970 cm^{-1} was assigned to aliphatic groups (C−H stretching). The absorption peak at 1470 cm^{-1} was assigned to the quaternary ammonium group, and the absorption bands appearing around 1035 and 1100 cm^{-1} were assigned the sulfur ($-SO_3^-$) stretching vibration bands. When the FTIR spectra of the CCM and poly(SBMA) homopolymer were compared, the peak intensity at 2875 and 1720 cm^{-1} increased due to the presence of DEGMA, AEMA, and CL in the final structure. In addition, the new peak at 1245 cm^{-1} corresponding to the C−O stretching confirms the successful addition of a cross-linker and DEGMA to the micellar structure.[17]

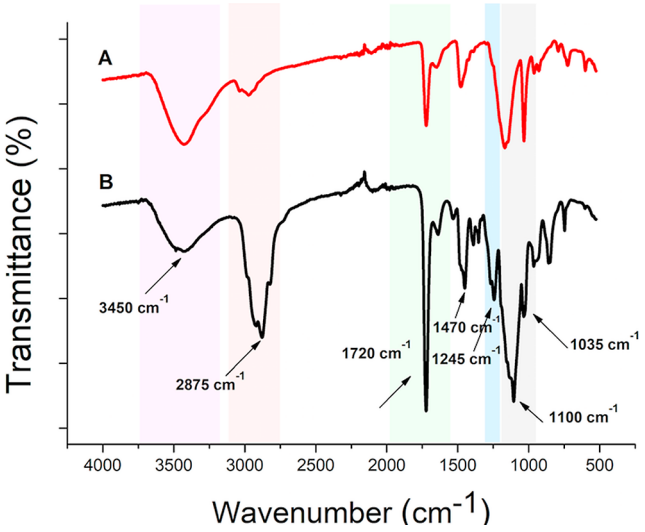

Figure 2. FTIR spectra for poly(SBMA) homopolymer (A) and CCM (B).

The formation of the homopolymer and CCM was also confirmed by ^{1}H-NMR analysis (Figure 3). In the homopolymer's spectrum, the polymer backbone and $\mathbf{CH_2}CH_2SO_3^-$ of SBMA sidechain signals were observed at 0.8−2.5 ppm. Also, $-CH_2\mathbf{CH_2}SO_3^-$, $-N^+(\mathbf{CH_3})_2$, $-N^+(CH_3)_2\mathbf{CH_2}$, $-OCH_2\mathbf{CH_2}N$, $-O\mathbf{CH_2}CH_2N$ of the SBMA side chain were shown at 2.96−3.13, 3.22−3.38, 3.80−4.00, 4.46−4.65 ppm, respectively. The RAFT end group signal was shown at 7.49−8.20 ppm. The degree of polymerization of the SBMA block was determined to be 75 from the integration of the end group signals between δ 7.50−8.20 ppm relative to the polymer peaks 3.07 and 3.32 ppm, as demonstrated in the study of Doncom et al.[18] [PSBMA, Mn (1H-NMR) = 15.884 kDa and Mn(SEC) = 18.3 kDa].

In the CCM's spectrum, the polymer backbone and $-\mathbf{CH_2}CH_2SO_3^-$ of the SBMA sidechain signals were observed at 0.8−2.5 ppm. Also, $-CH_2\mathbf{CH_2}SO_3^-$, $-N^+(\mathbf{CH_3})_2$, $-N^+(CH_3)_2\mathbf{CH_2}$, $-OCH_2\mathbf{CH_2}N$, $-O\mathbf{CH_2}CH_2N$ peaks of the SBMA sidechain were shown at 2.96−3.13, 3.22−3.38, 3.80−4.00, 4.46−4.65 ppm, respectively. The $-O\mathbf{CH_3}$, $-OCH_2\mathbf{CH_2}O$, and $-COO\mathbf{CH_2}CH_2O$ peaks corresponding to the DEGMA sidechain were shown at 3.38−3.53, 3.55−3.80, and 4.46−4.65 ppm, respectively. The $-COO\mathbf{CH_2}CH_2O$, $-COOCH_2\mathbf{CH_2}O$ peaks of the cross-linker sidechain were shown at 3.55−3.80 and 4.46−4.65 ppm, respectively. The $-CONH\mathbf{CH_2}CH_2$ and $-CONHCH_2\mathbf{CH_2}$ peaks corresponding to the AEMA sidechain were shown at 3.55−3.80 and 4.09−4.31, respectively. The signal of CTA end groups was not seen in the ^{1}H-NMR spectrum of CCM due to the higher molecular weight of CCMs and the RAFT end group remaining in the internal structure of the micelle.

TEM was used for the size and morphology analysis of a selected micelle (CCM3). According to the TEM results, the

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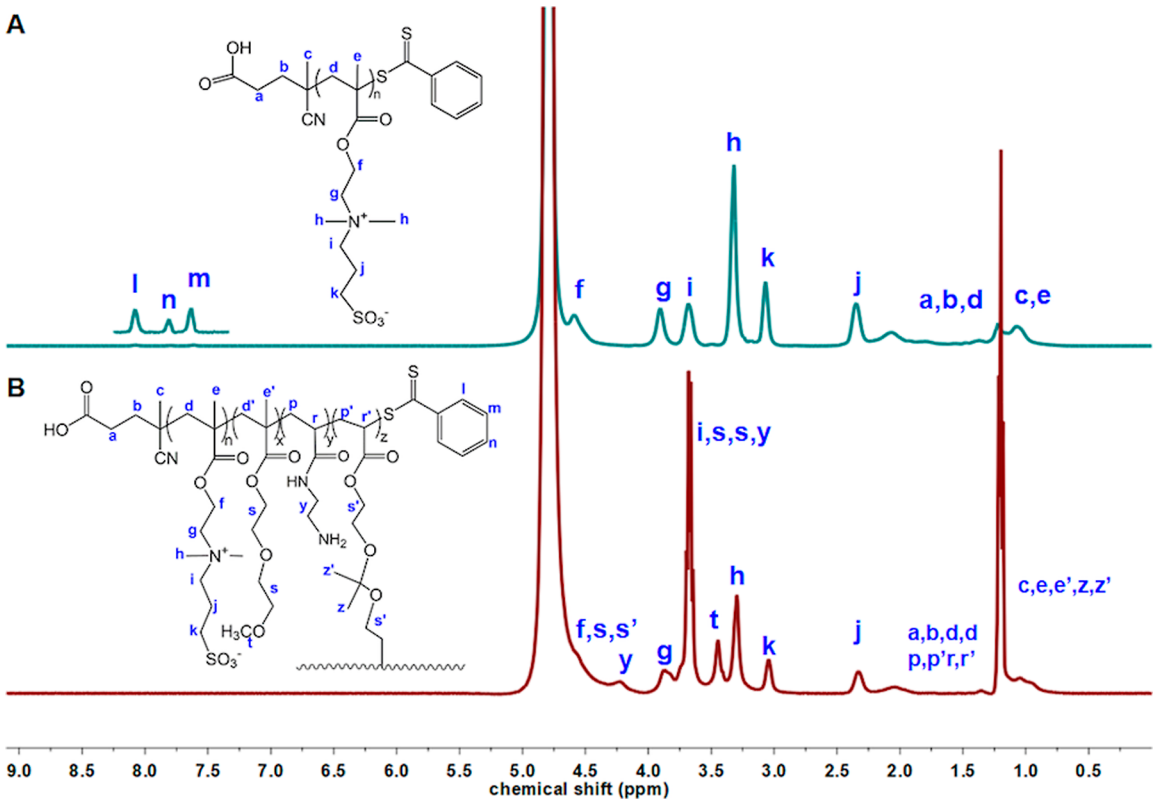

Figure 3. ^{1}H-NMR spectrum of poly(SBMA) (A) and CCM (B) in 0.5 M NaCl in D_2O.

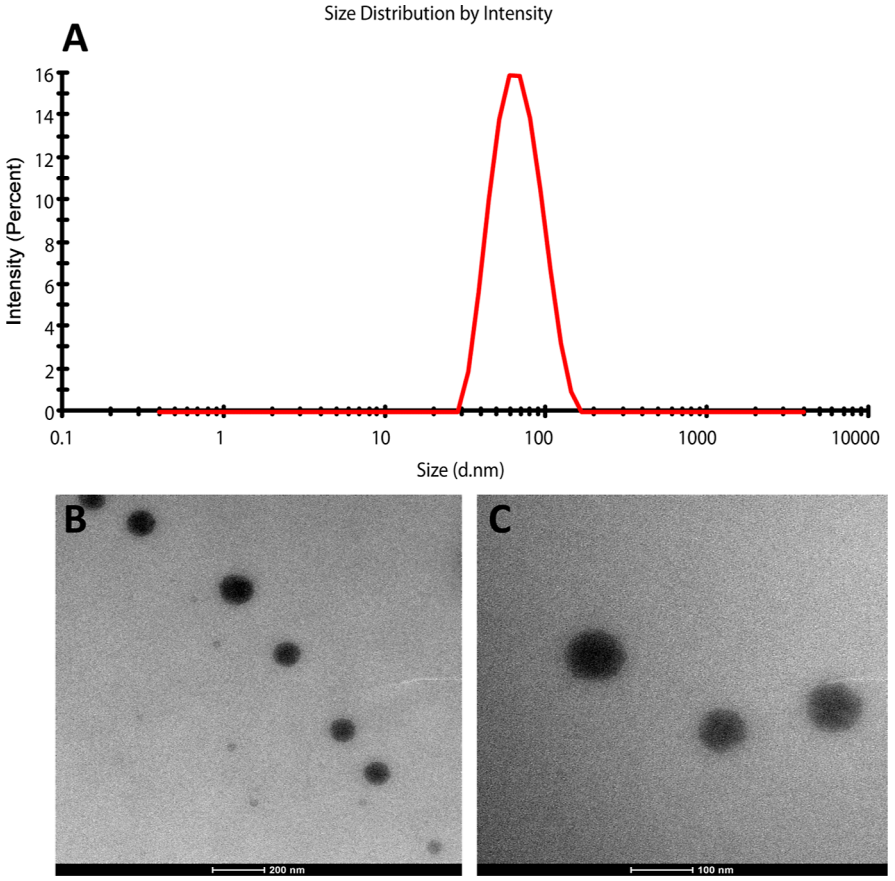

Figure 4. DLS result of CCM3 (A), TEM results of CCM3 on 200 (B) and 100 nm (C) scale.

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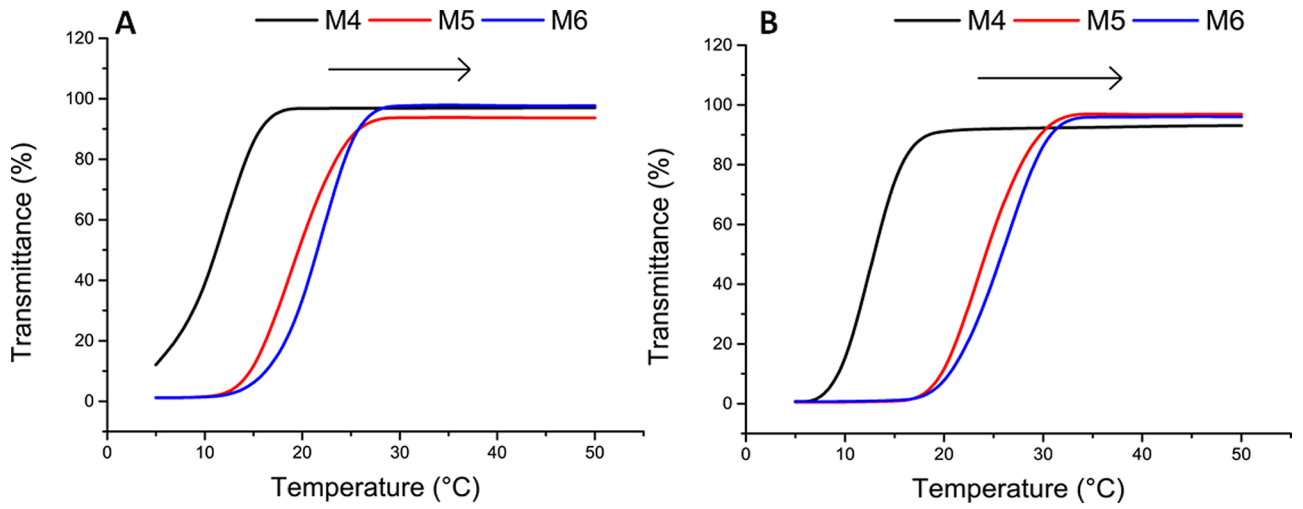

Figure 5. Effect of the chain length on the temperature sensitivity of homopolymers (M4, M5, and M6) in aqueous solutions with 5 (A) and 10 mg/mL (B).

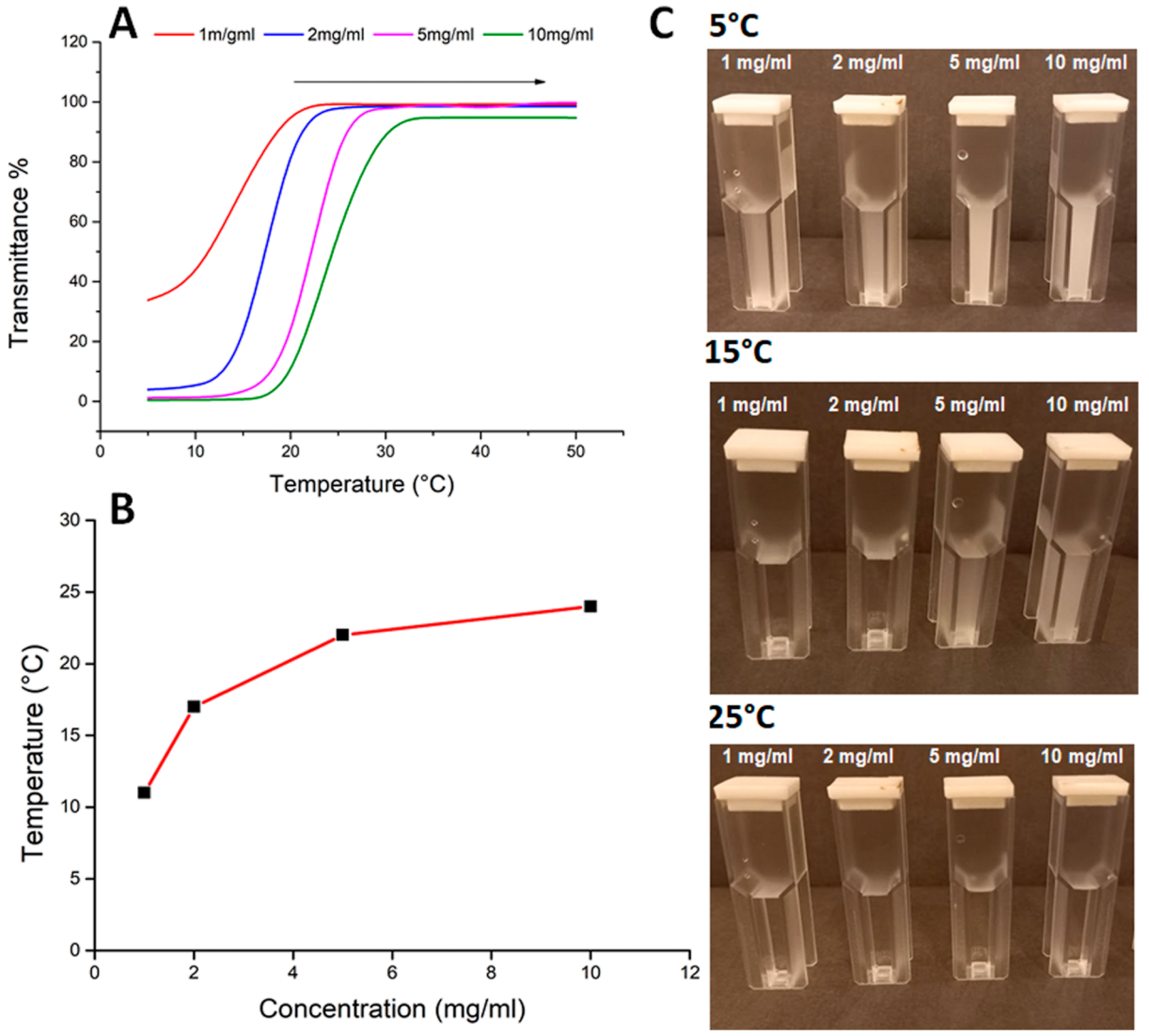

Figure 6. Representative transmittance versus temperature plot with a varying M6 homopolymer concentration (A). Changes in cloud points with different concentrations of M6 (B), and images of transmittance changes based on the concentration and temperature of M6 homopolymer solutions (C).

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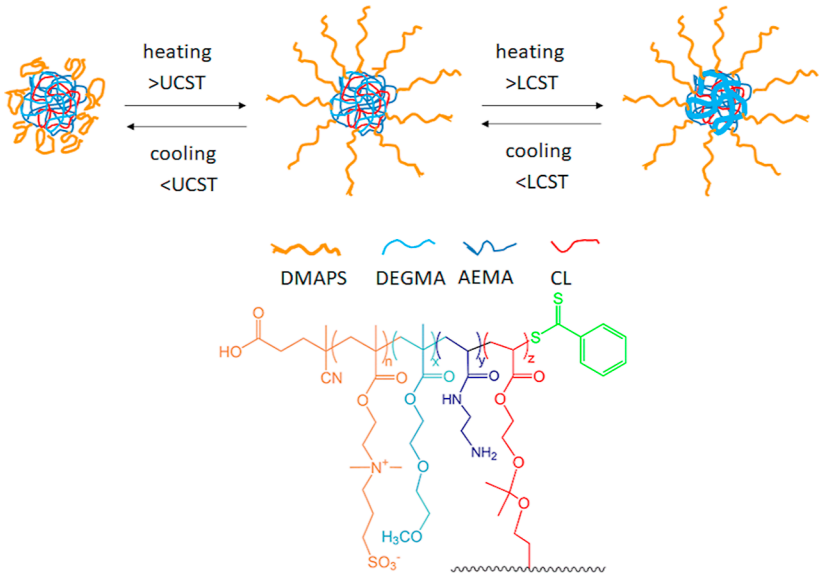
Scheme 3. Structure of CCM and Molecular Changes During UCST and LCST Transitions



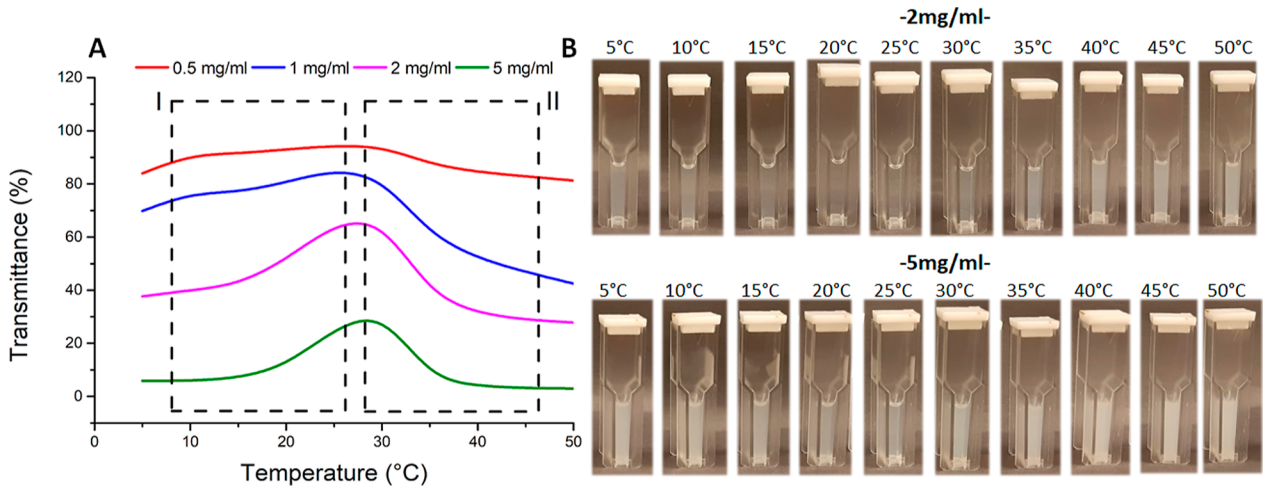


Figure 7. Transmittance versus temperature changes in CCM6 at different concentrations and UCST−LCST transitions (A) and transmittance change images of CCM6 at varying temperatures for 2 and 5 mg/mL solutions (B).

micelles were obtained with a very low size distribution (88.5 ± 5.6 nm) (Figure 4B,C). This result was also supported by the DLS analysis, and the intensity size distribution of micelles was 65.5 ± 6.2 nm (Figure 4A).

3.2. Solution Characteristics. *3.2.1. Molecular Weight and Concentration Dependence on the Temperature Sensitivity of Poly(SBMA) Homopolymers.* In order to investigate the effect of the concentration and chain length on the temperature sensitivity of homopolymers, the transmittance of the samples was measured. The cloud point measurements in aqueous solutions were performed by turbidimetry using a UV/Vis spectrophotometer with a temperature control unit. Cloud point was taken as the point at which 50% of the light was transmitted through the solution.[5]

Here, when the transmittance was measured for the poly(SBMA) solution with different chain lengths at 5 and 10 mg/mL concentrations, a shift to the higher temperature side was observed, and the cloud point increased with the increase in the molecular weight or chain length of the SBMA homopolymer. The transition temperature increased from 12 to 26 °C by the increase in the molecular weight from 31.5 to 75.7 kDa at 5 mg/mL polymer concentrations (Figure 5A). At a higher concentration (10 mg/mL), these temperature values changed from 11 to 22 °C for the same molecular weight range (Figure 5B). According to Kim et al.'s study, a similar temperature−polymerization degree (DP) relation was observed in which a drastic phase transition occurred (Kim, Langmuir, 2020). They obtained slower phase transition in higher DPs (>95) due to the low solubility of the polymers. Papadakis et al. reported that the UCST value of PSPP [homopolymer of 3-(*N*,*N*-dimethyl-*N*-(3-methacryl-amidopropyl) ammonio propanesulfonate] was dependent on the molecular weight in 5% aqueous solutions.[11]

Then, different concentrations (1−10 mg/mL) of an aqueous solution of poly(SBMA) (M6, Mn: 75,7, Mw: 79,2) were prepared, and the change in cloud points was observed by the transmittance measurement. As seen in Figure 6A,B, the cloud point increased with the polymer concentration, and the transition temperature increased from 11 to 26 °C at the concentration of 1 to 10 mg/mL. These transitions were also observed by the naked eye, as seen in Figure 6C, confirming

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temperature sensitivity. Our results are consistent with the reports in the literature, which showed that the transition temperature could be controlled by adjusting the sulfobetaine homopolymer chain length and the concentration.[6,19,20]

3.2.2. Concentration-Dependent Temperature Sensitivity Behavior of CCMs. Here, we investigated the effect of concentration on the temperature responsiveness of CCMs. The micelles with a double temperature sensitivity behavior were synthesized by RAFT polymerization by using an M6 homopolymer. These micelles are expected to have both LCST and UCST transitions due to DEGMA units in the core and SBMA in the shell, respectively (Scheme 3). To investigate temperature sensitivity and overall phase behavior of the CCMs, aqueous solutions of CCMs were prepared at different concentrations (0.5−5 mg/mL), and temperature-dependent transmittance was measured.

The expected two regimes (I, II) were observed in Figure 7A. At region I, CCMs exhibited a UCST transition dependent on the concentration, as expected due to the SBMA shell.[11,19,20] At higher temperatures (region II), sharp and clear transitions (LCST) were observed for CCMs. The UCST and LCST behavior of CCM6 were observed also with the naked eye, as seen in Figure 7B. This behavior was reported as "schizophrenic" in the literature.[11] Qui et al. also reported that poly(NIPA)- and sulfobetaine-based copolymers formed aggregates that are strongly dependent on salt and polymer concentrations.[11,21] When we compare the transmittance temperature change of M6 and CCM6, sharper transitions were observed for homopolymers. Although these experiments were conducted at the same concentrations of homopolymers and micelles, the amount of SBMA is different. In the homopolymer case, the whole sample is composed of only SBMA, while SBMA chains form the shell part of the micellar structure in the CCM case. This probably causes a weaker UCST transition in cross-linked micelles compared to homopolymers.

Following UCST and LCST analysis, we also performed the size measurement for the CCMs depending on the temperature. Here, we have used two different micelles (CCM3 and CCM6) having SBMA with different chain lengths to examine the effect of the zwitterionic block. Additionally, the effect of concentration was investigated, but we did not put any data here because almost no difference in size was observed. According to Figure 8 and Table 2, the size of the micelle increases with the molecular weight, as expected. For CCM3, the micelle size was slightly increased between 20 and 25 °C due to UCST transition and SBMA chain extension depending on the temperature and chain solubility increase. At higher temperatures, between 30 and 35 °C, where the DEGMA chain collapses due to LCST transition, micelle sizes are increased. Note that SBMA chains are soluble and extended, while DEGMA chains are expected to be collapsed in this region. Here, we obtained a higher micelle size probably because of continuing SBMA chain extension. We can say that the contribution of SBMA chains on the sizes seems more effective than the DEGMA chain shrinkage because a smaller core size is expected due to DEGMA chain collapse. This behavior is exhibited more significantly in the CCM6 case, in which the SBMA chain length is longer.

4. CONCLUSIONS

Sulfobetaine homopolymers of various degrees of polymerization were synthesized by the RAFT technique. The concentration and chain length dependency on the temperature sensitivity of homopolymers in aqueous solutions were investigated. The cloud point values increased with the increase in the molecular weight or chain length of the SBMA homopolymer. Also, the cloud point is elevated with the polymer concentration, and the transition temperature increased from 11 to 26 °C at the concentration from 1 to 10 mg/mL. Then, CCMs with an SBMA shell and a DEGMA core were prepared by RAFT polymerization in the presence of a degradable cross-linker. These micelles exhibited a double temperature responsiveness with LCST and UCST transitions due to the SBMA and DEGMA units, as expected. The size of the micelles increases with the molecular weight of the SBMA unit, which was located on the shell part of the micelles. We observed a slight increase in the micelle size due to the UCST transition and also a more significant increase caused by the LCST transition of DEGMA units.

According to these results, the transition temperatures of a polymer solution can be adjusted by changing the concentration and chain length of the polymeric units in the homopolymers and also CCMs. Thus, these controllable micellar nanosized structures demonstrating both USCT and LCST could offer an ease of drug loading and a sustained release profile.[22] Since, at the above UCST, the shell part of CCMs is soluble, it creates a hydrophilic cloud for the micelle nanoparticle to prevent nonspecific adsorption in the bloodstream, which is one of the most important criteria for a drug-

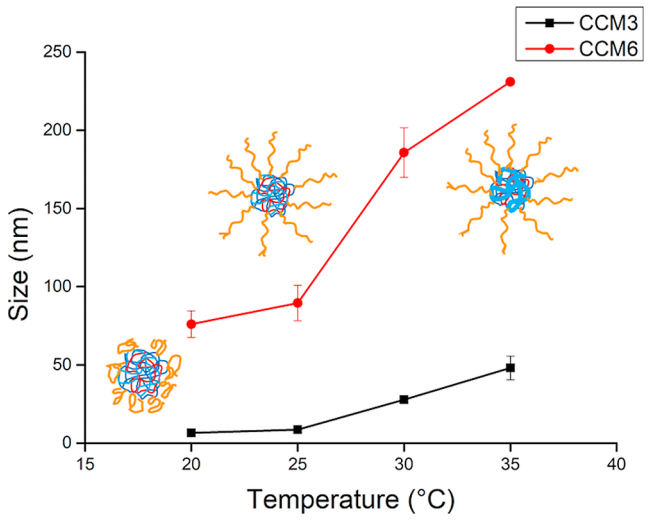

Figure 8. Change in the micelle size depending on the temperature for CCM3 and CCM6 with 1 mg/mL concentration.

Table 2. Average Hydrodynamic Diameter of Copolymer Micelles by DLS

temperature (°C)	average hydrodynamic diameter (nm)	PDI
	CCM3	
20	6.55 ± 2.37	0.396
25	8.53 ± 1.63	0.337
30	27.83 ± 2.08	0.547
35	48.07 ± 7.51	0.427
	CCM6	
20	76.03 ± 8.46	0.238
25	89.55 ± 11.32	0.209
30	185.76 ± 15.90	0.065
35	231.03 ± 0.92	0.011

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carrying micelle system. Below the LCST, the core part of our nanocarrier is swollen due to hydrogen bonds formed between molecules of water and the functional groups of the polymer components. Also, it makes the core ready to be loaded with drug molecules. Above the LCST, a hydrophilic−hydrophobic transition occurs, and the polymer core becomes insoluble, leading to the squeezing-out of internal water molecules. It results in the release of the encapsulated molecules from the medium. In conclusion, due to their dual-responsive characteristics, the CCMs show promising potential to be used as smart drug carriers for controlled delivery.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Bansal, K. K.; Upadhyay, P. K.; Saraogi, G. K.; Rosling, A.; Rosenholm, J. M. Advances in Thermo-Responsive Polymers Exhibiting Upper Critical Solution Temperature (Ucst). *eXPRESS Polym. Lett.* **2019**, *13*, 974−992.

(2) Rzaev, Z. M. O.; Dinçer, S.; Pişkin, E. Functional Copolymers of N-Isopropylacrylamide for Bioengineering Applications. *Prog. Polym. Sci.* **2007**, *32*, 534−595.

(3) Seuring, J.; Agarwal, S. Non-Ionic Homo- and Copolymers with H-Donor and H-Acceptor Units with an UCST in Water. *Macromol. Chem. Phys.* **2010**, *211*, 2109−2117.

(4) Donovan, M. S.; Sumerlin, B. S.; Lowe, A. B.; McCormick, C. L. Controlled/"living" Polymerization of Sulfobetaine Monomers Directly in Aqueous Media via RAFT. *Macromolecules* **2002**, *35*, 8663−8666.

(5) Willcock, H.; Lu, A.; Hansell, C. F.; Chapman, E.; Collins, I. R.; O'Reilly, R. K. One-Pot Synthesis of Responsive Sulfobetaine Nanoparticles by RAFT Polymerisation: The Effect of Branching on the UCST Cloud Point. *Polym. Chem.* **2014**, *5*, 1023−1030.

(6) Kim, D.; Matsuoka, H.; Saruwatari, Y. Formation of Sulfobetaine-Containing Entirely Ionic PIC (Polyion Complex) Micelles and Their Temperature Responsivity. *Langmuir* **2020**, *36*, 10130−10137.

(7) Hong, L.; Wang, Z.; Wei, X.; Shi, J.; Li, C. Antibodies against Polyethylene Glycol in Human Blood: A Literature Review. *J. Pharmacol. Toxicol. Methods* **2020**, *102*, 106678.

(8) Isoglu, A. I.; Ozsoy, Y. Advances in Micelle-Based Drug Delivery: Cross-Linked Systems. *Curr. Top. Med. Chem.* **2017**, *17*, 1469−1489.

(9) Bayram, N. N.; Ulu, G. T.; Topuzoğulları, M.; Baran, Y.; İşoğlu, S. D. HER2-Targeted, Degradable Core Cross-Linked Micelles for Specific and Dual PH-Sensitive DOX Release. *Macromol. Biosci.* **2021**, *2100375*, 2100375.

(10) Lu, Y.; Zhang, E.; Yang, J. Strategies to Improve Micelle Stability for Drug Delivery. *Nano Res.* **2018**, *11*, 4985−4998.

(11) Papadakis, C. M.; Müller-Buschbaum, P.; Laschewsky, A. Switch It Inside-out: "Schizophrenic" Behavior of All Thermoresponsive UCST-LCST Diblock Copolymers. *Langmuir* **2019**, *35*, 9660−9676.

(12) Vishnevetskaya, N. S.; Hildebrand, V.; Niebuur, B.; Grillo, I.; Filippov, S. K.; Laschewsky, A.; Müller-Buschbaum, P.; Papadakis, C. M. Aggregation Behavior of Doubly Thermoresponsive Polysulfobetaine-*b*-Poly(*N*-Isopropylacrylamide) Diblock Copolymers. *Macromolecules* **2016**, *49* (17), 6655−6668.

(13) Sun, H.; Chen, J.; Han, X.; Liu, H. Multi-Responsive Hydrogels with UCST- and LCST-Induced Shrinking and Controlled Release Behaviors of Rhodamine B. *Mater. Sci. Eng. C* **2018**, *82*, 284−290.

(14) Morimoto, N.; Yamamoto, M. Design of an LCST-UCST-Like Thermoresponsive Zwitterionic Copolymer. *Langmuir* **2021**, *37*, 3261−3269.

(15) Bhuchar, N.; Sunasee, R.; Ishihara, K.; Thundat, T.; Narain, R. Degradable Thermoresponsive Nanogels for Protein Encapsulation and Controlled Release. *Bioconjugate Chem.* **2012**, *23*, 75−83.

(16) Bulmus, V.; Chan, Y.; Nguyen, Q.; Tran, H. L. Synthesis and Characterization of Degradable p(HEMA) Microgels: Use of Acid-Labile Crosslinkers. *Macromol. Biosci.* **2007**, *7*, 446−455.

(17) Wu, J.; Xiao, Z.; Chen, A.; He, H.; He, C.; Shuai, X.; Li, X.; Chen, S.; Zhang, Y.; Ren, B.; Zheng, J.; Xiao, J. Sulfated Zwitterionic Poly(Sulfobetaine Methacrylate) Hydrogels Promote Complete Skin Regeneration. *Acta Biomater.* **2018**, *71*, 293−305.

(18) Doncom, K. E. B.; Willcock, H.; O'Reilly, R. K. The Direct Synthesis of Sulfobetaine-Containing Amphiphilic Block Copolymers and Their Self-Assembly Behavior. *Eur. Polym. J.* **2017**, *87*, 497−507.

(19) Sun, W.; An, Z.; Wu, P. UCST or LCST? Composition-Dependent Thermoresponsive Behavior of Poly(N-Acryloylglycinamide-Co-Diacetone Acrylamide). *Macromolecules* **2017**, *50*, 2175−2182.

(20) Wei, M.; Gao, Y.; Li, X.; Serpe, M. J. Stimuli-Responsive Polymers and Their Applications. *Polym. Chem.* **2017**, *8*, 127−143.

(21) Lertturongchai, P.; Ibrahim, M. I. A.; Durand, A.; Sunintaboon, P.; Ferji, K. Synthesis of Thermoresponsive Copolymers with Tunable UCST-Type Phase Transition Using Aqueous Photo-RAFT Polymerization. *Macromol. Rapid Commun.* **2020**, *41*, 1−6.

(22) Karimi, M.; Zangabad, P. S.; Ghasemi, A.; Amiri, M.; Bahrami, M.; Malekzad, H.; Ghahramanzadeh Asl, H.; Mahdieh, Z.; Bozorgomid, M.; Ghasemi, A.; Boyuk, M. R. T.; Hamblin, M. R. Temperature-Responsive Smart Nanocarriers for Delivery of Therapeutic Agents: Applications and Recent Advances. *ACS Appl. Mater. Interfaces* **2016**, *8*, 21107−21133.