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Micelles self-assembled from thermoresponsive 2-hydroxy-3-butoxypropyl starches for drug delivery

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ABSTRACT

A new class of thermoresponsive polymers, 2-hydroxy-3-butoxypropyl starches (HBPS), was synthesized by changing the hydrophobic–hydrophilic balance of starch using butyl glycidyl ether as hydrophobic reagent. The lower critical solution temperatures (LCSTs) of HBPS can be adjusted by varying the molar substitution (MS) of hydrophobic groups in the range of 4.5–32.5 °C. In water, HBPS can self-assemble into micelles below the LCST, and the micelles are deformed and aggregate into more polar and larger objects above the LCST. The drug loading HBPS micelles showed thermoresponsive controlled release, namely, the drug release is accelerated dramatically above the LCST.

1. Introduction

Recently, micelles formed by the assembly of thermoresponsive polymers in aqueous solution have been extensively investigated as potential intelligent drug delivery system and offer many attractive characteristics (Rapoport, 2007; Wei, Cheng, Zhang, & Zhuo, 2009). Compared with traditional polymeric micelles, the main advantage of thermoresponsive polymeric micelles is that these micelle systems can achieve on–off drug release of incorporated drugs in response to temperature change. Meanwhile, starch and its derivatives have emerged as one of the most promising biomaterials for drug carriers due to their biodegradability and biocompatibility. For example, hydrophobic starches such as palmitoylated starch acetate (Tan, Xu, Li, Sun, & Wang, 2010) and propyl starch (Santander-Ortega et al., 2010) can be used as nanoparticulate drug carriers. In addition, nano-sized micelles self-assembled from hydrophobically modified starch which might find use as a potential drug carrier, were reported (Besheer, Hause, Kressler, & Mader, 2007; Lu, Zhang, Wang, & Chen, 2011). Thus starch derivatives with thermoresponsivity and self-assembly properties should allow the design of ideal material for drug carriers.

It has been well realized that thermoresponsivity of water-soluble polymers can be obtained by controlling the hydrophobic–hydrophilic balance of the polymeric chains (Jia, Chen, Zhu, & Yan, 2006; Khutoryanskaya, Mayeva, Mun, & Khutoryanskiy, 2008; Ohya, Toyohara, Sasakawa, Arimura, & Ouchi, 2005). Even though starch is insoluble in cold water, modification of starch by grafting an appropriate amount of hydrophobic groups on starch chains can generate water soluble polymers by disrupting the inter- and intra-molecular hydrogen bonds of starches (Funke & Lindhauer, 2001). More importantly, the balance of hydrophilic/hydrophobic of starch chain can be tailored by controlling the degree of substitution of hydrophobic group. Therefore, it should theoretically be possible to synthesize thermoresponsive starch derivatives by controlling the hydrophobic–hydrophilic balance of the starch chains. However, starch-based thermoresponsive polymer and micelles self-assembled from thermoresponsive starch derivatives have not been reported up to now.

According to the above research frame, thermoresponsive 2-hydroxy-3-butoxypropyl starches (HBPS) were synthesized by using butyl glycidyl ether as hydrophobic reagent. The resultant HBPS exhibit tunable lower critical solution temperature (LCST), at the same time maintain the ability to form micelles below the LCST. The micellar characteristics of HBPS were investigated by using fluorescence techniques and dynamic light scattering (DLS). Finally, the controlled drug release behaviors of the resulted micelles were studied under different temperatures.

2. Experimental

2.1. Materials

Corn starch (food-grade) was supplied by Huangrong Chemical Factory (Changchun, PR China). Butyl glycidyl ether (>99%) was purchased from Beijing Chemistry Factory (Beijing, PR China). Other

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reagents and solvents were commercially available and used without further purification.

2.2. Degradation of starch

30 g corn starch was suspended in 100 mL methanol in a 250 mL, three-necked flask. 4 mL of conc. (36.5%, w/w) HCl was added. And the mixture was heated to 45 °C, in a water bath under stirring. After 4 h, the degraded starch was filtered from the solution and washed with 80% (v/v) acetone to remove the acid. The starch was then dried under vacuum at 50 °C.

2.3. Synthesis of 2-hydroxy-3-butoxypropyl starches

Methanol/HCl degraded corn starch (4.05 g, 25 mmol of anhydroglucose units (AGUs)) was suspended in distilled water (10 mL) in a 100 mL, three-necked flask. NaOH (0.5 g, 12.5 mmol) was added, and the mixture was heated to 75 °C, in a water bath under stirring. After 1 h, a predetermined amount of butyl glycidyl ether (BGE) was added to the flask. The reaction was carried out at 75 °C for 5 h. The suspension was then cooled in ice water and neutralized to pH 7.0 with 1 M HCl. Next the product was subsequently precipitated by the addition of acetone, and washed with 90% (v/v) acetone three times. The products were purified by dialysis in deionized water for two days, followed by freeze-drying, and then dried in vacuum oven at 50 °C for 5 h.

2.4. Characterization

The molecular weights and molecular weight distribution of acidified starch and HBPS were measured on an Agilent Technologies 1200 series gel permeation chromatograph equipped with two columns (ultrahydrogel 1000 7.8 mm × 300 mm and ultrahydrogel 250 7.8 mm × 300 mm). Sample was dissolved in 1 mL of eluent (concentration 0.1%, w/w). Injection volume was 100 μL. H_2O was used as the eluent at a flow rate of 1 mL/min at 20 °C. Polysaccharide (Polymer Laboratories Inc.) was used for calibration. ^1H NMR and ^{13}C NMR-spectra were recorded at room temperature on a Varian INOVA 400 spectrometer. The degraded starch and HBPS were dissolved in DMSO-d_6 containing a few drops of D_2O. LCSTs were measured with a UV–vis spectrophotometer (PerkinElmer Lambda 35, America). The transmittance of HBPS in aqueous solution (1%, w/w) was measured at 500 nm under heating rate of 0.5 °C/min. The size and size distributions of micelles were evaluated by dynamic light scattering (DLS) using Nanoparticle Size Measurement (Malvern Nano-ZS90, Britain). The concentration of the polymer was 10 mg/mL. Samples were filtered through a 0.45 μm filter before measurement. The *Z*-average diameters of the micelles were given by the instrument. Fluorescence spectra were recorded on a spectrofluorophotometer (JASCO FP-6500, Japan) by using pyrene as a hydrophobic fluorescent probe. Pyrene solution in methanol (3.0×10^{-4} M, 50 μL) was added into a 25 mL vial, and the methanol was evaporated. Then necessary amount of HBPS solution was added to get pyrene concentration 6×10^{-7} M. The solutions containing pyrene were kept for 24 h at room temperature before measurements. Excitation spectra were monitored at 390 nm, and excitation spectra were recorded ranging from 300 to 360 nm. Emission spectra were obtained by exciting the pyrene solution at 339 nm. Slit widths for both excitation and emission sides were maintained at 3.0 nm. The scanning speed was set at 50 nm/min. Measurements were performed at 20 °C.

2.5. Drug loading and in vitro drug release (Li et al., 2006)

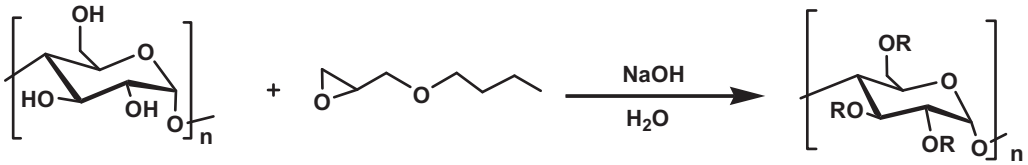
Typically, HBPS-1 (50.0 mg) and prednisone acetate (50.0 mg) were dissolved in 10 mL DMF. The solution was put into a dialysis tube and subjected to dialysis against 1000 mL of distilled water, which was renewed every 3 h during the course of initial 12 h to remove the free drug, for 24 h at 4 °C. After dialysis, the dialysis tube was directly immersed into 400 mL distilled water. Aliquots of 3 mL were withdrawn from the solution periodically. The volume of solution was held constant by adding 3 mL distilled water after each sampling. The amount of prednisone acetate released from micelles was measured at different temperatures through the LCST using UV absorbance at 242 nm. The concentration of prednisone acetate in distilled water (*c*) was obtained based on the standard curve: c (μg/mL) = c (mg/L) = $A \times 34.4601$, where A is the UV absorbance at 242 nm. The cumulative drug release was calculated from the relationship: cumulative drug release % = $(M_t/M_0) \times 100$, where M_t is the amount of drug released from micelles at time t and M_0 is the amount of drug loaded in HBPS-1 polymeric micelles. M_0 was estimated by subtracting the amount of unloaded drug from the feed drug amount (50.0 mg). The amount of unloaded drug was analysed by measuring the absorbance at 242 nm of dialyzate after drug loading. It was found that around 10.5 wt% of the feed drug, prednisone acetate, was loaded into HBPS-1 micelles (M_0 = 5.25 mg).

3. Results and discussion

3.1. Synthesis of 2-hydroxy-3-butoxypropyl starches

The general synthetic route for the preparation of HBPS is shown in Scheme 1. HBPS with different MS were prepared, among them, characteristics and solution properties of four HBPS samples with thermoresponsivity are summarized in Table 1.

A typical ^1H NMR spectrum of HBPS is shown in Fig. 1, together with a spectrum of degraded corn starch. Peak assignment for substituent was straightforward (see Fig. 1 and inset), where the triplet at 0.75 ppm (peak f) belongs to the methyl group, the peaks at 1.2 ppm (peak e) and 1.4 ppm (peak d) were the methylene group of butyl (except $-CH_2O-$). The peaks between 3.00 and 4.00 ppm (peak c) corresponded to the six protons of the anhydroglucose units (AGUs) and seven protons of the O–CH_2–CHOH–CH_2–O–CH_2-group of the substituent. The peaks at 5.1 ppm (Fig. 1(A)) and 5.1–5.4 ppm (Fig. 1(B)) were assigned to the anomeric proton (H1) of degraded corn starch and HBPS, the clear broadening of H1's peak for HBPS is due to the substitution at O-2 position. The down field-shift indicates successful etherification. The molar substitution (MS) was defined as the molar ratio of butyl glycidyl ether substituent to



R = H or -$CH_2CHOHCH_2OCH_2CH_2CH_2CH_3$, according to MS

Scheme 1. Synthesis pathway of the thermoresponsive HBPS.

Table 1
Preparation[a] and characterization of HBPS.

Sample	BGE:AGU[b]	MS[c]	M_w ($\times 10^5$)[d]	PDI[d]	Efficiency (%)	LCST (°C)[e]	CMC[f]
HBPS-1	0.46	0.32	3.8171	1.94	69.6	32.5	125.5
HBPS-2	0.62	0.40	4.8969	2.79	64.5	26.5	55.0
HBPS-3	0.92	0.52	6.2664	1.59	56.5	15.5	16.0
HBPS-4	1.23	0.63	7.2596	3.36	51.2	4.5	4.0

[a] AGU, anhydroglucose unit. Weight average molar masses of acidified starch were 3.68×10^5 g/mol (Pd: 30.44) determined by GPC. Amount of degraded corn starch in all reaction was 4.05 g.
[b] Molar ratio.
[c] MS, molar substitution of butyl glycidyl ether determined by ^{1}H NMR.
[d] Determined by GPC.
[e] Determined by the UV–vis spectroscopy measurement.
[f] Determined by the fluorescence excitation spectra of pyrene.

AGU of the starch molecules. ^{1}H NMR was used for the calculation of MS according to Eq. (1):

$$\mathrm{MS} = \frac{(I_{\mathrm{CH_3}}/3)}{(I_{\mathrm{H1}})} \quad (1)$$

where I_{CH_3} is the integral for the methyl group peak at 0.75 ppm (peak f), while I_{H1} is the integral for the anomeric proton (H1) of HBPS between 4.90 and 5.5 ppm (peaks a and b).

Fig. 2 shows the ^{13}C NMR spectrum and the assignment of the signals of degraded corn starch and HBPS. The peaks for carbon atoms of the CH_3- and CH_2-group of butyl (except –CH_2O–) were at 13.7 ppm (peak g), 18.8 ppm (peak f), and 31.3 ppm (peak e), respectively. The peaks at 60.4 ppm (peak d), 100.4 ppm (peak a) and 96.4 ppm (peak b) were assigned to C-6 (unsubstituted O-6 position), C-1 (unsubstituted O-2 position) and C-1 (influence by substituted O-2 position), respectively. Meanwhile, peak assignments for other signals at 65–85 ppm (peak c) were not easy due to the overlap among different peaks.

3.2. Thermoresponsive behavior of HBPS

As expected, the transparent aqueous solution of HBPS becomes turbid at a specific temperature as the temperature is increased, and becomes transparent again when the temperature decreases, indicating that the HBPS do exhibit the LCST behavior. Fig. 3(A) is a typical photograph of aqueous solutions of HBPS-1. The solution was transparent at 20 °C, but it was turbid at 40 °C. The LCST, defined as the temperature corresponding to 50% transmittance of 1.0 wt% aqueous solution of samples at 500 nm during the heating process, was 32.5 °C. Fig. 3(B) shows phase transition curves

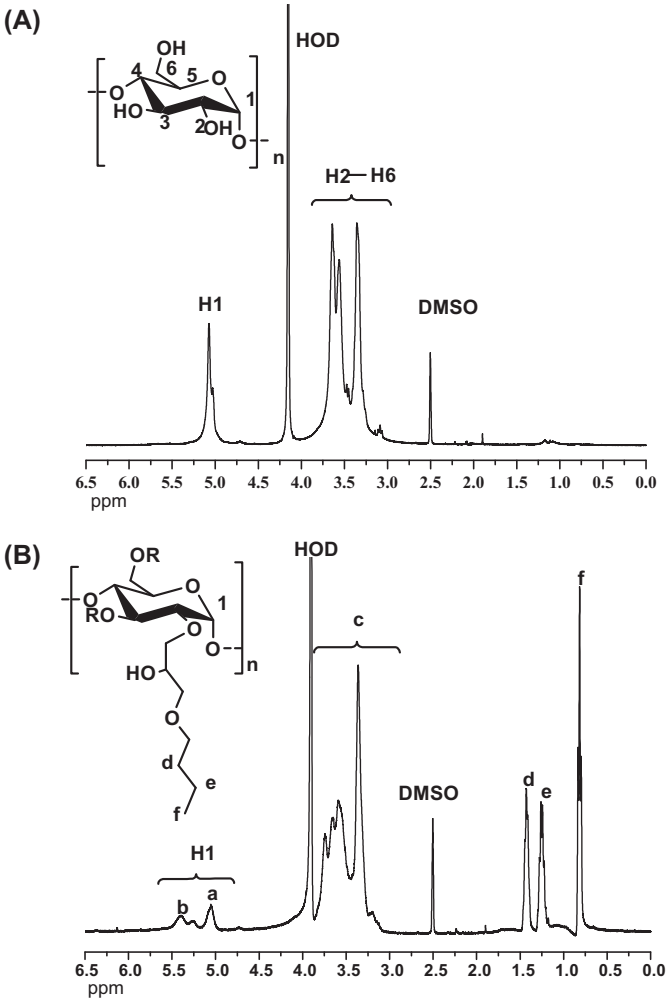


Fig. 1. ^{1}H NMR spectra of degraded corn starch (A) and HBPS (B).

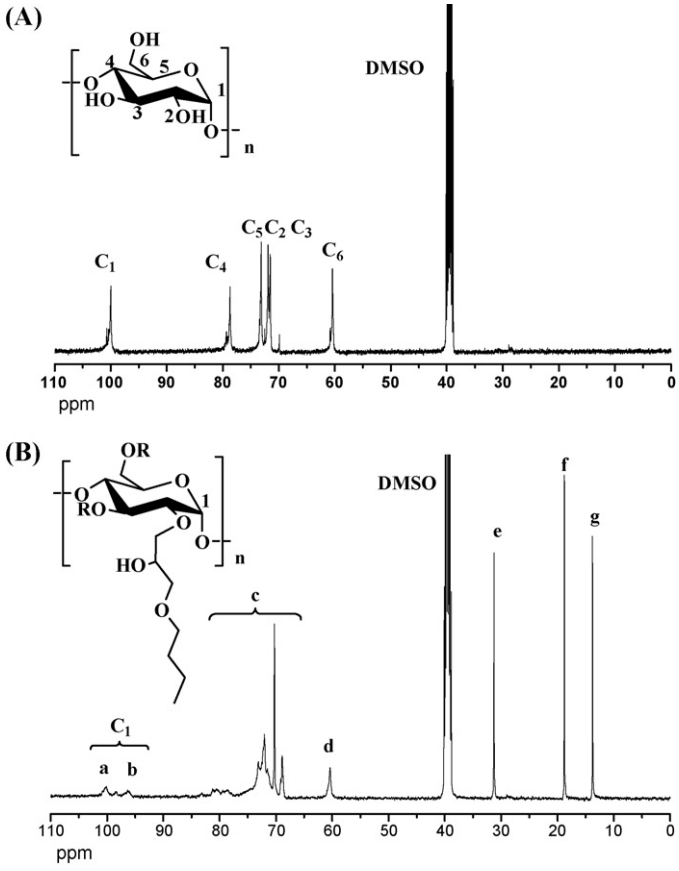


Fig. 2. ^{13}C NMR spectra of degraded corn starch (A) and HBPS (B).

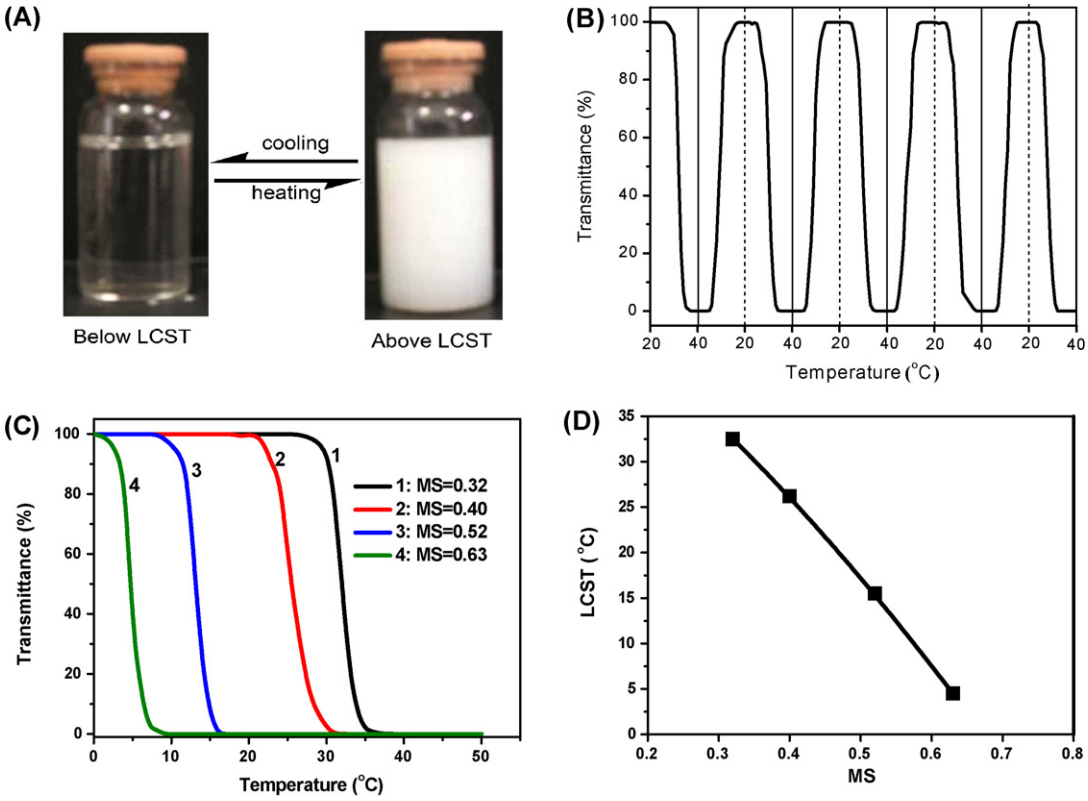


Fig. 3. (A) Photographs of HBPS-1 aqueous solution at 20 °C and 40 °C. (B) Reversible changes of optical transmittance against temperature fluctuation for 1.0 wt% aqueous solutions of HBPS-1 at 500 nm with a heating/cooling rate of 0.5 °C/min. (C) Transmittance changes for 1.0% (w/v) aqueous solutions of HBPS 1–4 at 500 nm with a heating rate of 0.5 °C/min. (D) Effect of molar substitution (MS) on LCST.

of the HBPS-1 aqueous solution for heating and cooling cycles. As can be seen, the transmittance values of the solution in the heating cycle (or cooling cycle) are almost equal to each other in the multiple-cycle experiments, reflecting that the highly sensitive phase separation is reversible. Fig. 3(C) displays the effect of molar substitution (MS) on phase transition behavior. From the plots of LCST values against MS (Fig. 3(D)), it indicates that the LCST decreases linearly with increasing MS, and an increase of the MS from 0.32 to 0.63 resulted in a decrease in the LCST from 32.5 to 4.5 °C. It revealed that the LCSTs of HBPS could be controlled by varying the MS value.

It is worthy to note that the HBPS with MS from 0.32 to 0.63 have good thermoresponsivity, the HBPS with $MS_{HB} \leq 0.25$ are completely water-soluble and HBPS with $MS_{HB} \geq 0.72$ are insoluble, both of them have no LCST behavior during the heating process from 0 to 100 °C. These results suggest that the hydrophobic groups and the MS play a vital role in the thermoresponsivity and the LCST of starch derivatives. An appropriate amount of hydrophobic groups not only improved cold water solubility of starch derivatives by disrupting the inter- and intra-molecular hydrogen bonds of starches, but, more important is the fact that the hydrophobic-groups provided a favorable intramolecular hydrophilic/hydrophobic balance for successful starch based thermoresponsive polymer formation.

3.3. Micelle formation behavior of HBPS below the LCST

The amphiphilic nature of HBPS, carrying hydrophobic-alkyl groups in hydrophilic starch chain, provides an opportunity to form micelles in water. The micelle forming behavior of HBPS was

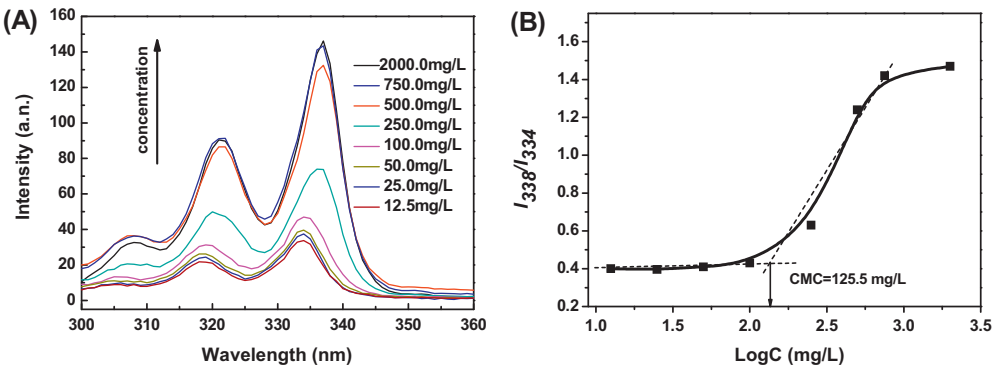


Fig. 4. (A) Excitation spectra of pyrene (6×10^{-7} M) in water in the presence of increasing concentrations of HBPS-1. Measurement was performed at 25 °C. (B) Plot of I_{338}/I_{334} in the excitation spectra vs concentration of HBPS-1.

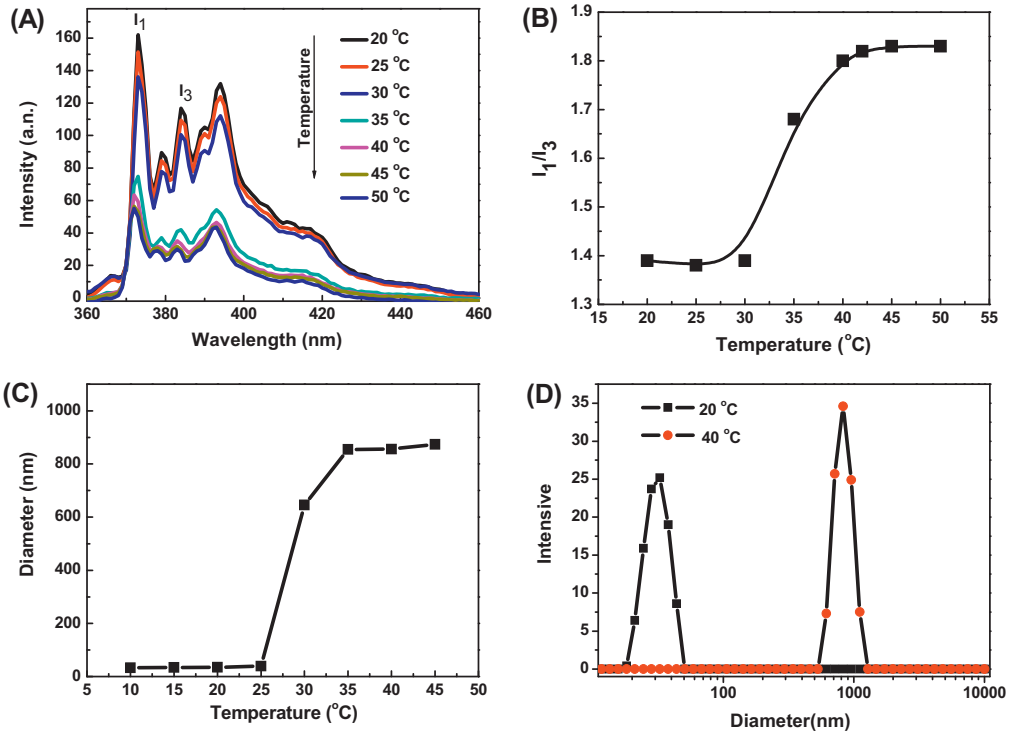


Fig. 5. (A) Typical fluorescence emission spectra of pyrene (6×10^{-7} M) obtained for 2.0 g/L aqueous solution of HBPS-1 at different temperatures. (B) Plots of I_1/I_3 as a function of temperature. (C) Temperature dependence of diameter of HBPS-1 (10 mg/mL). (D) Particle size distribution of HBPS-1 (10 mg/mL) in water at 20 °C and 40 °C.

studied by fluorescence spectroscopy using pyrene as a probe (Wang, Morinaga, Sudo, & Endo, 2011). Fig. 4(A) shows a series of the excitation spectra of pyrene in the presence of the HBPS-1 with various concentrations below the LCST (25 °C). By increasing the concentration of polymer from 12.5 to 2000 mg/L, an increase in fluorescence intensity and a red-shift of the low-energy band from 334 to 338 nm in the excitation spectra can be clearly detected, reflecting the partitioning of pyrene from the aqueous media into the hydrophobic cores of micelles self-assembled from HBPS-1 (Yuan, Du, Wang, & Wang, 2010). The critical micelle concentrations (CMCs) of HBPS determined by the fluorescent excitation spectra of pyrene (Fig. 4(B)) are listed in Table 1 (Lee & Huang, 2008). Taken together, these results indicate that HBPS are able to self-assemble into micelles below the LCST, and the hydrophobic core region of micelles can aid the encapsulation and solubilization of hydrophobic drugs.

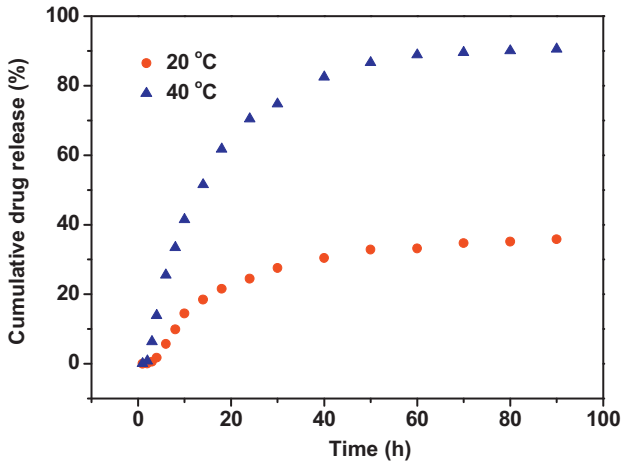


Fig. 6. Drug release behavior of thermoresponsive drug-loading HBPS-1 micelles at 20 °C and 40 °C.

3.4. Thermoresponsive structural changes of HBPS-1 micelles

It has been demonstrated in literature that micelles self-assembled from thermoresponsive polymers are able to change the micelle structure in response to temperature, and can achieve on–off drug release (Chung, Yokoyama, Aoyagi, Sakurai, & Okano, 1998; Chung, Yokoyama, & Okano, 2000). In this paper, typically, the structural changes and drug release properties of HBPS-1 micelles as a function of temperature through the LCST were investigated because the LCST of HBPS-1 is close to body temperature, and similar to that of poly(*N*-isopropylacrylamide) (PNIPAM), which is one of the most studied thermoresponsive polymers with LCST of 32 °C (Fujishige, Kubota, & Ando, 1989; Pelton, 2000).

Thermoresponsive structural changes of HBPS-1 micelles were investigated by the relative intensity of the first and the third emission peaks (I_1/I_3) in the emission spectrum of pyrene (Chung et al., 2000). Fig. 5(A) shows the fluorescence behavior exemplified using the spectra of pyrene at different temperatures of the HBPS-1 solution (2.0 g/L > CMC). Fig. 5(B) shows the temperature dependence of I_1/I_3 ratios obtained for the aqueous solution. Interestingly, I_1/I_3 ratio was found to increase with increasing temperatures. In the temperature range of 20–30 °C, I_1/I_3 ratios remain almost constant at 1.39, indicating that the pyrene was located in the hydrophobic core region of micelles. Upon further increase of temperatures, a dramatic increase in I_1/I_3 values occurs at around 35 °C. A similar 'abnormal' polarity change was also observed for hydrophobically modified PNIPAM (Cao et al., 2005; Chung et al., 2000; Ringsdorf, Venzmer, & Winnik, 1991; Schild & Tirrell, 1991), and was attributed to micelle structural deformation and formed polymer-rich phase in which hydrophobic substituents are randomly distributed above the LCST (Ringsdorf et al., 1991). However, because the polarity of starch backbone was much larger than that

of PNIPAM backbone, above the LCST, the polarity of polymer-rich phase of HBPS-1 (I_1/I_3 = about 1.83) was significantly higher than that of hydrophobically modified PNIPAM (I_1/I_3 = about 1.5) (Cao et al., 2005; Schild & Tirrell, 1991). The high polarity of HBPS-1's polymer-rich phase and micelle structural deformation will certainly stimulate the release of solubilized hydrophobic drugs from the micelles above the LCST.

In order to further verify the thermoresponsive structural changes of HBPS-1 micelles, we investigated the changes of average micelle diameter in aqueous solution as a function of temperature using DLS. As the temperature increased, a dramatic increase of *Z*-average diameter of micelle from 35 to 800 nm was detected in the temperature range 25–35 °C (Fig. 5(C)). This temperature agreed well with the result (32.5 °C) of turbidity measurements. Fig. 5(D) shows the size and size distributions of HBPS-1 at 20 and 40 °C, respectively. At 20 °C, the aqueous solution of HBPS-1 is clear, *Z*-average diameter of micelle is 35.5 nm and PDI is 0.404. At 40 °C, aqueous solution of HBPS-1 becomes turbid, *Z*-average diameter is 855.6 nm and PDI is 0.151. An abrupt increase in *Z*-average diameter, and narrow size distribution as judged by PDI above LCST reflected that intermicellar aggregation inducing micelle structural deformation was induced in the micellar solution of HBPS-1 above LCST (Kujawa, Tanaka, & Winnik, 2006).

3.5. Controlled drug release

In this study, the drug release behavior of HBPS-1 micelles was investigated by using prednisone acetate, an anti-inflammatory drug with a very low solubility in water, as a model drug. Because the LCST of HBPS-1 micelle was determined to be 32.5 °C, the *in vitro* drug release profile from the HBPS-1 micelles was evaluated in distilled water at both a lower and a higher temperature (20 and 40 °C). The controlled drug release from HBPS-1 micelles was examined in distilled water and release data are shown in Fig. 6. At 20 °C (below the LCST), about 38% of the drug is released from the micelles in around 100 h, about 62% of the drug still remains in the core of the micelles due to the stable micelle structure. At 40 °C (above the LCST), about 90% of the drug is released from the micelles in around 100 h. The drug release is accelerated dramatically above the LCST, corresponding well with the temperature-induced micelle structural deformation and intermicellar aggregation of HBPS-1 confirmed by fluorescence spectra and DLS, as described above.

4. Conclusions

New thermoresponsive HBPS were synthesized and characterized. The LCST of HBPS can be adjusted by controlling the molar substitution of 2-hydroxy-3-butoxypropyl group. DLS and fluorescence spectroscopy have shown that HBPS were able to self-assemble into micelles below the LCST, while above the LCST micelles aggregate into more polar and larger objects. The *in vitro* drug release experiment shows that the micelles can be useful as intelligent drug delivery system. Micelles self-assembled from thermoresponsive HBPS, which have some remarkable features such as backbone's good biodegradability and biocompatibility, show very promising applications in biomedical fields. Moreover, the same approach can be taken using hydrophobic groups other than butyl group and extended easily to the preparation of various types of starch based polymers with thermoresponsivity and self-assembly properties. The research is going on in our group.

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