Tuning of Lower Critical Solution Temperature of Thermoresponsive 2-Hydroxy-3-Alkoxypropyl Hydroxyethyl Cellulose by Alkyl Side Chains and Additives

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Two kinds of thermoresponsive 2-hydroxy-3-alkoxypropyl hydroxyethyl celluloses (HAPEC) were prepared by grafting butyl and isopropyl glycidyl ethers onto hydroxyethyl celluloses (HEC). The HAPEC was characterized by ^{1}H NMR, ^{13}C NMR, and 2D HSQC NMR. The lower critical solution temperature (LCST) of HAPEC can be tuned by changing the molar substitution (MS). The LCST decreased with the increasing MS of the alkyl chains. The HAPEC concentration, salt concentration, and organic solvent concentration had a marked influence on LCST. In addition, the differences of thermoresponsive properties between the two kinds of HAPECs were investigated. 2-Hydroxy-3-butoxypropyl hydroxyethyl cellulose (HBPEC), which has longer hydrophobic side chains, demonstrated a lower LCST when both HBPEC and 2-hydroxy-3-isopropoxypropyl hydroxyethyl cellulose (HIPEC) possessed similar MS values. HBPEC, which has longer hydrophobic side chains, exhibited thermoresponsive flocculation behavior, and the critical flocculation temperature (CFT) was adjusted in the range from 27.3 to 51.2 °C by changing the molar substitution.

Keywords: Cellulose ethers; Thermoresponsive; LCST; Alkyl side chains; Additives

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INTRODUCTION

Thermoresponsive polymers that undergo a coil-to-globule transition in aqueous solution show lower critical solution temperature (LCST) behavior as the temperature rises (Hinrichs *et al.* 2017). Thermoresponsive polymers with this property have been developed in a wide range of applications, such as drug delivery (Wu *et al.* 2016), targeted drug release (Chen *et al.* 2013), and tissue engineering (Wang *et al.* 2013). It is important to understand how the polymer structure and additives, such as inorganic salts and organic solvents, are responsible for LCST behavior. Generally, the monomeric units of thermoresponsive polymers exhibit a common structural feature of having both hydrophilic and hydrophobic groups. Therefore, the modification of the chemical structure of the polymer has commonly been used to tune the LCST. Homopolymers and copolymers of poly(*N*-isopropylacrylamide) (PNIPAM) are the most heavily studied and typical thermoresponsive polymers; thus, they have received remarkable attention (Shen *et al.* 2013). The LCST of poly(*N*,*N*-dialkylacrylamide) can be controlled in a range of 0 to 100 °C by changing the type of disubstituted alkyl group on the nitrogen atom (Fischer *et al.*

2011). Additionally, the LCST of PNIPAM copolymers can be controlled in the range of 32.8 to 45.3 °C by grafting different end groups (Xia *et al.* 2006). Polysaccharide-based polymers show analogous phenomena, and polysaccharide grafted to different polymers exhibits various LCST. For example, the LCST of cellulose-*g*-copolymers could be tuned by grafting poly(*N*,*N*-diethylacrylamide) and PNIPAM side chains in the range of 18 to 26 °C and 22 to 26 °C, respectively (Hufendiek *et al.* 2014). Another efficient method to tune the LCST is by changing the molecular weight of thermoresponsive polymers. Chitosan-*g*-poly(*N*-vinylcaprolactam) aqueous solutions presented LCST between 26 and 44 °C by transforming the molecular weight of poly(*N*-vinylcaprolactam) from 4.2 to 46.6 kDa (Fernandez-Quiroz *et al.* 2015). Thermoresponsive properties of polysaccharide-based polymers originate primarily from their thermoresponsive synthetic polymer components. In other words, the introduction of polysaccharides improves the biodegradability of the polymers (Zhang *et al.* 2019) but does not change their thermoresponsive properties.

The addition of small molecule agents into the polymer solutions is a good method to alter the polymer-water interaction or directly interaction with polymer chains (Guner and Demirel 2012). Therefore, the thermoresponsive behavior can be adjusted by adding small molecule additives. Moreover, adding small molecule additives is a convenient and fast method to tune the LCST of thermoresponsive polymers. In general, changing the salt concentration can affect the thermoresponsive behavior of polymers, which leads a salting-out of thermoresponsive polymer aqueous solutions. NaCl is the most common inorganic salt used to adjust the LCST of synthetic polymers and polysaccharide-based polymers (Yang *et al.* 2016). The presence of organic solvents, such as methanol (Maeda and Takaku 2010), ethanol (Backes *et al.* 2017), and isopropanol (Yao *et al.* 2016), can affect the phase separation of polymer/water systems, and thus it can influence the LCST behavior. The polymer chains collapse when a small amount of alcohol is added into the polymer aqueous solutions, and the further addition of alcohol causes polymer chain reswelling (Dhara and Chatterji 2000). Nevertheless, there are few reports on the change of thermoresponsive behavior of polysaccharide-based polymers by small molecule additives.

In the present work, two different thermoresponsive 2-hydroxy-3-alkoxypropyl hydroxyethyl celluloses (HAPEC) were synthesized by using an etherification reaction of hydroxyethyl celluloses with butyl and isopropyl glycidyl ethers. The HAPEC exhibits reversible thermoresponsive property, and the LCST can be tuned to a wide temperature range. The effect of alkyl substitution degree on LCST was investigated. The phase separation behaviors of HAPECs with different alkyl side chains were compared. Moreover, the effects of the polymer solution concentration and different additives (NaCl and the organic solvents) on the LCST behavior of HAPEC were investigated. Therefore, the thermoresponsive behavior of HAPEC with different alkyl chains was investigated, and the effects of different factors on LCST were summarized systematically. The study results possess a certain reference value to design thermoresponsive polymers and tune LCST of them.

EXPERIMENTAL

Materials

Hydroxyethyl cellulose (HEC) was obtained from Sigma-Aldrich (St. Louis, MO, USA, MSOH = 2.5, MW = 2.5×10^5 g/mol). Butyl glycidyl ether (BGE) (>99%) and isopropyl glycidyl ether (IPGE) (> 99%) were obtained from Tokyo Chemical Industry

Co., Ltd. (Tokyo, Japan). Methanol, ethanol, isopropanol, butanol, and other reagents were commercially available, analytical grade, and used without further purification.

Synthesis of 2-Hydroxy-3-Alkoxypropyl Hydroxyethyl Cellulose

First, 2.0 g of HECs (7.3 mmol of anhydroglucose units AGU) were dissolved in 12 mL of deionized water in a 100-mL three-necked flask, and then 0.8 g of NaOH aqueous solutions (40 wt.%) were added. The mixture was placed in a 70 °C water bath with stirring for 1 h. BGE (3.2 g, 4.0 g, 4.8 g, 5.6 g, 6.4 g) or IPGE (2.9 g, 3.6 g, 4.3 g, 5.0 g, 5.7 g) was added to the three-necked flask drop by drop. The etherification reaction was conducted at 90 °C for 9 h. After the etherification reaction was completed, the system was cooled to room temperature, and the mixture was neutralized to pH 7 by adding 1 M HCl. The HAPECs were purified by using dialysis tube (8000~14000) for 72 h in deionized water, and the products were dried by rotary evaporator and lyophilization.

Characterization

^{1}H NMR, ^{13}C NMR, and 2D HSQC NMR spectra were collected by using a Varian INOVA 500 spectrometer (Palo Alto, USA), with 60 mg of HAPECs dissolved in 1 mL DMSO-*d*$_6$.

The LCST values of HAPECs were measured by using a Mettler Toledo T90 (Zurich, Switzerland) with a temperature-controlled LAUDA RP200. In this work, photometric electrode of Mettler Toledo T90 was chosen, and 10 mL of HAPEC aqueous solution was added into the titration cup. The transmittance of thermoresponsive HAPEC in aqueous solution was defined at 590 nm under 1 °C/min. The LCST value was determined as the temperature at which the optical transmittance of the polymer solution was 50%. The critical flocculation temperature (CFT) values of the HAPECs were measured using a Cary Series UV-Vis spectrophotometer (Palo Alto, CA, USA). The CFT value was determined as the temperature at the peak of the variation of absorbance with the temperature (Tian *et al.* 2015).

RESULTS AND DISCUSSION

Synthesis and Characterization of HAPEC

2-Hydroxy-3-butoxypropyl hydroxyethyl cellulose (HBPEC) and 2-hydroxy-3-isopropoxypropyl hydroxyethyl cellulose (HIPEC) were synthesized as illustrated in Fig. 1. To evaluate the MS and LCST of different thermoresponsive polymers, the LCST is summarized in Table 1.

Figures S1 through S4 (see Appendix) show the ^{1}H NMR and ^{13}C NMR of HAPEC.

^{1}H NMR of HBPEC: δ 0.86 as (-CH_3), δ 1.30, 1.46 as (-CH_2-), and δ 2.70~4.00 as (-O(CH_2CH_2O)$_x$–CH_2–CHOH–CH_2–O–CH_2-).

^{13}C NMR of HBPEC: δ 13.64 as (-CH_3), δ 18.81,31.06 as (-CH_2-).

^{1}H NMR of HIPEC: δ 1.0 as (-CH_3), δ 2.70~4.00 as (-O(CH_2CH_2O)$_x$–CH_2–CHOH–CH_2–O–CH_2-).

^{13}C NMR of HIPEC: δ 27.3 as (-CH_3).

These results indicate successful etherification. The 2D HSQC NMR spectra (Figs. S5 and S6) further confirm the assignment of ^{1}H and ^{13}C of HAPEC/DMSO-*d*$_6$, illustrating the one-bond correlation between proton and carbon.

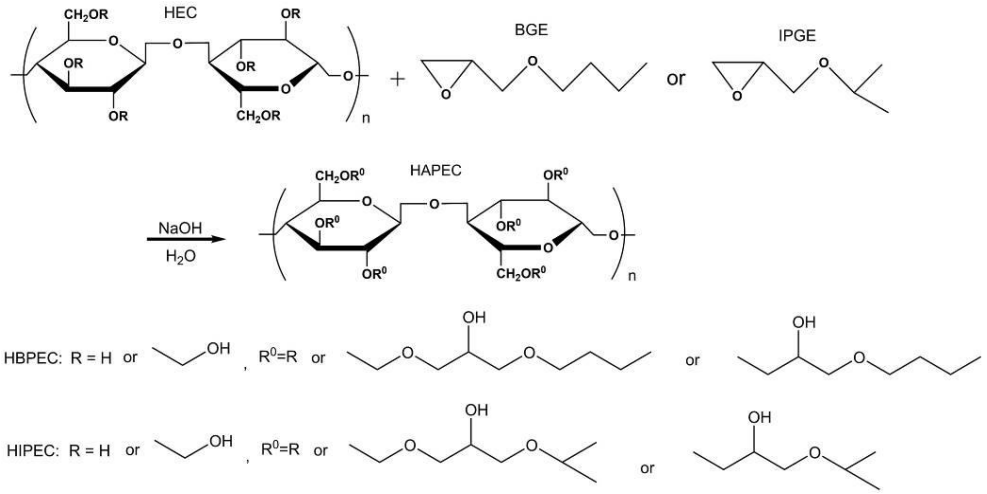

Fig. 1. Synthetic pathway of HBPEC and HIPEC

Table 1. Preparation and Characteristic of HBPEC and HIPEC

Sample	n(AGE): n(AGU) [a]	MS [b]	RE (%) [c]	LCST (°C) [d]	CFT (°C) [e]
HBPEC-1	2.0	1.04±0.04	52.0±3.1	37.9±1.5	51.2±2.1
HBPEC-2	2.5	1.23±0.03	49.2±1.2	31.5±1.1	43.9±2.3
HBPEC-3	3.0	1.61±0.11	53.7±3.4	27.0±1.7	38.5±1.7
HBPEC-4	3.5	2.04±0.01	58.3±0.3	22.4±0.7	32.7±2.9
HBPEC-5	4.0	2.23±0.03	55.8±0.8	18.4±0.9	27.3±2.4
HIPEC-1	2.0	1.21±0.04	60.5±1.9	55.6±0.9	-
HIPEC-2	2.5	1.51±0.06	60.4±2.4	45.5±1.5	-
HIPEC-3	3.0	2.01±0.03	67.0±1.1	36.9±1.1	-
HIPEC-4	3.5	2.36±0.10	67.4±2.7	29.5±1.6	-
HIPEC-5	4.0	2.88±0.11	72.0±2.6	20.6±1.8	-

[a]Mole ratio of etherifying agent to glucose unit of cellulose.
[b]MS, determined by ^{1}H NMR. See calculation formula in Supplementary Materials.
[c]RE, reaction efficiency. See calculation formula in Supplementary Materials.
[d]Determined by Mettler Toledo T90 with a temperature-controlled LAUDA RP200.
[e]Determined by Cary Series UV-Vis spectrophotometer.
AGE: Alkyl glycidyl ether; AGU: Anhydroglucose units; MS: Molar substitution; RE: Reaction efficiency; LCST: Lower critical solution temperature; CFT: Critical flocculation temperature; HBPEC: 2-Hydroxy-3-butoxypropyl hydroxyethyl cellulose HIPEC: 2-Hydroxy-3-isopropoxypropyl hydroxyethyl cellulose

Effect of MS on the LCST of HAPEC

Figure 2(a-b) show the variation of optical transmittance with temperatures in 10 g/L aqueous solutions of HBPEC and HIPEC. The transmittance of HBPEC and HIPEC aqueous solutions with different MS decreased sharply when the temperature was near the LCST. At lower temperature, the strong hydrogen bonds were formed between the hydrophilic HEC backbones of HAPEC and water molecules, which induced the HAPEC to dissolve in water (Nun *et al.* 2017). As the temperature increased to LCST, hydrophobic interactions among the alkyl groups become dominant, resulting in phase separation (Fang *et al.* 2017). The relationship between LCST and MS is shown in Fig. 2(c). Increasing the MS of HBPEC from 1.04 to 2.23 induced a notable decrease of the LCST from 37.9 to 18.4 °C. Furthermore, as the MS of HIPEC increased from 1.21 to 2.88, the LCST decreased

from 55.6 to 20.6 °C. As shown in Fig. 2(c) and Table 1, HBPEC with longer alkyl chain showed lower LCST when both the HBPEC and HIPEC had a similar MS. For example, HBPEC-2 (MS=1.23) and HIPEC-1 (MS=1.21) exhibited an LCST of 31.5 °C and 55.6 °C, respectively. Compared with HIPEC, HBPEC displayed dominant intramolecular hydrophobic interactions, thus leading to lower LCST.

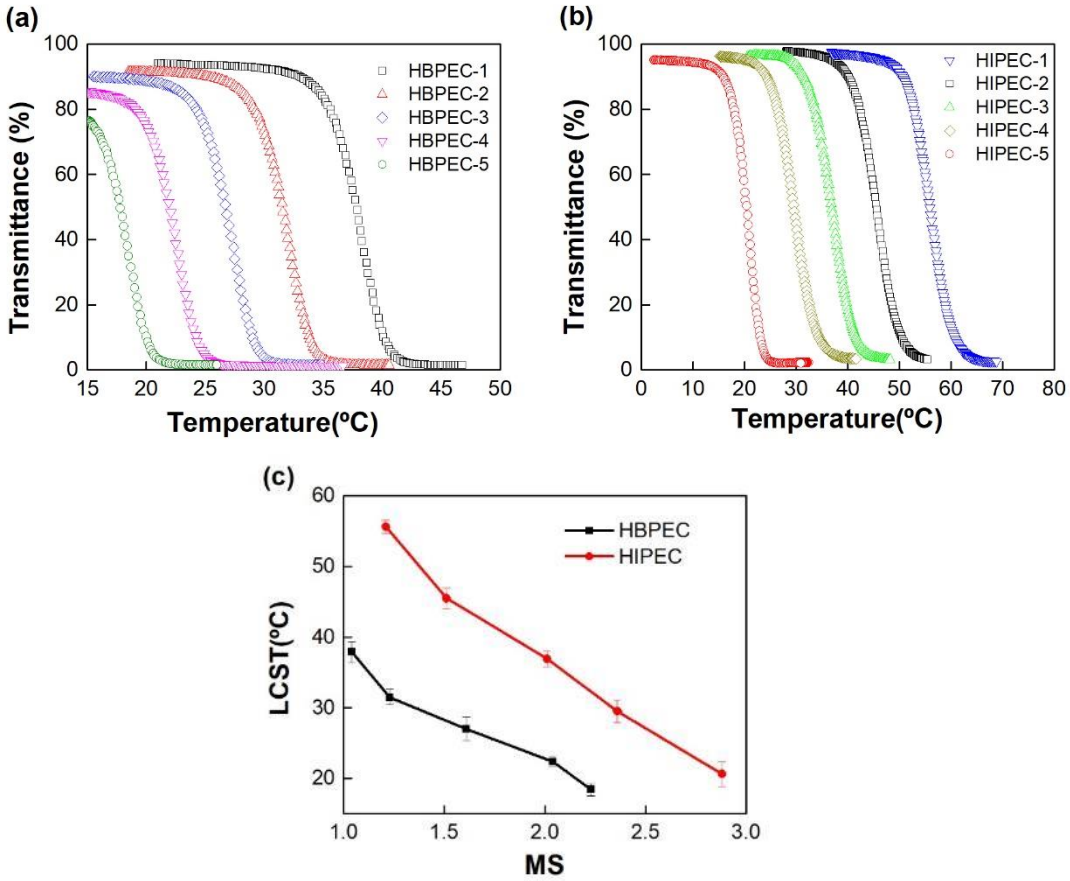

Fig. 2. Transmittance changes for aqueous solutions of HBPEC (a) and HIPEC (b) (10 g/L); (c) Effect of MS on LCST

Thermoresponsive Hysteresis Study

HBPEC-2 and HIPEC-1, which have similar MS with different alkyl side chain lengths, were investigated for comparing their thermoresponsive behavior. As Fig. 3 illustrates, HAPEC showed reversible phase separation during the heating and cooling cycle. The LCST of HAPEC in the cooling process was higher than that of the heating process; this phenomenon demonstrates hysteresis of LCST in the heating-and-cooling process. The hysteresis can be attributed to the incomplete disruption of additional hydrogen bonds between HAPEC chains, thereby leading to higher LCST (Chen *et al.* 2005).

The $LCST_{cooling}$ of HBPEC presents obvious hysteresis because the hydrophobic association of HBPEC side chains is stronger, and the molecule chains coil after heating. In comparison, the hydrophobic interaction of HIPEC chains is weaker, and the molecule chains are stretchable after the temperature increases. Hence, HIPEC exhibits slight hysteresis in the cooling process (Ding and Zhang 2006).

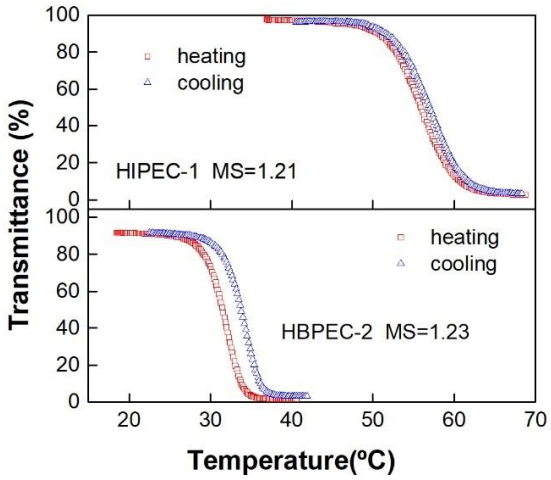

Fig. 3. Transmittance changes for HBPEC-2 and HIPEC-1 aqueous solutions (10 g/L) during heating and cooling

Effect of HAPEC Aqueous Solution Concentration on LCST

The effects of HAPEC concentrations (1 to 10 g/L) on LCST are shown in Fig. 4. As the HBPEC-2 and HIPEC-1 aqueous solution concentrations decreased, the LCST of HBPEC and HIPEC solutions increased (Fig. 4(a-b)).

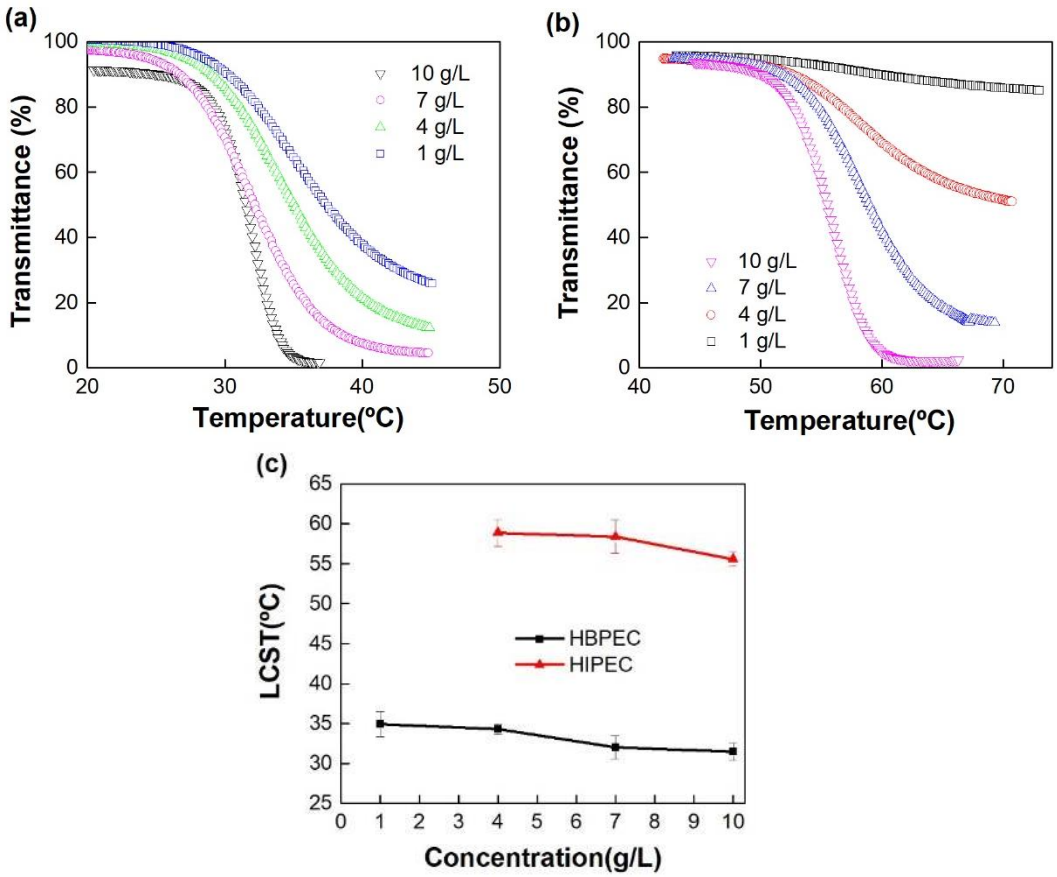

Fig. 4. Transmittance changes for aqueous solution of HBPEC-2 (a) and HIPEC-1 (b) with different concentrations; (c) Effect of HBPEC-2 and HIPEC-1 concentrations on LCST

As shown in Fig. 4(c), when the concentrations ranged from 4 to 10 g/L, the LCST of HBPEC-2 and HIPEC-1 was controlled in the range of 31.5 to 34.3 °C and 55.6 to 58.9 °C, respectively. The LCST of HBPEC-2 and HIPEC-1 decreased by 2.8 °C and 3.3 °C, respectively. These results were similar to the variation of LCST of PNIPAM with the polymer concentrations. When the temperature is above the LCST, the formation of aggregates driven by hydrophobic interactions among hydrophobic alkyl groups causes phase separation in the aqueous solution. A lower concentration of HAPEC makes the hydrophobic interaction weaken; because of the decline in the quantity of alkyl groups per unit volume, more energy is required to offset the loss of entropy (Ju *et al.* 2014). It is worth noting that the effect of concentration on HBPEC-2 and HIPEC-1 were slightly different. HIPEC with lower alkyl length showed weaker hydrophobic interaction; therefore, there is a greater decrease in LCST for the HIPEC sample solution.

Effect of NaCl Concentrations on the LCST of HAPEC

It is important to control the LCST of thermoresponsive polymers conveniently and rapidly in different applications. In biomedicine, blood and bodily fluids need a certain number of electrolytes; thus, tuning the LCST by adding inorganic salts is important. The effects of NaCl concentrations on LCST of HAPEC were investigated by recording the transmittance of 10 g/L HBPEC-2 and HIPEC-1 aqueous solutions with different NaCl concentrations. As illustrated in Fig. 5, when the NaCl concentration ranged from 0.1 to 0.3 M, the LCST of HBPEC-2 and HIPEC-1 were tuned in the range of 25.1 to 31.5 °C and 52.1 to 55.6 °C, respectively. The effect of NaCl concentration on LCST was explained as follows. During dehydration, the hydration interactions between NaCl and water molecules are stronger than that between HAPEC molecules and water, which breaks the hydrogen bonds and causes dehydration of the polymer chains (Seo *et al.* 2012). However, the surface tension between polymer chains and water molecules increases linearly with NaCl concentration (Hiruta *et al.* 2015). The aggregation of HAPEC molecular chains reduces the specific surface and surface free energy. These two different mechanisms may contribute to the decrease of LCST with NaCl concentration.

Figure 5 also shows that when the NaCl concentration increased from 0 to 0.3 M, the LCST of HIPEC-1 and HBPEC-2 decreased by 3.5 °C and 6.4 °C, respectively.

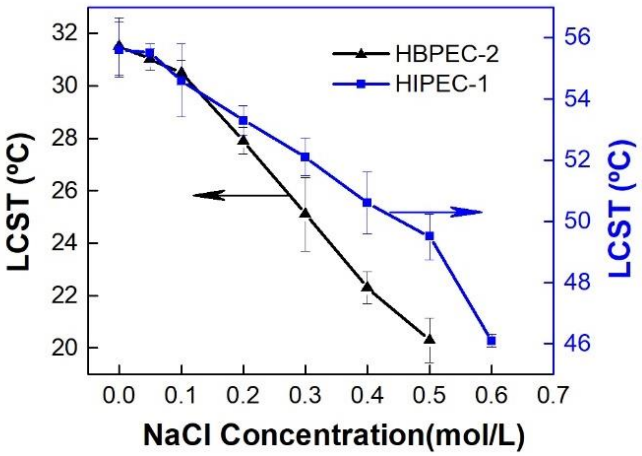

Fig. 5. Effect of NaCl concentrations on LCST

For HBPEC, the decrease of LCST with NaCl concentration was faster than HIPEC due to the differences in the length of alkyl side chains. The phase separation behavior of HBPEC with longer alkyl length was more susceptible to salting-out than HIPEC, thereby the LCST of HBPEC sample solution decreased greatly.

Effect of Organic Solvents on the LCST of HAPEC

The phase separation behavior of thermoresponsive polymer aqueous solutions can be changed by adding organic solvents, and it is a relatively convenient method to adjust the LCST of the thermoresponsive polymer. HBPEC-2 and HIPEC-1 at a concentration of 10 g/L were chosen to investigate the effects of methanol, ethanol, isopropanol, and butanol on LCST.

As shown in Fig. 6, when the concentrations of ethanol and isopropanol were increased from 0 to 30% v/v, the LCST of HBPEC-2 decreased from 31.5 to 17.6 °C and 1.5 °C, respectively. The HAPEC tends to form hydrogen bonds with organic solvents rather than water molecules. Therefore, the hydrophilic portion of HAPEC skeleton is shielded by organic solvent molecules, which hinders the formation of hydrogen bonds with water molecules, causing a lower solubility of HAPEC polymer. HAPEC in different alcohols shows different solubility; longer aliphatic chain of the alcohol results in less solubility. In addition, the alcohol with longer carbon length causes a stronger hydrophobic interaction between alcohol molecules and water; thus, the LCST decreases more rapidly. Generally, the LCST is decreased when the organic solvent concentration is in range of 0 to 20% v/v, with the effectiveness of organic solvents to reduce the LCST in the following order: butanol > isopropanol > ethanol > methanol (Ju *et al.* 2013).

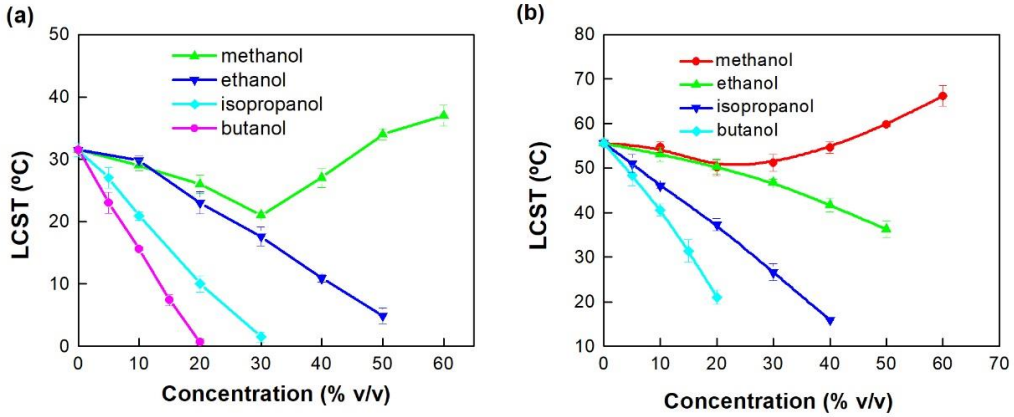

Fig. 6. Effects of organic solvents on LCST of HAPEC aqueous solutions (a) HBPEC-2, (b) HIPEC-1 (10 g/L)

The effect of methanol on LCST at high concentration was different from other alcohols. As the concentration of methanol increased to 60% v/v, the LCST of HBPEC-2 increased to 37.3 °C. When the concentration of alcohol was above the LCST minimum, the presence of additives in the second and higher solvation shells results in the solubility of HAPEC increasing rapidly (Lucht *et al.* 2017). When the concentration of methanol was above 30% v/v, excess methanol dissolved the HBPEC-2, which increased the LCST. Similarly, when the methanol concentration was above 20% v/v, the LCST of HIPEC-1 increased.

The Flocculation Behavior of HAPEC

In the presence of salt, some thermoresponsive polymers exhibit flocculation behavior when the temperature is above the LCST, which is called the critical flocculation temperature. The HBPEC showed flocculation behavior in a salt-free aqueous solution, but thermal flocculation was not observed in HIPEC. As shown in Fig. 7, the absorbance of HBPEC and HIPEC aqueous solutions increased rapidly with increasing temperature. As the temperature increased to LCST, the hydrophilic interaction between polymer and water molecules weakened, and the hydrophobic interaction was dominant among the polymer chains, causing the phase separation of polymer aqueous solutions (Tian *et al.* 2016). However, a sudden decline of absorbance of the HBPEC aqueous solutions occurred when the temperature was over 43.9 °C, resulting from the flocculation of HBPEC induced by the increased temperature.

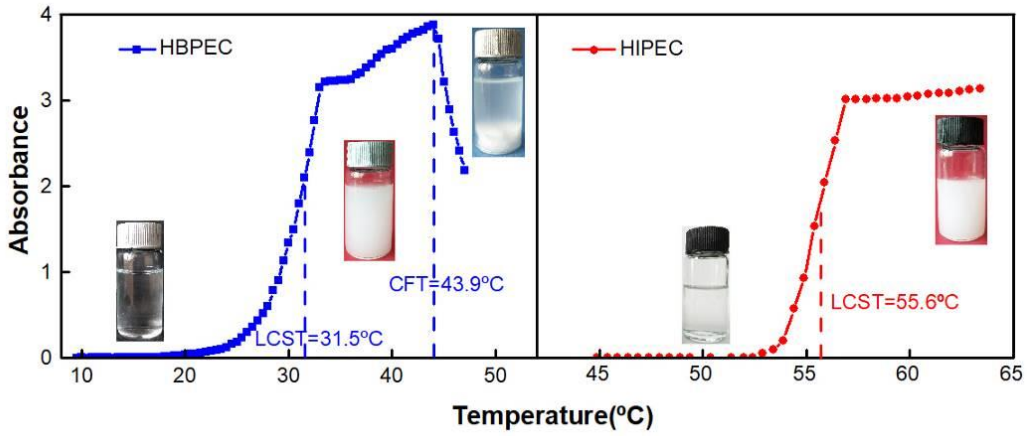

Fig. 7. Absorbance changes for HBPEC and HIPEC aqueous solutions (10 g/L)

CONCLUSIONS

1. In this work, thermoresponsive behavior of HBPEC and HIPEC was studied. The LCST of HBPEC and HIPEC was tuned in the range of 18.4 to 37.9 °C and 20.6 to 55.6 °C, respectively, by changing the MS. The LCST of HAPEC was modified by changing the HAPEC concentration. When the concentration ranged from 4 to 10 g/L, the LCST of HBPEC-2 and HIPEC-1 decreased by 2.8 °C and 3.3 °C, respectively.
2. The addition of NaCl and organic solvents could tune the LCST of HAPEC. The alcohols methanol, ethanol, isopropanol, and butanol were selected to investigate the relationship between organic solvents and LCST of HAPEC. All organic solvents reduced the LCST of HAPEC when their concentration was relatively low. The effectiveness of organic solvents to reduce the LCST observed the following orders: butanol > isopropanol > ethanol > methanol.
3. Compared with HIPEC, the HBPEC, which has longer hydrophobic side chains, exhibited thermoresponsive flocculation behavior when the temperature was above its CFT. In addition, the CFT was adjusted from 27.3 to 51.2 °C by changing MS in a range from 1.04 to 2.23.

4. As all the result confirmed, HBPEC, which has longer alkyl side chains, demonstrated more distinct thermoresponsive behavior and was more susceptible to additives. Interestingly, HBPEC possessed thermoresponsive flocculation behavior, but HIPEC aqueous solution showed no flocculation even at higher temperature.
5. The present study provided a method for tuning the LCST of cellulose-based thermoresponsive polymers. These kinds of polymers may be employed in applications where thermoresponsiveness is important.

ACKNOWLEDGMENTS

The authors are grateful for the support of the National Key R & D Program of China (2017YFD0701700), the Key Areas R & D Program of Guangdong Province (2019B020215001), the Project for the Ocean and Fisheries Department of Liaoning Province (201811), and the National Natural Science Foundation of China (No. 21376041, 31472312, and 31672673).

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Article submitted: May 24, 2019; Peer review completed: July 29, 2019; Revised version received: August 9, 2019; Accepted: August 12, 2019; Published: August 16, 2019.
DOI: 10.15376/biores.14.4.7977-7991

APPENDIX

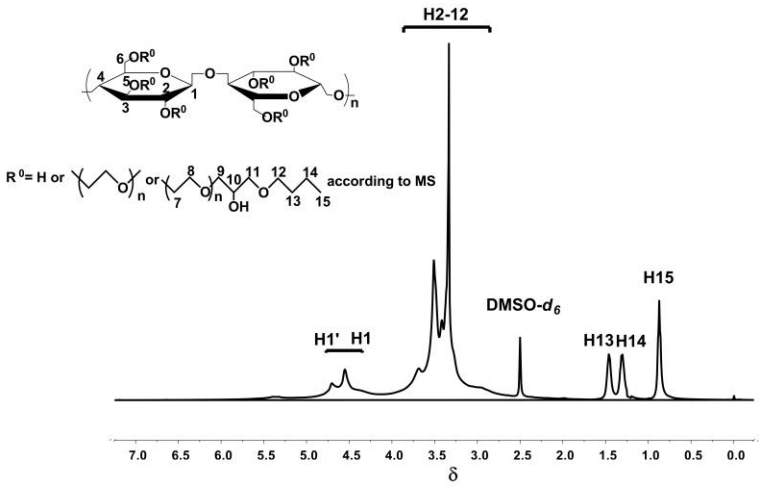

Fig. S1. ^{1}H NMR of HBPEC-2 recorded in DMSO-d_6

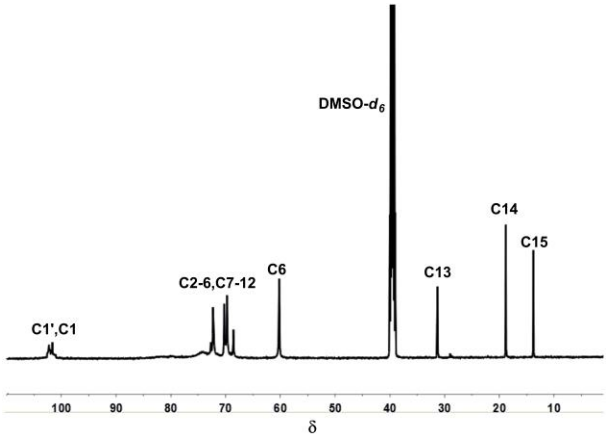

Fig. S2. ^{13}C NMR of HBPEC-2 recorded in DMSO-d_6

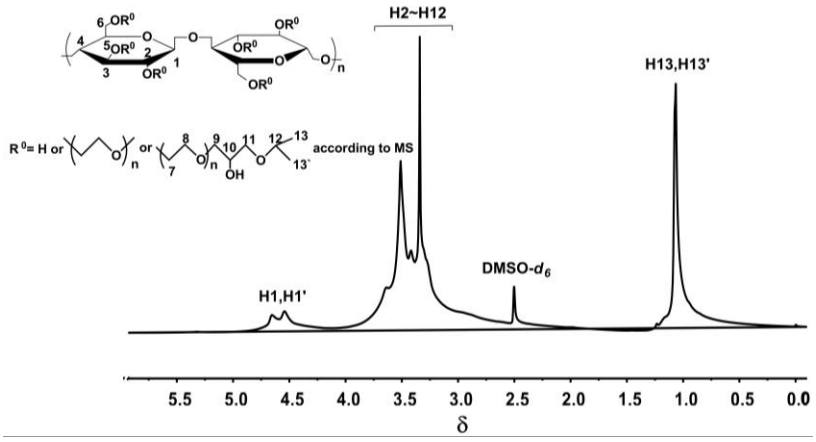

Fig. S3. ^{1}H NMR of HIPEC-1 recorded in DMSO-d_6

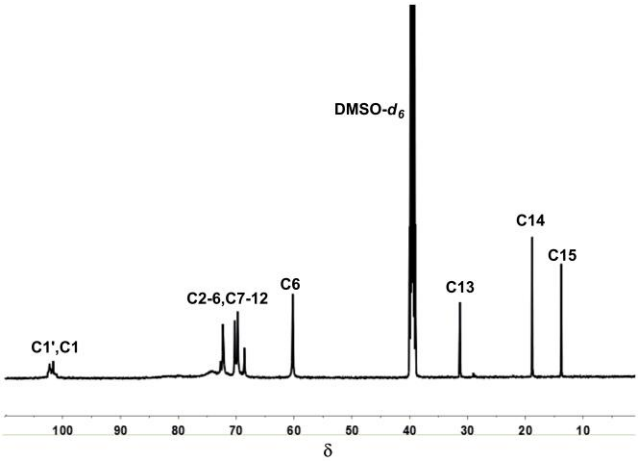

Fig. S4. ^{13}C NMR of HIPEC-1 recorded in DMSO-d_6

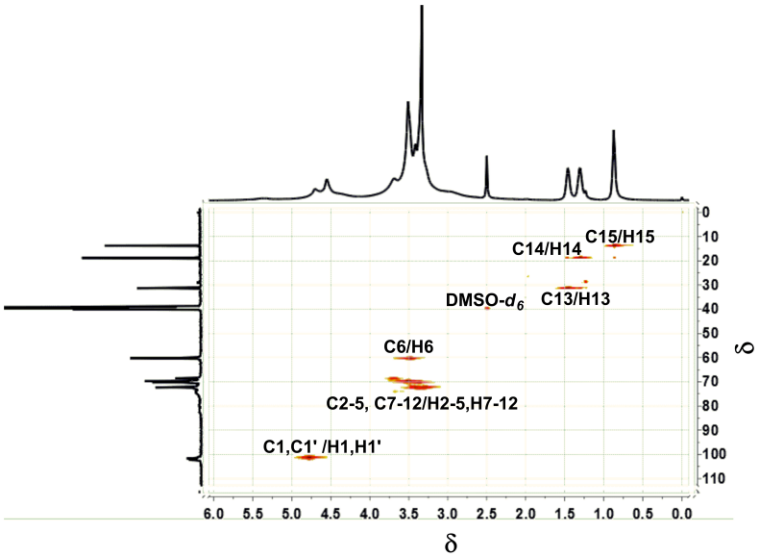

Fig. S5. 2D HSQC NMR of HBPEC-2 recorded in DMSO-d_6

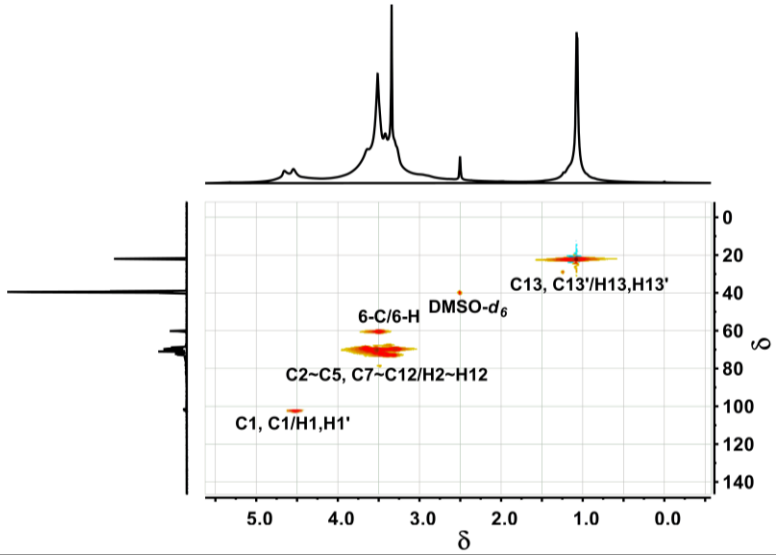

Fig. S6. 2D HSQC NMR of HIPEC-1 recorded in DMSO-d_6

The MS of HBPEC (1) and HIPEC (2) were calculated by the following formulas,

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

$$MS = I_{CH_3}/(6 \times I_{H1}) \quad (2)$$

where I_{CH_3} represents the integral area of terminal methyl, and I_{H1} represents the integral area of H1.

The reaction efficiency of HBPEC and HIPEC were calculated using the following formula,

$$RE = MS/[n(\text{BGE}):n(\text{AGU})] \times 100\% \quad (3)$$

where *MS* represents the molar substitution, *n*(AGE):*n*(AGU) represents the molar ratio of etherifying agent to glucose unit of cellulose.