Carbohydrate Polymers 108 (2014) 307–312

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Thermoresponsive starch derivates with widely tuned LCSTs by introducing short oligo(ethylene glycol) spacers

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ARTICLE INFO

Article history:
Received 15 October 2013
Received in revised form 17 January 2014
Accepted 17 February 2014
Available online 25 February 2014

Keywords:
Starch ethers
Thermoresponsivity
Oligo(ethylene glycol)

ABSTRACT

Water soluble, thermoresponsive 3-[2-butoxy(ethoxy)$_m$]-2-hydroxypropyl starch ethers (BE$_m$S) (m = 0, 1, or 2) were prepared by reacting degraded waxy maize starch with n-butyl glycidyl ether, 3-(2-n-butoxyethyl) glycidyl ether, and 3-[2-(2-n-butoxyethoxy)ethyl] glycidyl ether, respectively. The lower critical solution temperatures (LCSTs) of BE$_m$S could be tuned to a wide range from 17.5 °C to 55.0 °C by changing the side chain lengths of oligo(ethylene glycol) groups and their average molar substitution (MS). The LCSTs of BE$_m$S increase with increasing side chain length of oligo(ethylene glycol) groups when BS, BES, and BE$_2$S have similar MS values. By contrast, an increase in BE$_m$S concentration and addition of NaCl to the BE$_m$S solutions could lead to a decrease in the LCSTs of BE$_m$S. In addition, the effects of NaCl and BE$_m$S concentrations on the LCSTs become weaker when the side chain length of oligo(ethylene glycol) groups increase.

1. Introduction

Thermoresponsive polymers with lower critical solution temperatures (LCSTs) have attracted much attention because of their wide range of applications in sensors (Zareie, Boyer, Bulmus, Nateghi, & Davis, 2008), drug delivery (Schmaljohann, 2006), gene delivery (Merdan, Kopecek, & Kissel, 2002), tissue engineering (Xu, Flores, & McCormick, 2011), and separation processes (Bergbreiter, Zhang, & Mariagnanam, 1993). The LCSTs of thermoresponsive polymers should be controlled to a range of 20–40 °C in biomedical applications (Schmaljohann, 2006). Thus, a method for tuning the LCSTs of polymers should be developed. In addition, thermoresponsive polymers should be biocompatible and biodegradable for biomedical and biotechnology applications.

Thermoresponsive polymers generally exhibit a common structural feature of having both hydrophilic and hydrophobic groups in a delicate balance in monomeric units (Mori et al., 2005). Therefore, LCSTs could be tuned depending on the changes in these groups. For example, the LCSTs of poly(*N*-monoalkyl acrylamide) (Ito & Kubota, 1999; Kubota, Fujishige, & Ando, 1990; Maeda, Nakamura, & Ikeda, 2001), poly(*N*,*N*-dialkyl acrylamide) (Fischer, Zufferey, & Tahoces, 2011; Liu & Zhu, 1999; Xu, Jiang, & Liu, 2008), poly(2-alkyl-2-oxazoline)s (Bloksma et al., 2011), poly[oligo(ethylene glycol) methyl vinyl ether] (Ishizone et al., 2008), and poly[oligo(ethylene glycol) methyl ether methacrylates] (Ishizone et al., 2008) could be tuned by changing the type of hydrophobic alkyl or increasing the lengths of the hydrophilic oligo(ethylene glycol) groups. The biocompatibility and biodegradability of several thermoresponsive polymers are improved by grafting thermoresponsive polymer segments onto polysaccharides, which are non-toxic, biocompatible, and biodegradable. However, tuning the LCSTs of polysaccharide-based thermoresponsive polymers is difficult because the LCSTs of these polymers heavily depend on the LCSTs of the grafted thermoresponsive polymer segments and could not be tuned to a wide range. For example, pullulan-g-PNIPAAm (Ghimici & Constantin, 2011), dextran-g-PNVCL (Shi & Zhang, 2006), dextran-g-poly(NIPAAm-co-DMAAm) (Zhang, Srivastava, & Misra, 2007), and pullulan-g-PIPOZ (Morimoto, Obeid, Yamane, Winnik, & Akiyoshi, 2009), which are polysaccharide-based thermoresponsive polymers, have LCST values of 34, 34, 38, and 51.9 °C, respectively. These LCST values are very close to the LCSTs of corresponding grafted thermoresponsive polymer segments.

Compared with pullulan and dextran, which are produced by bacterial strains (Heinze, Liebert, Heublein, & Hornig, 2006), starch is derived from plants and has an abundant resource. We recently reported a thermoresponsive starch derivate by introducing hydrophobic butyl groups onto hydrophilic starch (2-hydroxy-3-butoxypropyl starches, HBPS) (Ju, Yan, & Zhang, 2012), and its LCST ranged from 4.0 °C to 32.5 °C. Lower LCSTs limit the applications of these biodegradable thermoresponsive starch

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http://dx.doi.org/10.1016/j.carbpol.2014.02.057
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derivates. Low LCST may be attributed to the smaller contribution of hydrophilic starch on the hydrophobic–hydrophilic balance of starch derivate. Hydrophilic oligo(ethylene glycol) groups were reported to contribute the thermosensitivity of the poly(methyl ether methacrylates) and poly(ethyl ether methacrylates) (Ishizone et al., 2008) by changing the hydrophilic and hydrophobic balance of methyl ether methacrylates and ethyl ether methacrylates. Therefore, in this paper, hydrophilic oligo(ethylene glycol) spacers were introduced into HBPS to synthesize 3-[2-butoxy(ethoxy)$_m$]-2-hydroxypropyl starch ethers (BE$_m$S). The thermoresponsive behavior of these spacers was investigated in detail, and results demonstrate that their LCSTs could be tuned to a wide temperature range. The effects of the lengths of the oligo(ethylene glycol) spacers on the thermoresponsive behavior were also examined in detail. The effects of NaCl concentration and the concentration of aqueous solutions on LCSTs were also determined.

2. Experimental

2.1. Materials

Waxy maize starch was obtained from the National Starch and Chemical Company (NJ, USA). Butyl glycidyl ether (>99%) was obtained from Tokyo Chemical Industry Co., Ltd. (Japan). 3-(2-Butoxyethyl) glycidyl ether and 3-[2-(2-butoxyethoxy)ethyl] glycidyl ether were synthesized in the laboratory. Other reagents and solvents were commercially available and used without further purification.

2.2. Preparation of degraded waxy maize starch

Waxy starch (60 g) was suspended in 150 mL of methanol in a 500 mL three-necked flask. Concentrated (36.5%, w/w) HCl (8 mL) was added. The mixture was heated to 50 °C in a water bath under stirring. After 4 h, the degraded starch was filtered from the solution and washed with 50% (v/v) acetone to remove the acid. The starch was then dried under an infrared lamp.

2.3. Preparation of BE$_m$S

Degraded waxy maize starch [4.0 g, 25 mmol of anhydroglucose units (AGUs)] was dissolved in deionized water (8 mL) in a 100 mL three-necked flask. NaOH (1.08 g, 40 wt% aqueous solution) was added, and the mixture was placed in a 70 °C water bath under stirring for 1 h. Afterward, a predetermined amount of alkyl glycidyl ether (AGE) was added to the flask. The reaction was conducted at 70 °C for 5 h. The suspension was then cooled in ice water and neutralized by increasing amounts of glacial acetic acid after adding 10 mL deionized water and 15 mL ethanol until the fuchsia color of phenothalin faded. The products were purified by dialysis in deionized water for 2 day, followed by separation with acetone and then drying for 5 h under an infrared lamp after filtration.

2.4. Characterization

^{1}H-NMR was recorded at room temperature using a Varian INOVA 400 spectrometer. The sample (10 mg) was dissolved with 0.5 mL D_2O/DClv (v/v% = 50%, DCl: wt% > 20%) and then incubated at 90 °C in a water bath for 2 h.

The LCSTs were determined using Mettler Toledo T90 equipped with a thermo controller (LAUDA RP200, Germany). The transmittance of the thermoresponsive, water-soluble products in aqueous solution (0.5%, w/w) was measured at 590 nm under the heating rate of 1 °C/min.

The molecular weights and molecular weight distribution 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). The sample was dissolved in 1 mL of eluent (concentration 0.5%, w/w). The injection volume was 50 μL. The H_2O was used as the eluent at a flow rate of 1 mL/min at 30 °C. Polysaccharide (Polymer Laboratories Inc.) was used for calibration.

The size and size distributions of micelles were evaluated by dynamic light scattering using nanoparticle size measurement (Malvern Nano-ZS90, Britain). The concentration of the polymer was 5 g/L. Samples were filtered through a 0.45 μm filter before measurement. The *Z*-average diameters of the micelles were provided by the instrument.

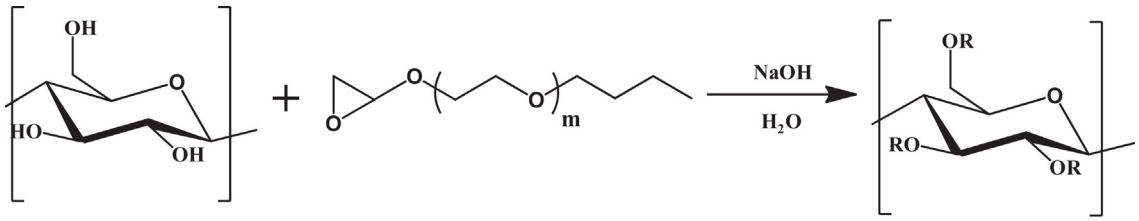
The critical micelle concentration of BE$_m$S was determined by fluorescence spectra (Hitachi F 7000, Japan) using pyrene as hydrophobic probe. Pyrene solution in methanol (3.0×10^{-4} M, 2 μL) was added to a 10 mL vial, and the methanol was evaporated. Afterward, the necessary amount of BE$_m$S solution was added to obtain 1.2×10^{-7} M pyrene concentration. The solutions containing pyrene were ultrasonically treated for 15 min and allowed to stand overnight.

3. Results and discussion

3.1. Synthesis and characterization of BE$_m$S

The general synthetic route for the preparation of BE$_m$S with different MS values is shown in Scheme 1. The characteristics and solution properties of different samples with thermoresponse are summarized in Table 1. Gel permeation chromatograms given as supplementary data Figs. S1–S11. MS is calculated using the following formula: $MS = (I_{CH_3}/3)/I_{H1}$.

The typical ^{1}H-NMR spectra of BS-1, BES-2, and BE$_2$S-4 are shown in Fig. 1. Peak assignments for BS-1, BES-2, and BE$_2$S-4 were straightforward (Fig. 1 and inset). The triplet at 0.90 ppm is attributed to the methyl group, and the peaks at 1.35 and 1.58 ppm are attributed to the methylene group of butyl (except —CH_2O—). The peaks at 4.5 ppm to 5.5 ppm were assigned to the anomeric protons (H1) of BS-1, BES-2, and BE$_2$S-4. The difference of the peaks between 3.0 ppm and 4.0 ppm is correlated with the different side chain lengths of the oligo(ethylene glycol) groups in their



R= H or -$CH_2CHOHCH_2O(CH_2CH_2O)_mCH_2CH_2CH_2CH_3$, according to MS
m=0 or1 or 2.

Scheme 1. Synthetic pathway of BE$_m$S.

Table 1
Preparation[a] and characterization of BE_mS.

Sample	AGE:AGU[b]	MS[c]	M_w = (1.23×10^6 g/mol)[d]	PDI[d]	Efficiency (%)	LCST (°C)[e]
BS-1	0.75	0.68	1.180	6.76	90.7	23.6
BS-2	0.63	0.57	0.259	1.87	91.2	28.0
BS-3	0.50	0.46	0.340	1.88	92.0	41.8
BES-1	1.00	0.85	2.370	9.93	85.0	23.1
BES-2	0.75	0.64	0.170	1.25	85.3	31.1
BES-3	0.50	0.49	0.288	1.57	98.0	46.0
BE_2S-1	1.50	1.31	4.800	6.90	87.3	17.5
BE_2S-2	1.25	1.13	2.790	7.31	90.4	23.7
BE_2S-3	1.00	0.94	0.727	3.04	94.0	28.7
BE_2S-4	0.75	0.65	0.174	1.34	86.7	34.5
BE_2S-5	0.50	0.42	0.442	1.83	84.0	55.0

[a] AGU, anhydroglucose unit. The average molar weight of DWS was 1.23×10^6 g/mol (PDI: 3.98) as determined by GPC. The amount of DWS in all reactions was 4.0 g.
[b] Molar ratio.
[c] MS, determined by ^{1}H-NMR.
[d] Determined by GPC.
[e] Determined by Mettler Toledo T90 and LAUDA RP200.

structures. Most of the molecular weights of BE_mS were less than the molecular weights of unmodified degraded waxy maize starch ($M_w = 1.23 \times 10^6$ g/mol), which could be attributed to the degradation of DWS under alkaline conditions. However, considering that the LCSTs of BS-1, BES-1, BE_2S-1, and BE_2S-2 were lower than the testing temperature, aggregations may have occurred during testing, resulting in an increase in the molecular weights.

3.2. Thermoresponsive behavior of BE_mS

The thermoresponsive behavior of BE_mS in aqueous solutions was investigated at a concentration of 5 g/L (0.5 wt%). Fig. 2A–C show the variations in the transmittance of 0.5 wt% aqueous solutions of different samples at 590 nm at the heating rate of 1 °C/min. The results demonstrate the thermoresponsiveness of BE_mS. The LCSTs of BE_mS decrease as the MS increases (Fig. 2D). For example, the LCSTs of BS decreased from 41.8 °C to 23.6 °C as the MS increased from 0.46 to 0.68. Additionally, changing the lengths of hydrophilic oligo(ethylene glycol) groups on BE_mS also could tune LCST. For instance, the LCSTs of BES and BE_2S, which have only one and two oligo(ethylene glycol) groups in their side chains, respectively, can be tuned in the range of 23.1–46.0 °C and 17.5–55.0 °C, respectively, which are wider than the range for BS. The results demonstrate that LCSTs of BE_mS can be adjusted to a wider temperature range by introducing hydrophilic oligo(ethylene glycol) spacers into BS.

Considering that both the MS and length of oligo(ethylene glycol) groups heavily influence the LCSTs of BE_mS, studying the effects of the lengths of oligo(ethylene glycol) groups on LCSTs will only be meaningful when BS, BES, and BE_2S have similar MS values. Fig. 3 shows that BS-1 (MS = 0.68), BES-2 (MS = 0.65), and BE_2S-4 (MS = 0.64) have LCSTs of 23.6, 31.3, and 34.5 °C, respectively. Their LCSTs increase with increasing lengths of the oligo(ethylene glycol) groups, similar to the thermoresponsive water-soluble polymers with oligo(ethylene glycol) groups (Aoshima, Oda, & Kobayashi, 1992; Bloksma et al., 2011; Yamamoto, Pietrasik, & Matyjaszewski, 2008). More hydrophilic oligo(ethylene glycol) groups increase the difficulty of the dissociation of water molecules around the polymer chains during phase transition. Therefore, more energy will be needed to compensate for the loss of entropy because of the collapse of the polymer chains (Hua, Jiang, Li, & Zhao, 2006). Longer pendants of oligo(ethylene glycol) lead to stronger interactions between the starch derivate and water molecules, thereby leading to higher LCSTs.

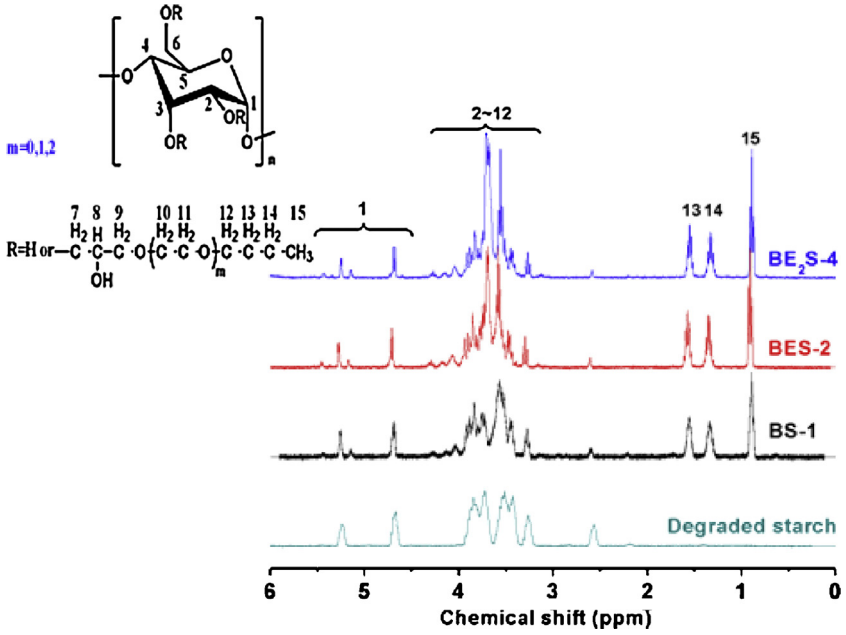


Fig. 1. ^{1}H-NMR of BS-1, BES-2, and BE_2S-4.

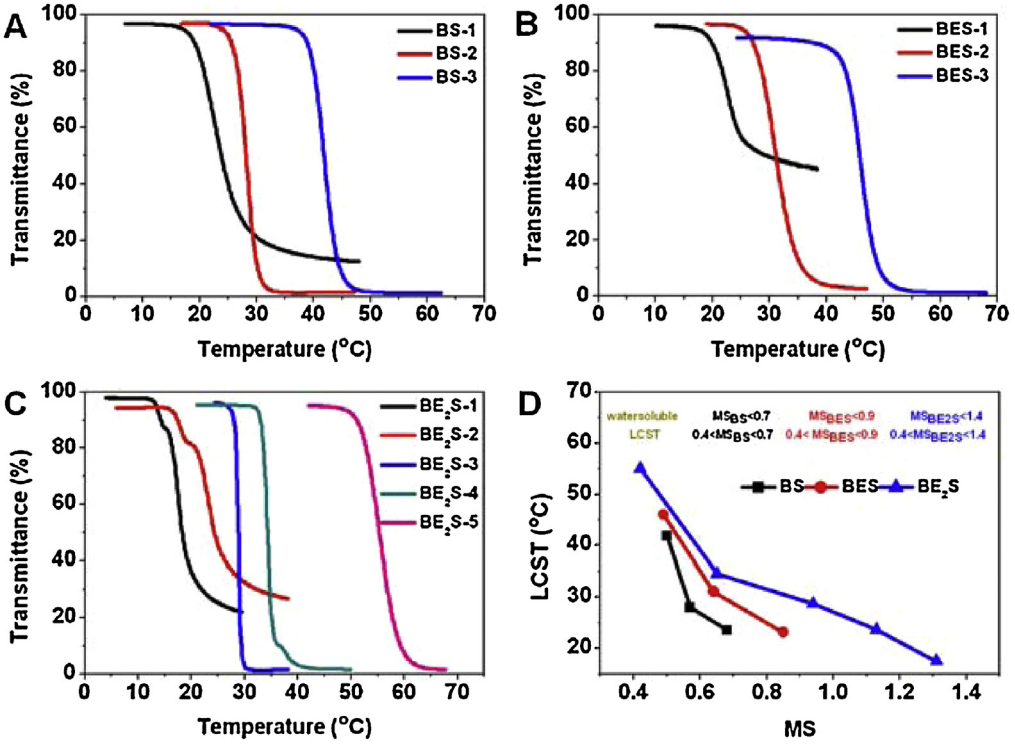


Fig. 2. (A–C) Variations in the transmittance of 0.5 wt% aqueous solutions of the different samples at 590 nm at the heating rate of 1 °C/min. (D) Effects of MS on LCSTs.

3.3. Effect of concentration on thermoresponsive behavior

Thermoresponsive polymers are diluted during application, thereby resulting in loss of thermoresponsiveness. Therefore, the effect of the lengths of oligo(ethylene glycol) groups on the concentration resistance of BE_mS was examined. The effect of thermoresponsive starch derivate concentration on LCSTs was determined for BS-1, BES-2, and BE_2S-4, which have corresponding LCSTs of 23.6, 31.3, and 34.5 °C at a concentration of 5 g/L. The concentration dependence of the LCST was also investigated in the range of 0.1–0.5 wt%, and the results are plotted in Fig. 4D. Obviously, lower concentrations of BS-1, BES-2, and BE_2S-4 result in higher LCSTs and broader phase transitions (Fig. 4A–C). These observations may be related to an aggregation kinetic effect, i.e., lower concentration of starch derivates leads to longer times required for the formation of aggregates.

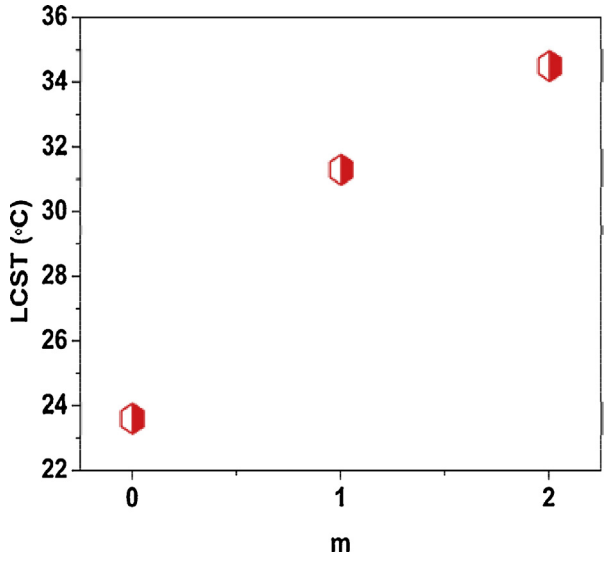


Fig. 3. Variations in the LCSTs with increasing lengths of oligo(ethylene glycol) groups (m).

Fig. 4(D) shows that the LCSTs of BES-2 and BE_2S-4 decreased by 3.9 and 1.7 °C, respectively, when the concentration varied from 1 g/L to 5 g/L. Notably, as the side chain lengths of oligo(ethylene glycol) groups increase, the effect of concentration on thermoresponsive behavior becomes weaker, i.e., an increase in length of oligo(ethylene glycol) groups could enhance the anti-dilution ability of BE_mS. A similar phenomenon could be observed for poly-di(ethylene glycol) methyl ether methacrylate and poly-tri(ethylene glycol) methyl ether methacrylate (Hua et al., 2006; Yamamoto et al., 2008) or poly-α-hydro-ω-(4-vinylbenzyl)tris(oxyethylene) and poly-α-hydro-ω-(4-vinylbenzyl)tetrakis(oxyethylene) (Hua et al., 2006).

3.4. Effect of NaCl concentration on thermoresponsive behavior

To investigate the effect of NaCl concentration on the LCSTs of BE_mS, the variations in the transmittance of 5 g/L aqueous solutions of BS-1, BES-2, and BE_2S-4 with different NaCl concentrations (no salt, 0.01, 0.05, 0.10, and 0.20 mol/L) during heating were recorded (Fig. 5A–C).

Fig. 5D shows the LCSTs recorded for BS-1, BES-2, and BE_2S-4 with increasing NaCl concentrations. The addition of electrolytes is known to change the normal hydrogen bonding water structure. The addition of NaCl to aqueous BE_mS solutions changes the properties of the hydration layer and could disrupt the highly oriented water molecules that surround the polymer (Eeckman, Amighi, & Moes, 2001; Maeda, Takenouchi, Yamamoto, & Aoyagi, 2006). The presence of NaCl leads to partial dehydration of BE_mS and consequently to a decrease in the LCST. For example, at 5 g/L, the LCST of BS-1 in 0.2 mol/L NaCl solution is 9.7 °C lower than the LCST of deionized water. Interestingly, as shown in Fig. 5D, LCSTs of BES-2 and BE_2S-4 in 0.2 mol/L NaCl solution decreased by 8.3 and 3.8 °C,

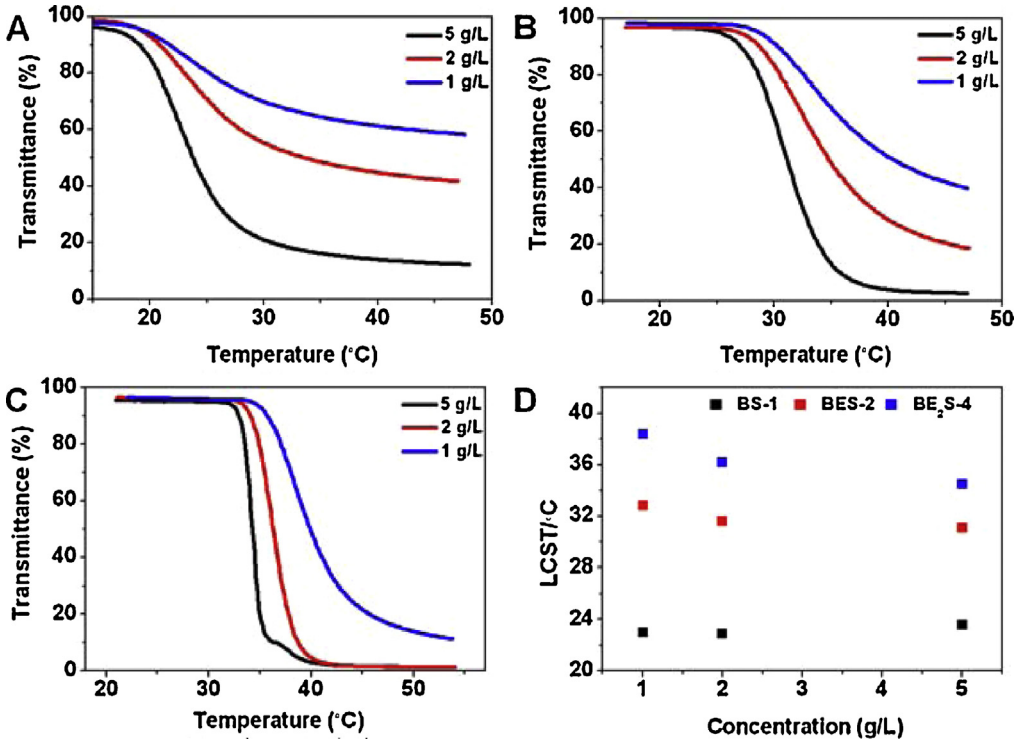


Fig. 4. (A–C) Variations in the transmittance of 0.1% (w/v) to 0.5% (w/v) aqueous solutions of the different samples with changes in temperature. (A–C: BS-1, BES-2, and BE_2S-4, respectively). (D) Effect of sample concentration on LCSTs.

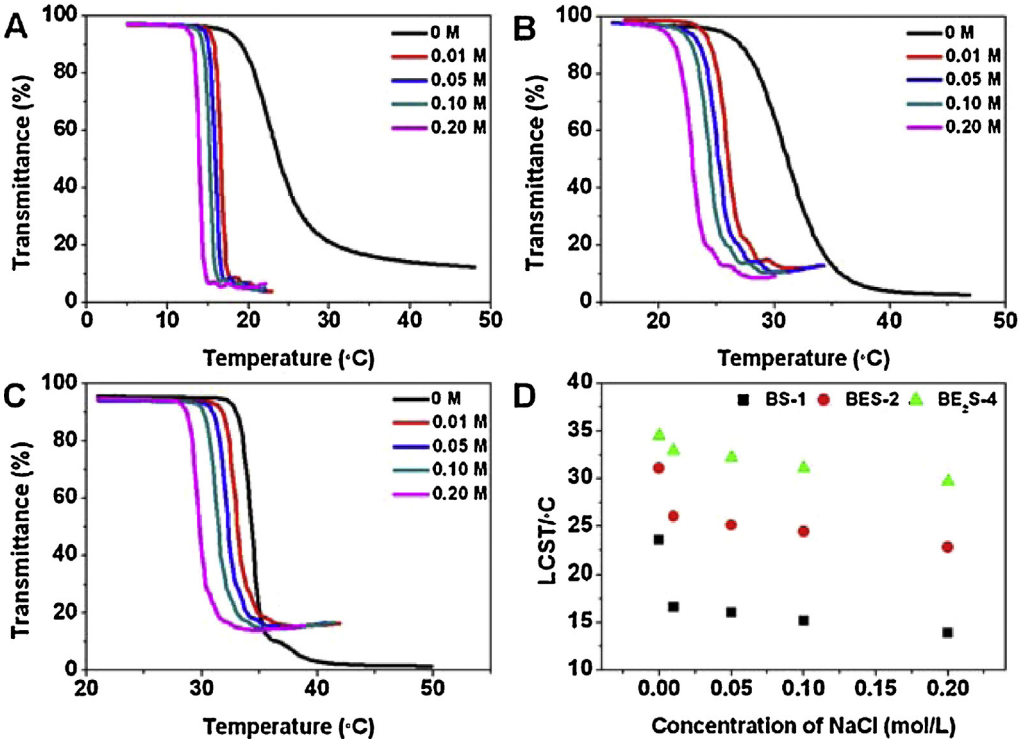


Fig. 5. (A–C) Variations in the transmittance of 0.5% (w/v) aqueous solutions of different samples with different NaCl concentrations during heating. (A–C: BS-1, BES-2, and BE_2S-4, respectively). (D) Effect of NaCl concentration on LCSTs.

respectively, compared with the LCST of deionized water. The results indicate that the presence of more oligo(ethylene glycol) groups in thermoresponsive starch derivates results in a smaller decrease in the LCSTs at the same NaCl concentration.

4. Conclusion

A method to obtain thermoresponsive polysaccharides derivates with appropriate MS was developed based on the principle of hydrophilic and hydrophobic balance. The method was used to prepare thermoresponsive starch derivates (BE_mS) bearing oligo(ethylene glycol) group side chains. The LCST of BS (BE_mS, $m = 0$) could be tuned to the range of 23.6–41.8 °C by changing its MS from 0.46 to 0.68. Furthermore, after the introduction of hydrophilic oligo(ethylene glycol) groups, the LCSTs of BES and BE_2S could be tuned to the wider ranges of 23.1–46.0 °C and 17.5–55.0 °C, respectively.

The LCSTs of BE_mS could be influenced by the addition of NaCl and shift to lower temperatures as the NaCl concentration is increased in the BE_mS solutions. The effects of BE_mS concentration and addition of NaCl on the LCSTs of BS-1, BES-2, and BE_2S-4, which have similar MS values, were also investigated. The results show that as the lengths of oligo(ethylene glycol) groups increased, the LCSTs also increased. The anti-dilution ability and NaCl resistance were enhanced simultaneously.

Acknowledgements

The authors gratefully acknowledge the financial support from the National Science Foundation of China (No. 207762027 and 21076033), the Key Program of National Natural Science Foundation of China (20836001) and the Program for Changjiang Scholars and Innovative Research Team in University (IRT0711).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.02.057.

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