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Novel pH- and temperature-responsive polymer: Tertiary amine starch ether

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ABSTRACT

A novel double pH- and temperature-responsive tertiary amine starch ether (TAS) has been developed. Synthesis was performed by grafting dipropyl or dibutyl epoxypropylamine onto hydroxyethyl starch. The cloud point temperatures (T_C) of TAS could be tuned to a wide range from 26 to 72.8 °C by changing the alkyl chain length, their average molar substitution (MS), and pH value of the solution. The T_C of TAS increases with decreasing the alkyl chain length, MS, and pH value of the solution. A linear relationship occurs between the T_C and the pH, indicating well-tunable T_C. These TAS also showed single pH-sensitive property due to the existence of tertiary amino and hydrophobic alkyl groups. The synthetic strategy presented here could be employed in the preparation of other novel biomaterials with dual pH- and temperature-responsive properties from a variety of polysaccharides.

1. Introduction

Polymers responding to more than one stimulus, in particular to temperature and pH, have received much attention. These dual functional systems have great importance in biological applications and can mimic the responsive macromolecules found in nature (Chen & Hoffman, 1995; Klaikherd, Nagamani, & Thayumanavan, 2009). In the past decade, various types of pH- and temperature-responsive polymers have been prepared. Among them, tertiary amine-containing polymers are some of the most frequently investigated pH- and temperature-responsive polymer (Deen, 2012; Han et al., 2013; Jung, Song, Lee, Jeong, & Lee, 2011; Xiao et al., 2014). The most widely studied example of this type of polymer is poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMA) and its related copolymers (Han et al., 2013; Plamper et al., 2007). PDMA has antibacterial, hemostatic, and anticancer activity (Rawlinson et al., 2010). It has been employed in the preparation of new drug or gene delivery systems because of excellent biocompatibility (Newland et al., 2013; Peng et al., 2011). The cloud points of PDMA can be easily turned by adjusting the pH of the aqueous solution. This is possible since the protonation degree of the tertiary amine group in PDMA varies depending on pH of the aqueous solution, and the hydrophobic–hydrophilic balance of PDMA change significantly at different pH. The analogous poly(*N*,*N*-diethylaminoethyl methacrylate) (PDEA) (Schmalz, Hanisch, Schmalz, & Muller, 2010) and poly(*N*-ethylpyrrolidine methacrylate) (PEPyM) (Gonzalez, Elvira, & Roman, 2005) homopolymers also exhibit pH- and temperature-responsive properties due to the presence of ionizable amine groups and hydrophobic *N*-alkyl groups. Contrarily, the cloud points of PDEA and PEPyM are low compared to that of PDMA. For example, at pH 7 the cloud point of PDMA is 80 °C, whereas the value for PDEA is 40 °C. Furthermore, PDEA exhibits pH-dependent solubility and is soluble in acidic solution as a weak cationic polyelectrolyte. At 25 °C, PDEA phase-separates out at a neutral pH, whereas PDMA is soluble over the whole pH range. These differences can be attributed to the more hydrophobic alkyl substituents at the amino group of PDEA.

In recent years, pH- and temperature-responsive polysaccharides have raised interest due to their degradable potential and biocompatibility. A popular preparation strategy is the grafting of stimuli-responsive polymers onto a polysaccharide backbone. Various dual sensitive grafted polysaccharide derivatives have been researched, such as cellulose-graft-poly(*N*,*N*-dimethylamino-2-ethyl methacrylate) (cellulose-g-PDMA) (Sui et al., 2008), hydroxypropyl cellulose-graft-poly(*N*,*N*-dimethyl aminoethyl methacrylate) (HPC-g-PDMA) (Ma et al., 2010b, hydroxypropylcellulose-graft-poly(4-vinyl pyridine) (HPC-g-P4VP) (Ma, Kang, Liu, & Huang, 2010a), chitosan-graft-poly[2-(*N*,*N*-dimethylamino)ethyl methacrylate] (CS-g-PDMA) (Yuan et al., 2011). However, the main drawback of most techniques reported is an unwanted homopolymer produced with the graft copolymer (Sui et al., 2008). On the other hand, the polysaccharides render stimuli-responsiveness due to the LCST transition of the stimuli-responsive polymers

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grafted onto polysaccharide backbone. Thus, the LCST of stimuli-responsive polysaccharides is usually similar to that of grafted stimuli-responsive polymers and difficult to adjust.

In this work, a new strategy has been proposed for preparing pH- and temperature-responsive polysaccharides. Additionally, a new kind of pH- and temperature-responsive tertiary amine containing polymer, namely tertiary amine starch ethers (TAS), has been developed. Starch is the second most abundant polysaccharide produced by plants. Starch and its derivatives have emerged as one of the most promising biomaterials for drug carriers due to their biodegradability and biocompatibility. It has been demonstrated that thermo-responsive starch can be synthesized by controlling the hydrophobic–hydrophilic balance of starch derivatives (Ju, Cao, & Zhang, 2013; Ju, Yan, & Zhang, 2012). Epoxypropyl tertiary amines contain ionizable amino groups, two hydrophobic alkyl chains, and highly reactive epoxy groups. It is expected that starch modified by epoxypropyl tertiary amine would be sensitive to temperature and pH changes due to the existence of ionizable amine groups, a hydrophilic starch backbone, and hydrophobic *N*-alkyl groups. Based on this, a novel class of pH- and temperature-responsive TAS has been successfully prepared by a simple etherification reaction between epoxypropyl tertiary amine and hydroxylethyl starch (HES). The hydrophobic–hydrophilic balance of TAS can be tailored by controlling the alkyl chain lengths, their average molar substitution (MS), and the pH of the aqueous solutions, resulting in tunable LCST within a wide range. Scheme 1 illustrates the outline of the TAS synthesis by an etherification reaction. In this paper, the effects of the alkyl chain lengths, MS, and the pH of the aqueous solutions on tuning the cloud point temperatures (T_C) of TAS by spectrophotometry are discussed in detail.

2. Experimental

2.1. Materials

Hydroxyethyl starch (MSOH = 0.5, 200 kDa) was purchased from Hustlife Scitech (Wuhan PR China). All other reagents were used as received, without further purification.

2.2. Synthesis of dialkyl epoxypropylamine (DAEPA)

The synthesis of DAEPA was prepared based on existing reports (Gilman et al., 1946). In a 2 L three-necked flask placed in an ice-water bath, 465 g epichlorohydrin (5.03 mol) and 15 mL water were mixed by vigorous stirring, while the temperature was maintained below 15 °C. 3.96 mol di-*n*-propylamine/di-*n*-butylamine was added in drop-wise and reacted for 48 h with continuous stirring, during which the temperature was maintained at 15–20 °C. At the end of the reaction, the mixture was cooled with an ice-water bath. Cooled 500 g sodium hydroxide solution (40 wt%) was added slowly, maintaining the temperature below 20 °C. The mixture was stirred vigorously for 40 min and poured into 200 mL water. The upper organic layer was separated; the lower aqueous layer was extracted with 50 mL ether 3 times. The ether extracts were combined to the upper layer then dried with anhydrous sodium sulfate. The final product was received after distillation under reduced pressure.

Dipropyl epoxypropylamine: 48.7%, b.p. 92.0 ~ 93.6 °C, 25 mmHg. Massspectrum ACPI: *m*/*z* (relative intensity) 158 (100%) [$(M+H)^+$, $C_9H_{19}NO$]; ^{1}H NMR (400 MHz, (CD3)2SO): δ 0.90 (t, 6*H*), δ 1.46 (m, 4*H*), δ 2.57–2.29 (m, 6*H*),δ 2.73–2.63 (m, 2*H*), δ2.95–2.91 (m, 1*H*).

Dibutyl epoxypropylamine: 58.0%, b.p. 90 ~ 93 °C, 4 mmHg. Massspectrum ACPI: *m*/*z* (relative intensity) 186 (100%) [$(M+H)^+$, $C_{11}H_{23}NO$]; ^{1}H NMR (400 MHz, (CD3)2CO): δ 0.92 (t, 6*H*), δ1.47–1.30 (m, 8*H*), δ2.57–2.29 (m, 6*H*), δ2.73–2.63 (m, 2*H*), δ2.95–2.91 (d, 1*H*).

2.3. Synthesis of TAS

An amount of 4.0 g HES (MSOH 0.5, 200 kDa, 21.7 mmol anhydroglucose units AGU), a volume of 16 mL DMSO, and an amount of 0.5 g (40 wt%) NaOH (10 mmol) were added to a 100 mL three-necked flask. After the HES suspended completely under stirring, a predetermined amount of DAEPA was added to the flask in drop wise. The reaction was carried out for 10 h, and the temperature was maintained at 90 °C. At the end of the reaction, the mixture was cooled to room temperature and neutralized to pH 3.0 with 6 M HCl. After the products were precipitated by the addition of acetone, they were purified by dialysis in deionized water for two days followed by freeze-drying.

2.4. Characterization

^{1}H-NMR spectra were executed on a Varian INOVA 400 spectrometer. The temperature was kept at 25 °C, and D_2O used as solvent. All of the titration measurements were carried out with a Mettler Toledo T90 titrator. The transmittance was measured at 590 nm. The concentration of the TAS was 1.0 g/L. The solutions were adjusted to pH ≈ 3.8 with 0.1 M HCl, and then titrated to pH ≈ 10.5 with 0.1 M NaOH at 20 °C while transmittance was measured at the same time. The temperature was controlled by a LAUDA E200 water bath, and the heating gradient was fixed to 1.0 °C/min when it was needed. The cloud point temperatures (T_C) are defined as the temperature at which the transmittance of the solution becomes 50%. Several solutions were prepared in Briton–Robinson buffer solution for the measurement of UV–vis spectra in different pH. The molecular weights and molecular weight distribution of TAS and HES were measured on an Agilent Technologies 1200 series gel permeation chromatograph equipped with ultrahydrogel 1000 column. Sample was dissolved in 1 mL of eluent (concentration 0.5%, w/w). The H_2O was used as the elu-ent at a flow rate of 1 mL/min at 30 °C. Polysaccharide (Polymer Laboratories Inc.) was used for calibration.

3. Results and discussion

3.1. Synthesis and characterization of TAS

Convenient etherification of starch was used to prepare TAS (Scheme 1). HES was used in this work due to its fine solubility, biodegradability, and biocompatibility. TAS were synthesized in homogeneous system, which employed DMSO as solvent and NaOH as catalyst to achieve a homogeneous distribution of substituent. TAS with different MS and alkyl were prepared: TAS with dipropyl (DP-TAS) and dibutyl (DB-TAS) show the tunable dual pH- and temperature responsive properties.

The characteristics and solution properties of four DP-TAS and four DB-TAS samples are summarized in Table 1. A typical ^{1}H NMR spectrum of TAS and the spectrum of HES are shown together in Fig. 1. The MS was calculated using the ratio of the integral of $-CH_3$ in the dialkylamino groups to the six times of the integral of H1 in the AGU by ^{1}H NMR (in Fig. 1). In GPC analysis, the most observed molecular weights for TAS was less than that of hydroxyethyl starch ($M_W = 2.69 \times 10^5$ g/mol) except DP-TAS 1.12. These measurements showed a decrease with increasing MS, which can be attributed to the degradation of HES under alkaline conditions and the formation of an entity of reduced hydrodynamic dimension. The latter occurs due to hydrophobic interaction in the inner domain comparing to the parent HES.