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Synthesis and characterization of bacterial cellulose sulfates using a SO_3/pyridine complex in DMAc/LiCl

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ABSTRACT

Various sodium bacterial cellulose sulfates (SBCS) were prepared via the homogeneous sulfation of bacterial cellulose (BC) with a SO_3/pyridine (Py) complex in a dimethyl acetamide/lithium chloride solution. The SBCSs were characterized using Fourier transform infrared spectroscopy, X-ray diffraction (XRD), carbon nuclear magnetic resonance spectroscopy, gel permeation chromatography, elemental analyses, and thermal gravimetric analyses. A variety of conditions (including various amounts of SO_3/Py, temperatures, and reaction times) were utilized to obtain SBCSs with DS values that ranged from 0.10 to 1.50. The XRD profiles indicated that the SBCSs had a cellulose II analog polymorphous structure. The differences between BC and microcrystalline cellulose (MC) were studied in their respective reactions. BC is more reactive than MC in both the sulfation and depolymerization processes. The order of reactivity for C—OH is C6 > C2 > C3 for both BC and MC. Cellulose sulfates with DS values >0.31 were soluble in deionized water.

1. Introduction

Cellulose is the most abundant renewable biopolymer on earth; both the parent material and its derivatives are popular commercial products in many fields, such as the food, textile, medicinal, environmental, and paper industries (Heinze, Liebert, Pfeiffer, & Hussain, 2003; Hoenich, 2006; Kim et al., 2008; Klemm, Heublein, Fink, & Bohn, 2005; Klemm, Philipp, Heinze, Heinze, & Wagenknecht, 1998, chap. 4). Various microorganisms, such as bacteria (*genera Aerobacter*, *Acetobacter*, *Achromobacter*, *Agrobacterium*, *Alacaligenes*, *Azotobacter*, *Pseudomonas*, *Rhizobium*, and *Sarcina*), fungi (*Saprolegnia* and *Dictystelium disoideum*) and algae (*Vallonia*), produce bacterial cellulose (BC), which is an exocellular polysaccharide (Chen, Cho, & Jin, 2010; Ross, Mayer, & Benziman, 1991).

BC is widely studied in the contexts of food, pulp, biomaterials, and the environment because it possesses unique structural properties, such as high degrees of polymerization (DP: 2000–6000), high chemical purity (>98%), a high Yang's modulus, a high water-absorption capacity, and a small, ultrafine, reticulated fiber structure (thickness: 3 ± 4 nm; width: 70 ± 80 nm) (Jonas & Farah, 1998; Retegi et al., 2010). BC derivatives have recently attracted more attention. These derivatives are generally processed through the use of additives during cultivation or chemical modification (Cai & Kim, 2010; Cheng, Catchmark, & Demirci, 2009; Ciechańska, 2004). Several chemical methods to modify BC have been reported, such as carboxymethylation (Yu, Yin, Ji, Yong, & Lin, 2010), acetylation (Ifuku et al., 2007; Geyer et al., 1994), phosphorylation (Oshima, Kondo, Ohto, Inoue, & Baba, 2008), succinylation (Yin et al., 2011), benzoylation (Wang, Luo, Peng, & Pei, 2008), acylation and carbanilation (Barthel & Heinze, 2006). BC is more reactive than microcrysatalline cellulose (MC) because of its finer fibril morphology (Tabuchi, Watanabe, Morianga, & Yoshinaga, 1998), and its order of acetylation reactivity in ionic liquid is O6 > O3 > O2, which differs from that of plant cellulose (PC), O6 > O2 > O3 (Schlufter, Schmauder, Dorn, & Heinze, 2006). However, compared to plant cellulose, BC does not receive the attention it deserves, particularly with respect to chemical modifications.

Cellulose sulfate, which is a half ester of cellulose, is widely studied in the biotechnology and pharmaceutical fields because it exhibits a number of biological effects, including antiviral, anticoagulant, antibacterial, and antioxidant activity (Groth & Wagenknecht, 2001; Heinze, Daus, Gericke, & Liebert, 2010; Wang, Li, Zheng, Normakhamatov, & Guo, 2007). Cellulose sulfate can also form polyelectrolyte complexes (Gericke, Liebert, & Heinze, 2009). Cellulose sulfate can be prepared heterogeneously, quasi-homogeneously, or homogeneously through the use of various sulfating agents, which include sulfuric acid, SO_3, chlorosulfuric acid, SO_2Cl_2, and SO_3 complexes (e.g., SO_3/DMF, SO_3/Py, etc.)

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(Baumann, Richter, Klemm, & Faust, 2000; Heinze, Liebert, & Koschella, 2006; Klemm et al., 2005; Wang, Li, Xiao, & Wu, 2009; Yao, 2000). Homogeneous sulfation reactions are common procedures for the preparation of cellulose sulfate with high DS, regular distribution, good solubility, and low chain degradation. Cellulose sulfate can be prepared in homogeneous processes either indirectly from intermediates, such as unstable cellulose esters and ethers (Richter & Klemm, 2003), or directly from cellulose (Gericke et al., 2009; Zhang, Brendler, Geissler, & Fischer, 2011).

When preparing cellulose sulfate directly from cellulose, the solvent system must be carefully chosen to ensure efficient sulfation and to mitigate chain degradation. Gericke et al. (2009) successfully prepared cellulose sulfates with DS that ranged from 0.13 to 1.46 in 1-butyl-3-methylimidazolium chloride (BMIMCl)/N,N-dimethylformamide (DMF) and sulfation agents (mol ratio to AGU of 0.7–3). The reaction occurs predominantly at the primary hydroxyl groups. When using BMIMCl without DMF as the co-solvent, water soluble cellulose sulfates were not formed efficiently due to the high viscosity of the ionic liquid/cellulose mixture, which caused poor miscibility, inadequate sulfating agent distribution, and an uneven distribution of the resultant sulfate groups along the cellulose chains. DMAc/LiCl is a heavily investigated solvent for the homogeneous modification of cellulose (Heinze & Petzold-Welcke, 2012; Liebert, 2010; McCormick & Callais, 1987). Performing the sulfation reaction in DMAc/LiCl is a disappointing approach for the preparation of water soluble cellulose sulfate because the reaction mixture coagulates during the reaction (Klemm et al., 1998, chap. 4; Wagenknecht, Phillipp, & Keck, 1985). Recently, Zhang, Brendler, et al. (2011) reported that using chlorosulfuric acid as a sulfating agent in DMAc/LiCl resulted in homogeneous sulfation throughout the reaction. Water-soluble cellulose sulfates with diverse DS were prepared using this process. Thus, the success of this homogeneous sulfation reaction in DMAc/LiCl resulted from the low molecular weight of the starting cellulose. BC is a high molecular weight cellulose. Research concerning the sulfation of BC in DMAc/LiCl would provide information regarding the sulfation of cellulose in DMAc/LiCl.

The chemical modification of BC and the advantages of cellulose sulfation in DMAc/LiCl, particularly concerning whether the mixture will coagulate, are not well understood; thus, this paper aimed to investigate the sulfation of BC with SO_3/Py in DMAc/LiCl. The reaction parameters that influenced the DS values and cellulose sulfate solubility in deionized water were evaluated. The structure of the SBCSs were characterized using elemental analyses (EA), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), carbon nuclear magnetic resonance spectroscopy (^{13}C NMR), gel permeation chromatography (GPC), and thermal gravimetric analyses (TG). The distribution of —SO_3H on sodium bacterial cellulose sulfates (SBCSs) and the differences between BC and MC in this reaction were also investigated.

2. Experimental

2.1. Materials

Bacterial cellulose (BC) was prepared according to the procedure in our previous report (Yin et al., 2011). BC gels were washed with running tap water for 24 h to remove any residue on their surfaces and stirred in a 1% (w/v) NaOH solution for 24 h at 70 °C. The BC gels were washed with distilled water until the filtrate was neutral and were then submerged into ethanol overnight to remove any organic impurities. The purified BC gels were dried under vacuum at 80 °C after several washes with distilled water. The dried BC materials were then crushed into a 40 mesh powder and dried under vacuum for 4 h at 115 °C before being stored for future modification. LiCl·H_2O was dried under vacuum at 120 °C over potassium hydroxide. SO_3/Py was purchased from the Aladdin Reagent Co. (China, Mainland) and used as received. MC (Avicel PH-101) was purchased from Sigma–Aldrich and dried under vacuum for 4 h at 115 °C prior to use.

2.2. Preparation of sodium bacterial cellulose sulfate (SBCS)

First, 14.48 g of LiCl and 160 mL of DMAc were measured into a three-neck flask. The mixture was heated at 110 °C until the LiCl dissolved completely. Next, 2 g of BC (12.3 mmol) were measured into the flask, and the mixture was stirred for 5 h at room temperature under nitrogen. The BC dissolved completely after being kept at room temperature (27 °C) for 12 h. Subsequently, 23.56 g of SO_3/Py (148.0 mmol) were added into the BC solution and reacted at 27 °C for 6 h. The product was precipitated in anhydrous ethanol and washed four times with ethanol. The precipitate was separated into two portions, one of which was directly dialyzed against deionized water and subsequently freeze-dried to obtain BC sulfate (BCS), and the other was immersed in 20 mL of 1 mol/L NaOH for 30 min and then dialyzed against deionized water. SBCS was obtained after lyophilization. The products were obtained in yields of 1.12 g (34%) and 1.09 g (30%) for the BCS and the SBCS, respectively. The DS for the SBCS was 1.33. The analyses results of the SBCS are as follows:

IR (KBr): ν = 3420 (OH), 2900 (CH), 1257 (S=O), 1060, 899 (C—O—C), 818 (C—O—S) cm^{-1}.
^{13}C NMR (ppm): δ = 102.3 (C-1), 100.3 (C-1′), 79.7 (C-2,3′), 78.2 (C-4), 73.8 (C-2), 72.7 (C-3), 72.5(C-5), 66.1 (C-6′), 59.9 (C-6).

2.3. Molecular weight (MW) determination of bacterial cellulose (BC)

The molecular weight of the BC was determined using the Mark–Houwink equation, which was described in a previous report (McCormick, Callais, & Hutchinson, 1985). Briefly, 0.50 g BC were dissolved in a 9% DMAc/LiCl solution and diluted to 1×10^{-4}, 2×10^{-4}, 3×10^{-4}, 4×10^{-4}, 5×10^{-4}, and 6×10^{-4} g/mL. The viscosities of the solutions were measured at 30 °C. The intrinsic viscosity of the solution was obtained from the reduced specific viscosity. The MW of the BC was calculated according to the Mark–Houwink equation (1),

$$[\eta] = KM_w^{\alpha}, \quad (1)$$

where η is the intrinsic viscosity of the BC solution and $K = 0.0128 \times 10^{-2}$ cm^3/g; $\alpha = 1.19$.

2.4. Characterization of SBCS

The FTIR spectra were measured using a Bruker TENSOR27 spectrometer with the samples immobilized in KBr and a scanning width ranging 4000–500 cm^{-1}. The ^{13}C NMR spectra of the SBCSs were recorded on a Bruker AV-500 NMR Spectrometer in D_2O at 27 °C. Thermogravimetric analyses were performed using a TA Q600 Thermo Gravimetric Analyzer at 50–800 °C with a heating rate 10 °C/min. Liquid nitrogen was applied to cool the system. The freeze-dried samples were crushed into a fine powder. XRD diagrams were recorded with a Bruker-AXS D8 Advance X-ray Powder Diffraction System in reflection mode. Nickel-filtered Cu-K alpha radiation (λ = 0.15418 nm), which was generated at 40 kV and 40 mA, was collimated using two pinholes of 0.3 mm diameter; the scanning width ranged from 5° to 70°. The degree of crystallinity (CI) was calculated using Eq. (2). I_0 represents the maximum diffraction intensity from the baseline, and I_k is the intensity obtained

by subtracting the base level from I_0 (Zhang, Li, Zhang, & Shi, 1993):

$$\mathrm{CI} = n \times \frac{I_k}{I_0}, \quad n = 0.75. \tag{2}$$

The MW of the water soluble SBCSs were determined using GPC. The samples were dissolved separately in water (5 mg/mL), filtered, and analyzed with the GPC system, which was part of a Waters 515 instrument fitted with two connecting columns (Ultrahydrogel 120 and Ultrahydrogel 500, Waters, USA). The eluent was 0.1 mol/L NaCl aqueous buffer with a flow rate of 0.6 mL/min and a temperature of 40 °C. The measurements were monitored with a Waters 2414 refractive index detector. The GPC system was calibrated before sample analyses with dextran standards (MW: 196 kDa, 43.5 kDa, 9.9 kDa, 1.46 kDa, and 106 Da).

The EA results were obtained with a VarioMicro cube elemental analyzer. The DS values were calculated according to Eq. (3) (Gericke et al., 2009):

$$\mathrm{DS} = \frac{\mathrm{S\%} \cdot 162.1}{3207 - 102.1 \cdot \mathrm{S\%}}, \tag{3}$$

where DS represents the degree of sulfation; S% is the sulfur content in the SBCSs.

3. Results and discussion

3.1. Preparation and solubility of SBCSs

BC gels were modified with SO_3/Py in DMAc/LiCl. The DS values were calculated using the EA results. The effects of three parameters, reaction time, SO_3/Py amount, and temperature, on the DS and solubility of the SBCSs are listed in Table 1. As shown in Table 1, the DS values increase with increasing amounts of SO_3/Py and vary from 0.11 to 1.02 for the SBCSs prepared at room temperature (27 °C) for 2 h (SBCS1, SBCS4, SBCS7, and SBCS10). The reaction time also affected the results. When the amount of SO_3/Py was 1 or 3 mol/mol AGU, the highest SBCS DS was obtained after 2 h. For the reactions with 6 or 12 mol SO_3/Py per mol AGU, SBCS with high DS values were produced after 4 h. Temperature was the most significant factor affecting the SBCS DS values. As listed in Table 1, the DS significantly improved with increasing temperature. The DS values of SBCS9 (27 °C), SBCS13 (40 °C), and SBCS14 (50 °C) were 0.56, 1.23, and 1.50, respectively.

Under homogeneous conditions, the cellulose sulfate polymers showed good solubility in water. The solubilities of the sodium cellulose sulfates and the cellulose sulfates in deionized H_2O are also listed in Table 1, demonstrating that the sodium cellulose sulfates and the cellulose sulfates have a similar solubility in water. The samples with a DS of >0.31 all dissolved in water.

In contrast to the sulfation of cellulose with SO_3/DMF in DMAc/LiCl (Klemm et al., 1998, chap. 4; Wagenknecht et al., 1985), during which the reaction system coagulated, the sulfation of cellulose with SO_3/Py in DMAc/LiCl remained homogeneous during the entire process. MC was sulfated under same conditions as SBCS9 and SBCS12 to prepare sodium microcrystalline cellulose sulfates (SMCS). The DSs of SMCS1 and SMCS2 were lower than the values for SBCS9 and SBCS12. The results were consistent with the results of an earlier acetylation reaction of BC (Tabuchi et al., 1998), indicating that BC has better reactivity than MC. This differences might result from the use of different raw cellulose. Because the dissolved polymer is not molecularly dispersed but present in a colloidal state (Ramos, Morgado, Seoud, Silva, & Frollini, 2011), there is also colloidal aggregates of cellulose in the DMAc/LiCl solvent system (Aono, Tatsumi, & Matsumoto, 2006). BC has finer fibrils than PC. The finer fibrils improve the contact between cellulose, solvent and the reagents, which results in better dissolution and also higher reactivity.

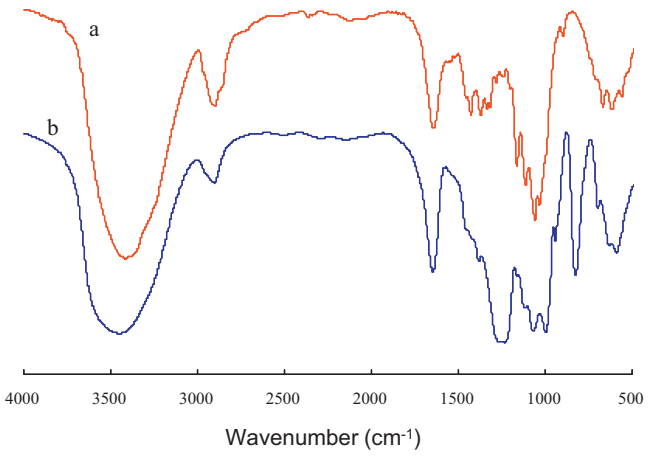

Fig. 1. FTIR spectra of (a) BC and (b) SBCS10 (DS of 1.02).

The direct homogeneous sulfation procedure for MC and spruce sulfite pulp (SSP) in BMIMCl/DMF (Gericke et al., 2009) utilized 3.0 and 1.4 mol/mol of SO_3/Py and AGU, respectively. The DSs of these cellulose sulfates, which were prepared from MC and SSP at 25 °C for 2 h, were 0.83 and 0.81, respectively. The cellulose sulfate prepared from MC in a homogeneous DMAc/LiCl system, which also included 4.5 mol chlorosulfuric per mol AGU at room temperature and a reaction time of 5 h, had a total DS of 1.85 (Zhang, Brendler, et al., 2011). Our current reaction system required more sulfating agent (12 mol sulfating agent per mol AGU) or higher temperature (>40 °C) when compared to the direct homogeneous sulfation of MC and SSP, obtaining a DS of approximately 1. The differences may have resulted from the hydrolysis of SO_3/Py; the high moisture content of the reaction, the hygroscopic nature of the reagents (i.e., BC, SO_3/Py, and DMAc/LiCl), and the ambient high humidity may have caused moisture absorption during the procedure. During the dissolution of BC in DMAc/LiCl at 110 °C, liquid beads were observed on the wall of the flask, which revealed the high moisture content in the reaction system. Significant polymer chain degradation occurred with sulfation, as discussed in Section 3.3, also indicating a high moisture content in the reaction mixtures.

3.2. Characterization of SBCSs

Fig. 1 depicts the FTIR spectra of BC and our SBCSs (a: BC, b: SBCS10 (DS of 1.02)). In the spectrum of BC, the absorbance values at approximately 3420, 2900, 1635, 1370, 1160, and 1060 cm^{-1} are characteristic of cellulose (Heinze et al., 2006; Ivanova, Korolenko, Korolik, & Zhbankov, 1989). The peaks at 3420 and 2900 cm^{-1} correspond to O—H and CH_2 stretches, respectively. The peak at 1060 cm^{-1} is attributed to the C—O—C from the pyranose rings' skeletal vibration, and the absorbance at 899 cm^{-1} is from the β-glucosidic linkages between the AGU. After modification, new peaks appear in the spectra of the SBCS. The peak at 818 cm^{-1} is attributed to the C—O—S stretching vibration t(C—O—S). The peak at 1257 cm^{-1} corresponds to the signal of the asymmetric stretching vibration tas(O=S=O) (Zhang, Brendler, & Fischer, 2010; Zhang, Peschel, Baucker, Groth, & Fischer, 2011). The presence of these peaks indicates the successful sulfation of the BC hydroxyl groups.

The ^{13}C NMR spectra of SBCS9 (a: DS of 0.56) and SBCS12 (b: DS of 1.33) in D_2O are shown in Fig. 2. The chemical shifts of C-1, C-4, C-2, C-3, C-5, and C-6 are located at 102.3, 78.2, 73.8, 72.7, 72.5, and 59.9 ppm, respectively (Zhang, Brendler, et al., 2011). In the SBCS9 spectrum, the signal for C-6, which has a sulfate group, shifts downfield (59.9–66.1 ppm), verifying the substitution at the

Table 1
Reaction conditions, elemental contents, and water solubilities of the cellulose sulfates.

Samples	Reaction conditions			Sulfur content (%)	DS	Solubility[a]	
	SO_3/Py (mol/mol AGU)	Time (h)	Temp. (°C)			SBCS	BCS
SBCS1	1	2	27	1.94	0.11	–	–
SBCS2	1	4	27	1.78	0.10	–	–
SBCS3	1	6	27	1.88	0.10	–	–
SBCS4	3	2	27	2.89	0.16	–	–
SBCS5	3	4	27	2.49	0.14	–	–
SBCS6	3	6	27	2.17	0.12	–	–
SBCS7	6	2	27	5.17	0.31	+	+
SBCS8	6	4	27	8.17	0.56	+	+
SBCS9	6	6	27	8.22	0.56	+	+
SBCS10	12	2	27	12.26	1.02	+	+
SBCS11	12	4	27	14.48	1.36	+	+
SBCS12	12	6	27	14.34	1.33	+	+
SBCS13	6	6	40	13.73	1.23	+	+
SBCS14	6	6	50	15.28	1.50	+	+
SMCS1[b]	6	6	27	7.27	0.48	+	+
SMCS2[b]	12	6	27	12.57	1.06	+	+

[a] Solubility in water: + soluble; –, insoluble.
[b] The solubility was measured for sodium microcrystalline cellulose sulfate and for microcrystalline cellulose sulfate.

C-6 position (Richter & Klemm, 2003; Zhang, Brendler, et al., 2011). In the SBCS12 spectrum, the signal for C-6 at 59.9 ppm disappears, indicating that C-6 has been completely sulfated and that the reactivity of the OH is higher at the C6 position than the other position. Additionally, a new peak, which is assigned to C-1, appears at 100.3 ppm due to the sulfation of the 2-O-position. Based on the integrals of the two peaks for C-1 (102.3 and 100.3 ppm), the partial DS_s at C-2 is 0.30. Another new peak at 79.7 ppm is attributed to C-2 and C-3 with their respective sulfate groups. Considering the total DS and DS_{S2}, the DS_{S3} is 0.03. The reactivity of C—OH prefers C6 > C2 > C3 because the three hydroxyl groups have different steric hindrance amounts (Roy, Semsarilar, Guthrie, & Perrier, 2009). The results are consistent with the data from the direct homogeneous sulfation using $ClSO_3H$/DMF in DMAc/LiCl (Zhang, Brendler, et al., 2011) and with SO_3/Py in BMIMCl (Gericke et al., 2009). In the report of Schlufter (2006), the C—OH reactivity of BC acetylation in BMIMCl was O6 > O3 > O2. Different interactions between the solvents and the hydroxyl moieties might lead to the different functionalization patterns. In the future, more other reactions should be investigated to figure out the principles of C—OH reactivity of BC.

The NMR spectra of the SMCS (c: SMCS1 (DS of 0.48); d: SMCS2 (DS of 1.06)) have similar peak assignments as the SBCS. When compared to the SBCSs prepared under the same conditions (a: SBCS9 (DS of 0.56); b: SBCS12 (DS of 1.33)), the SMCS has a lower DS, which agrees with the EA results.

The XRD patterns of BC and the SBCSs (a: BC; b: SBCS5; c: SBCS8; d: SBCS10; and e: SBCS14) are shown in Fig. 3. The profile of BC is a typical cellulose I XRD pattern, which exhibits high crystallinity (Tokoh, Takabe, Fujita, & Saiki, 1998). The CI of the samples was calculated based on the formula previously used for carboxymethyl cellulose (Zhang et al., 1993). The CI of the BC sample is 69.9%. Three main peaks can be identified at $2\theta = 14.5°$, 17°, and 22.5°, which are assigned, respectively to the $(\bar{1}\,1\,0)$, $(1\,1\,0)$, and $(2\,0\,0)$ reflection planes (Czaja, Romanovicz, & Brown, 2004). After modifying the SBCSs with lower DS values (SBCS5 (DS of 0.14) and SBCS8 (DS of 0.56)), all of the characteristic peaks disappear and a broad new peak at approximately 20° is observed. In the XRD patterns from the SBCSs with higher DS values (SBCS10 (DS of 1.02) and SBCS14 (DS of 1.50)), there are three peaks at

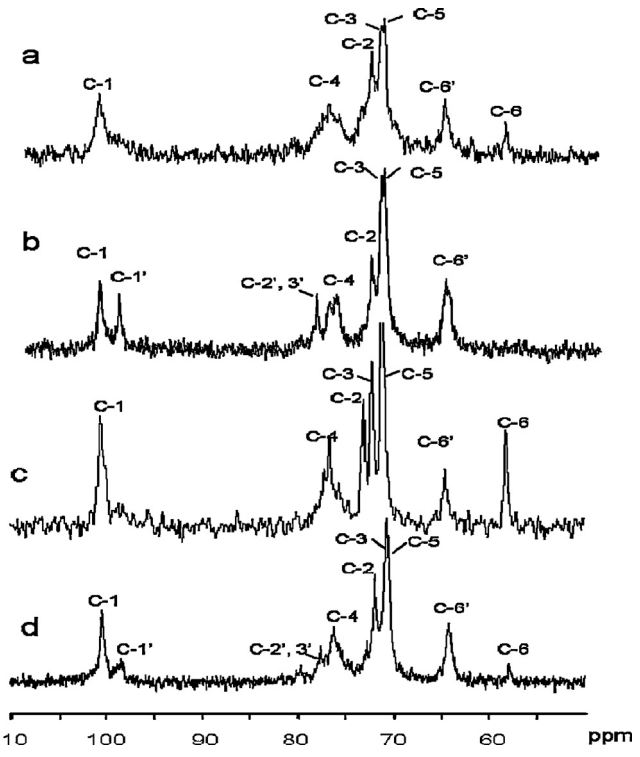

Fig. 2. ^{13}C NMR spectra of the cellulose sulfates: (a) SBCS9 (DS of 0.56), (b) SBCS12 (DS of 1.33), (c) SMCS1 (DS of 0.48), and (d) SMCS2 (DS of 1.06) in D_2O.

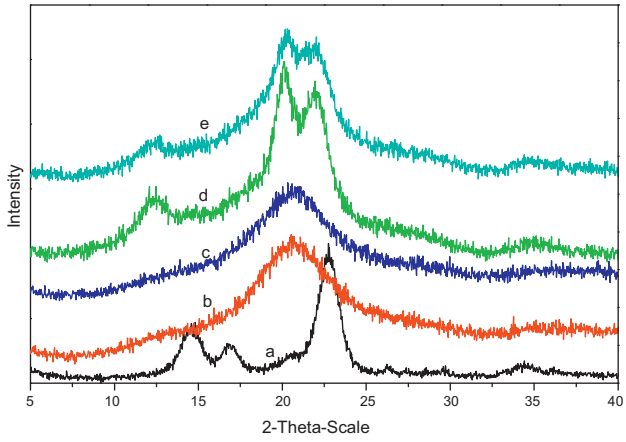

Fig. 3. XRD patterns of BC and the SBCSs: (a) BC, (b) SBCS5, (c) SBCS8, (d) SBCS10, and (e) SBCS14.

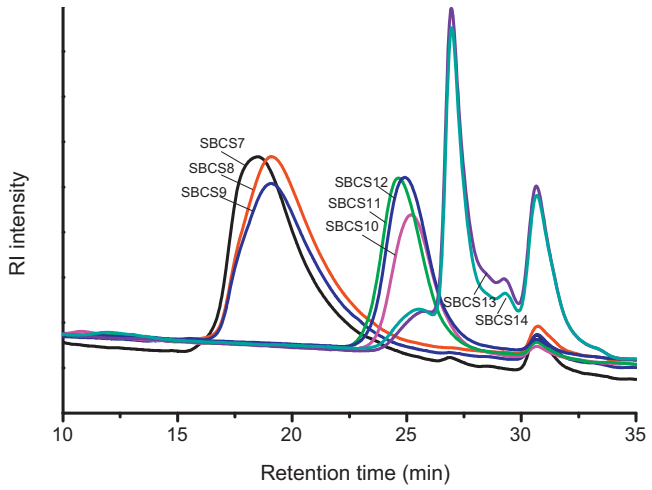

Fig. 4. GPC chromatographs of the water soluble SBCSs.

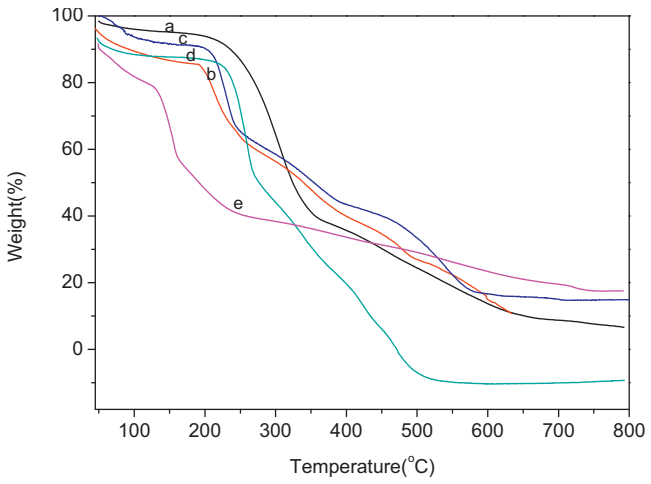

Fig. 5. TG thermograms for (a) BC, (b) SBCS5, (b) SBCS5, (c) SBCS8, (d) SBCS10, and (e) SBCS14.

approximately $2\theta = 12.1°$, 19.8°, 22.0°, which are the characteristic peaks for a cellulose II crystalline structure (Mitsuru, Tadafumi, & Kunio, 2003). These changes indicate that the crystalline regions of the original BC have been destroyed during the dissolution and homogeneous sulfation processes and that the more stable cellulose II polymorph forms over the course of SBCS precipitation and drying. These results are validated with the FT Raman measurements for the sulfation of microcrystalline cellulose (Zhang et al., 2010) and the XRD results for carboxymethyl cellulose (Pratik, 2012; Zhang et al., 1993). The substituent group and MW affect the reorganization of the polymer chains (Zugenmaier, 2008). The SBCSs with low DSs (SBCS5 (DS of 0.14) and SBCS8 (DS of 0.56)) do not possess obvious characteristics of the cellulose II polymorph and present only one broad peak, while the SBCSs with high DSs (SBCS10 (DS of 1.02) and SBCS14 (DS of 1.50)) express the pattern of a typical cellulose II polymorph. The CIs of SBCS10 and SBCS14 are 52.3% and 48.2%, respectively.

3.3. Molecular weight (MW) of water soluble SBCSs

The MWs of the water soluble SBCSs were measured via GPC, which used 0.1 mol/L NaCl as the eluent. Fig. 4 depicts the GPC chromatograms for the SBCSs, and Table 2 lists the DP_w and DP_n values of the samples. According to the Mark–Houwink equation, the DP of the raw BC is 2860.

In Fig. 4, one broad peak for SBCS7 (DS of 0.32), SBCS8 (DS of 0.56), and SBCS9 (DS of 0.57) are observed, and their respective DP_ns are 666, 480, and 507. The polydispersity (PDI) of SBCS7–SBCS9 is in the range of 2.20–2.38. These results indicate that some depolymerization occurs during sulfation and that longer reaction times generally result in more depolymerization. The depolymerization becomes more severe as the amount of SO_3/Py increases. The DP_n values for SBCS9 and SBCS12 decrease from 507 to 21 when the amount of SO_3/Py increases from 6 to 12 mol/mol AGU. Temperature also strongly affects the extent of depolymerization. As listed in Table 2, the DP_n values (7 for both samples) for SBCS13 (40 °C) and SBCS14 (50 °C) decrease when compared with SBCS9, which was prepared at 27 °C. In addition, the PDI falls in the range of 1.30–1.32 for SBCS10–SBCS14. MC also degraded during sulfation. The measured DP of MC is 380. The DP_n values are158 and 77 for SMCS1 and SMCS2, respectively. Compared with BC, the depolymerization of MC was not as severe. BC shows better reactivity, not only during sulfation but also during depolymerization because of its finer fibril morphology.

Table 2
MW distribution of the water soluble cellulose sulfates determined via GPC.

Samples	Mn (kDa)	MW (kDa)	DP_n	DP_w	DPI*
SBCS7	129.6	308.0	666	1582	2.38
SBCS8	105.1	240.0	480	1095	2.28
SBCS9	111.6	245.4	507	1115	2.20
SBCS10	5.8	7.5	21	27	1.30
SBCS11	7.5	9.9	24	31	1.32
SBCS12	6.6	8.6	21	27	1.31
SBCS13	2.0	2.7	7	9	1.31
SBCS14	2.2	2.9	7	9	1.31
SMCS1	34.8	101.4	158	460	2.91
SMCS2	20.8	73.5	77	272	3.53

* $DPI = DP_w/DP_n$.

The depolymerization of cellulose during sulfation occurs regardless of whether the reaction occurs directly or via intermediates; the result also commences whether the reaction proceeds homogeneously, quasi-homogeneously, or heterogeneously (Klemm et al., 1998, chap. 4; Richter & Klemm, 2003; Saake, Puls, & Wagenknecht, 2002; Zhang, Peschel, et al., 2011). The homogeneous sulfation of cellulose with SO_3/Py in DMAc/LiCl results in more severe depolymerization than in earlier reports. In the present system, much more sulfating agent is required to achieve high DS values, which also results in severe acidic degradation of the polymer chain (Gericke et al., 2009; Zhang, Peschel, et al., 2011). BC has a high MW, which is advantageous for the preparation of cellulose sulfate for polyelectrolyte complex formation. However, due to the severe depolymerization, the sulfation of BC with SO_3/Py in DMAc/LiCl is only practical for the preparation of SBCS with DSs of <0.6, which is still appropriate for polyelectrolyte complex preparation. In addition, solvent recycling remains a problem for the reactions in DMAc/LiCl. Sulfation of plant cellulose in ionic liquid/co-solvent is an important technique that offers many advantages, such as high efficiency and little polymer chain degradation (Gericke et al., 2009). To maintain BC's high MW, the sulfation of BC in ionic liquid/co-solvents might be considered in future research.

3.4. Thermal stability of SBCSs

The thermal stabilities of the SBCSs and BC with different DS values were measured using TG. The TG thermograms are shown in Fig. 5, and the thermal decomposition data are listed in Table 3. As shown in Fig. 5, the thermal stability of BC changed after sulfation and varied depending on the DS of the SBCs. The raw BC decomposed at 305 °C, while the decomposition of SBCSs was initiated at

Table 3
Decomposition temperatures of the BC and SBCSs.

Samples	DS	Decomposition temperatures (°C)			Weight loss (%)		
		T_1	T_2	T_3	I	II	III
BC		305	375		61.0	27.0	
SBCS5	0.14	213	350	478	27.2	20.5	12.2
SBCS8	0.56	231	359	540	32.1	16.6	26.6
SBCS10	1.02	260	334	426	42.8	22.0	16.1
SBCS14	1.50	155	194	500	28.0	13.0	21.4

155–260 °C and increased with their DSs. Three stages of decomposition occurred for the SBCSs at different temperatures (T_1, T_2, and T_3). The weight loss is highest in the first stage, which was due to the dehydration of glucose. Further decomposition, which was due to the release of volatile compounds, occurred at 194–359 °C. The continuous weight loss at approximately 426–540 °C was due to the degradation of the remaining residue. Crystallinity, crystal size, MW, and DS all impacted the thermal stability of cellulose and its derivatives (Tosh, 2011). In Fig. 2, the crystallinity of SBCS5, SBCS8, and SBCS10 increases with the DS, and the SBCSs with higher DSs have a higher T_1 in Table 3. Additionally, the cellulose degraded during sulfation. The MW affects both the CI and thermal stability. The DP_n values of SBCS10 and SBCS14 are 21 and 7, and the CIs are 52.3% and 48.2%, respectively. Compared to SBCS10, SBCS14 has lower thermal stability because of its lower CI and MW.

4. Conclusion

SBCSs with DS values that range from 0.10 to 1.50 were prepared through direct homogeneous sulfation of BC with SO_3/Py in DMAc/LiCl. The sulfation in this system revealed a regioselective preference for C6 > C2 > C3. The SBCSs with high DSs had a cellulose II analog polymorphous structure. The homogeneous sulfation of cellulose with SO_3/Py in DMAc/LiCl resulted in severe depolymerization due to the high moisture content of the reaction mixture. BC had higher reactivity than MC with regard to both sulfation and the concurrent depolymerization.

SBCS may be used to prepare polyelectrolyte complexes, which can be used in drug delivery or bioencapsulation systems. However, the present method is unsuitable for preparing SBCS with DS values of >0.6 because SBCSs with high DS values undergo extensive degradation; thus, they do not retain the high molecular weight necessary to form polyelectrolyte complexes. BC has the advantages of high purity and high DP. Sulfation of BC has not been reported until now. Other methods for sulfating cellulose, such as sulfation in ILs, acetosulfation, and sulfation via trimethylsilylation can be studied. These techniques could be used to prepare BC sulfates with high viscosity and regioselective substitution, which would help develop further applications of BC. Finally, the moisture content of the reaction system should be controlled to slow the depolymerization side process during the sulfation procedure.

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