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Synthesis and characterisation of cellulose sulfates regarding the degrees of substitution, degrees of polymerisation and morphology

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ABSTRACT

The synthesis and characterisation of cellulose sulfates were reported. Various cellulose sulfates with diverse degrees of substitution ascribed to sulfate groups (DS_S) between 0.21 and 2.59 were prepared through acetosulfation or direct sulfation of two celluloses. The number-average degrees of polymerisation (DP_n) of these cellulose sulfates were determined to be in the range of 59 and 232 via size exclusion chromatography (SEC). Accordingly, the molecular weight of cellulose was remarkably decreased during the sulfation. The use of high amount of sulfating agent and high sulfation temperature led to stronger reduction of the DP_n in comparison to low amount of sulfating agent and low temperature. The morphology of cellulose sulfate was analysed via scanning electron microscopy (SEM) and wide-angle X-ray diffraction (WAXD). Obtained cellulose sulfates demonstrated different surface properties from cellulose and became more amorphous than starting celluloses.

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1. Introduction

Cellulose sulfate is a half-ester of cellulose and has advantageous properties in comparison to cellulose, e.g. cellulose sulfates with a wide range of degrees of substitution ascribed to sulfate groups (DS_S) are water-soluble and proved to possess antivirus, anticoagulant and antibacterial properties (Groth & Wagenknecht, 2001; Schwarz-Albiez et al., 2007; Wang, Li, Zheng, Normakhamatov, & Guo, 2007; Yamamoto et al., 1991). Cellulose sulfates having high DS_S can promote the proliferation of 3T3 (3-day transfer, inoculum 3×10^5 cells) mouse fibroblasts in the presence or absence of fibroblast growth factor 2 (Peschel et al., 2010). Moreover, cellulose sulfate is a polyelectrolyte and can be used for the encapsulation of enzymes and cells (Dautzenberg, Schuldt, Lerche, Woehlecke, & Ehwald, 1999; Stadlbauer et al., 2006; Zhang, Yao, & Guan, 2005). For this purpose, cellulose sulfates with a DS_S between 0.3 and 0.8 and adequate viscosities are suitable.

Cellulose sulfate can be prepared heterogeneously in isopropyl alcohol with sulphuric acid or homogeneously with SO_3–pyridine complex in ionic liquids. Heterogeneous sulfation led to water-soluble cellulose sulfates with a total DS_S of about 0.7, but significant chain degradation was also observed (Lukanoff & Dautzenberg, 1994). Otherwise, homogeneous sulfation of cellulose resulted in water-soluble cellulose sulfates with only minor cellulose degradation (Gericke, Liebert, & Heinze, 2009; Saake, Puls, & Wagenknecht, 2002).

Furthermore, cellulose sulfate can also be synthesized quasi-homogeneously through direct sulfation or acetosulfation, which means that the suspension of cellulose turns into optically transparent solution during the reaction and the cellulose is dissolved in the reaction mixture. Water-soluble cellulose sulfates exhibiting diverse total DS_S and sulfation patterns can be prepared. The primary hydroxyl groups were found to be preferably sulfated during both sulfation processes (Hettrich, Wagenknecht, Volkert, & Fischer, 2008; Zhang, Peschel, Brendler, Groth, & Fischer, 2009). However, it is still not clear how the cellulose chains were altered during the quasi-homogeneous sulfation.

Besides the total DS_S, the molecular weight of sulfated polysaccharides is proposed to be another important feature for their biological activities, such as anticoagulant activity (Barbucci, Lamponi, Magnani, & Renier, 1998; Chaidedgumjorn et al., 2002; Rezaie, 2007; Yang, Du, Huang, Wan, & Li, 2002). It was shown that sulfated lacquer polysaccharides that are obtained from Lac tree (*Rhus vernicifera*) with high total DS_S and high molecular weights demonstrated good anticoagulant activities (Yang et al., 2002). Moreover, cellulose sulfate with high molecular weights displayed microbicidal activity against papillomavirus (Christensen et al., 2001).

Thus, in this report, various cellulose sulfates were prepared at first by acetosulfating and directly sulfating two celluloses having diverse degrees of polymerisation (*DP*). The DS_S, the *DP* and the morphology of prepared cellulose sulfates were analysed. The

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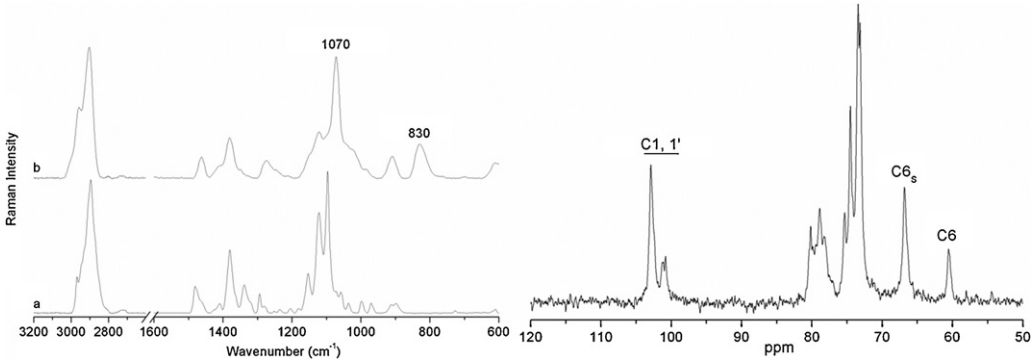

Fig. 1. Left: FT Raman spectra (3200–600 cm^{-1}) of: (a) cellulose and (b) CS4 (DS_S = 0.92) at RT. Right: ^{13}C NMR (120–50 ppm) of CS9 (DS_S = 0.97) in D_2O at RT.

effects of the sulfation parameters on the number-average degrees of polymerisation (DP_n) of cellulose sulfates were studied. The change in the morphology of cellulose after the sulfation was demonstrated.

2. Experimental

2.1. Materials

Microcrystalline cellulose (MCC) received from J. Rettenmaier & Söhne GmbH (Rosenberg, Germany) has an average Cuen-*DP* of 276 according to the manufacturer. Pulp V-81 (with 97.0% alpha cellulose, AC) containing 1.0% pentosans and 0.1% ash with a viscosity (in 0.5% cupri-diethylenediamine) of 7.2 cP and an average *DP* of 1180 was purchased from Buckeye Technologies Inc. (Memphis, USA). *N*,*N*-dimethylformamide (DMF) was freshly distilled and demineralised water was used in all experiments. Other chemicals were all of analysis grade and used as received. Dialysis membrane from Spectrum Laboratories Inc. (Rancho Dominquez, USA) has an approximate molecular weight cut off of 500 Da.

2.2. Sulfation of cellulose

The acetosulfation or direct sulfation of cellulose was carried out as described before (Zhang, Brendler, & Fischer, 2010).

Briefly, 1 g cellulose was suspended in 50 ml DMF for subsequent sulfation. The sulfating reagent, chlorosulfonic acid in DMF or the mixture of chlorosulfonic acid and acetic anhydride in DMF was dropped into the cellulose suspension. After being kept at 50 °C for 5 h, the solution was cooled down to RT and poured into a saturated ethanolic solution of anhydrous sodium acetate. Next to that, the resulting precipitate was collected through centrifugation. During the acetosulfation, deacetylation of the product using 1 M ethanolic solution of sodium hydroxide with subsequent washing was carried out and the deacetylated product was collected through centrifugation. Collected product was then washed with 4% sodium acetate solution in ethanol and dissolved in water. The pH value of the solution was adjusted to 8.0 with acetic acid/ethanol (50/50, w/w) and the solution was filtered, dialyzed against demineralised water and lyophilized.

2.3. Measurements

The contents of carbon, hydrogen and nitrogen were determined with Elemental Analyser vario EL from Elementar (Hanau, Germany). The content of sulphur was measured with Elemental Analyser Eltra CS 500 (Neuss, Germany). Total DS_S was calculated according to the equation: total DS_S = (S%/32)/(C%/72).

The ^{13}C NMR spectra were recorded at RT on Bruker DFX 400 spectrometer (Bruker, Etlingen, Germany) with a frequency of 100.13 MHz, 30° pulse length, 0.35 acq. time and a relaxation delay of 3 s. The scans of up to 20,000 were accumulated and D_2O was used for dissolving cellulose sulfates.

FT Raman spectra were recorded on a Bruker MultiRam spectrometer (Bruker) Ge diode as detector that is cooled with liquid-nitrogen. A cw-Nd:YAG-laser with an exciting line of 1064 nm was applied as light source for the excitation of Raman scattering. The spectra were recorded over a range of 3500–150 cm^{-1} using an operating spectral resolution of 3 cm^{-1} and a laser power output of 100 mW.

Molecular weights of cellulose sulfates in the form of mass and number-average degrees of polymerisation (DP_w and DP_n) are measured by size exclusion chromatography (SEC) with PSS Suprema 3000 and 100 Å columns (Polymer Standards Service GmbH, Mainz, Germany). The detection was carried out with a Waters 410 reflective index (RI) detector (Waters Corporation, Milford, MA, USA) and 0.1 mol/l NaCl aqueous buffer was used as mobile phase. The columns were calibrated with pullulan standards (Sigma–Aldrich, Buchs, Switzerland). Empower Pro software (Waters Corporation) was used for the analysis.

Scanning electron microscopy (SEM) images were obtained on a JEOL JSM-T330A scanning microscope (Jeol Ltd, Tokyo, Japan) at RT. Samples were coated with a 30 nm thick carbon and gold layer.

Wide-angle X-ray diffraction (WAXD) was carried out on a XRD 3003T/T X-ray diffractometer (GE Inspection Technologies, USA) with Cu Kα radiation from 6° to 36° using θ–θ total reflection technique.

3. Results and discussion

3.1. Sulfation of cellulose

All cellulose sulfates besides CS6 were prepared quasi-homogeneously and the CS6 was prepared heterogeneously because of using a very low amount of chlorosulfonic acid of 0.3 mol per mol anhydroglucose units (AGU) at 50 °C. Besides, CS10 was synthesized quasi-homogeneously with 0.3 mol chlorosulfonic acid per mol AGU, but at higher sulfation temperature of 70 °C. Comparing CS6 and CS10, high sulfation temperature of 70 °C facilitated the dissolution of cellulose during the acetosulfation, although the total DS_S was reduced at high temperature (Zhang et al., 2009). Both cellulose sulfates are water-soluble and exhibit the lowest DS_S of 0.24 and 0.21, respectively.

The introduction of sulfate groups into cellulose chains can be confirmed by FT Raman spectroscopy. As shown within the Raman spectrum of CS4 and cellulose (Fig. 1), the presence of new peaks at 1070 and 830 cm^{-1} is notable. These peaks are ascribed to symmetric stretching vibrations of O=S=O groups and stretching vibrations of C–O–S bonds (Cabassi, Casu, & Perlin, 1978; Zhang et al., 2010).

The presence of these peaks indicates successful sulfation of the hydroxyl groups of cellulose.

The DS_S of cellulose sulfates can be determined via elemental analysis or ^{13}C NMR spectroscopy (Table 1). Fig. 1 depicts the ^{13}C NMR spectrum of CS9. Within the ^{13}C NMR spectrum, $C6_S$ has a peak at 66.6 ppm after the sulfation of primary hydroxyl groups and the peak at 60.6 ppm ascribed to C6 without sulfate groups at 6-*O*-position is also visible. With sulfate groups at 2-*O*-position, the peak of C1 shifted downfield and was split into peaks attributed to C1 and C1′. The peak located at 103 ppm is derived from C1 and 100.8 ppm from C1′, respectively.

Based on the integrals of C6/$C6_S$ and C1/C1′, the partial DS_S at 6- and 2-*O*-position can be determined. The total DS_S of cellulose sulfate without sulfation at 3-*O*-position is equal to the sum of the partial DS_S (Zhang et al., 2009). The total DS_S of cellulose sulfate with sulfation at 3-*O*-position can be estimated via elemental analysis. In Table 1, the total DS_S of obtained cellulose sulfates are listed. According to the data, cellulose sulfates with various total DS_S were prepared and the total DS_S can be regulated by varying the sulfation conditions, e.g. the amount of the sulfating agent and sulfation temperature. Generally, the total DS_S increases with rising amount of chlorosulfonic acid, but decreases with higher sulfation temperature during both acetosulfation and direct sulfation.

3.2. DP of cellulose sulfates

Besides the DS_S, the *DP* value of sulfated polysaccharides is another important feature influencing their properties (Barbucci et al., 1998; Rezaie, 2007; Yang et al., 2002. Fig. 2 depicts SEC chromatograms of a few cellulose sulfates prepared from alpha cellulose (AC) and MCC through acetosulfation or direct sulfation. Table 1 lists the DP_w and DP_n of prepared cellulose sulfates. According to the data, cellulose was significantly degraded during acetosulfation and direct sulfation.

After the acetosulfation of AC having an average *DP* of 1180, obtained cellulose sulfates have only DP_n values between 107 and 232 (Fig. 2(a)). In the same way, cellulose sulfates from MCC having an average *DP* of 276 exhibited the DP_n in the range of 148–182 (Fig. 2(b)). The use of higher amount of chlorosulfonic acid resulted in stronger depolymerisation of cellulose. For instance, the DP_n values of CS3 and CS4 were lowered from 184 to 121 with an increase of chlorosulfonic acid from 0.85 to 3 mol per mol AGU. Moreover, higher reaction temperature led to stronger reduction of the DP_n comparing CS4 and 5 or CS6 and 10 (Table 1).

During direct sulfation, cellulose chains were also strongly degraded (Fig. 2(c)). According to Table 1, the DP_n values of CS13-16 are smaller than those of cellulose sulfates prepared through acetosulfation, although the direct sulfation was carried out at RT with a shorter duration. The reason may be the application of higher amount of chlorosulfonic acid which reduces the cellulose chains severely.

It has been demonstrated that cellulose was remarkably depolymerised during the sulfation using chlorosulfonic acid in DMF (Wang et al., 2007). Even under homogeneous conditions in N_2O_4-DMF solution, a strong decrease in the molecular weight of cellulose was observed (Philipp, Nehls, Wagenknecht, & Schnabelrauch, 1987). Severe reduction of the *DP* was also found during other sulfation processes, such as heterogeneous sulfation of cellulose (Saake et al., 2002).

In accordance with these findings, significant depolymerisation of cellulose during the acetosulfation or direct sulfation was visible in present report. The *DP* values of both AC and MCC were decreased to the range of 100–200 after the acetosulfation. The direct sulfation reduced the *DP* more strongly, although both sulfation processes were quasi-homogeneous. Moreover, the polydispersity – DP_w/DP_n – lies between 1.21 and 3.11, indicating wide distributions of the molecular weights of prepared cellulose sulfates (Fig. 2).

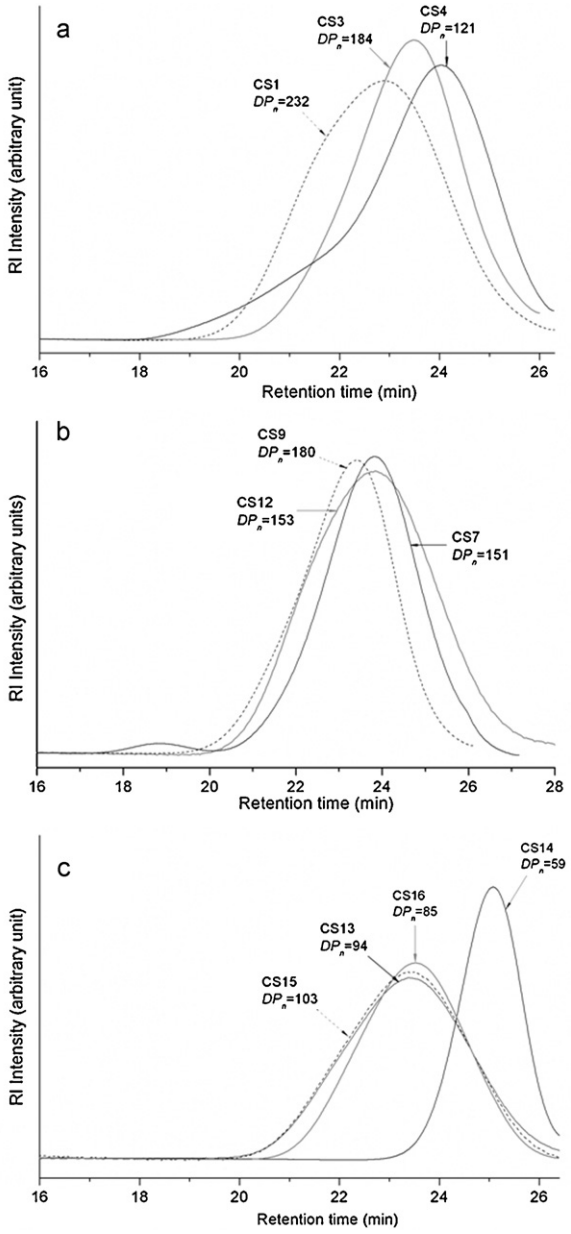

Fig. 2. SEC chromatograms of: (a) cellulose sulfates from AC by acetosulfation, (b) cellulose sulfates from MCC by acetosulfation and (c) cellulose sulfates from MCC by direct sulfation.

3.3. SEM

Besides the modification of cellulose on the molecular level, the surface property of cellulose was analysed after its sulfation.

After lyophilisation, cellulose sulfates formed optically white films or small aggregates. Fig. 3 displays the SEM images of cellulose and cellulose sulfates networks in two distinct magnifications. In the left row, the overall shapes of the cellulose and cellulose sulfates were illustrated. The right row depicts the same cellulose and cellulose sulfates in smaller scales to visualise their surfaces in detail. As shown in Fig. 3(a), MCC consists of microfibrils which are about 25 μm thick and in different lengths. Some aggregates of these microfibrils are also notable. After the sulfation, cellulose sulfates show completely different morphologies from cellulose as

Table 1
Synthesis and characterisation of cellulose sulfates.

Samples[a]	Starting materials	Molar ratio[b]	Reaction temperature (°C)	Total DS_S[c]	DP_w	DP_n	DP_w/DP_n
CS1	AC	0.85	40	0.66	597	232	2.57
CS2	AC	0.55	50	0.38	336	182	1.85
CS3	AC	0.85	50	0.53	361	184	1.96
CS4	AC	3	50	0.92	372	121	3.07
CS5	AC	1.5	70	0.48	177	107	1.66
CS6	MCC	0.3	50	0.24	567	182	3.11
CS7	MCC	0.55	50	0.43	281	151	1.86
CS8	MCC	0.85	50	0.47	424	173	2.45
CS9	MCC	3	50	0.97	365	180	2.03
CS10	MCC	0.3	70	0.21	286	148	1.93
CS11	MCC	0.7	70	0.34	423	151	2.80
CS12	MCC	0.85	70	0.41	382	153	2.50
CS13	MCC	4.5	RT	2.15	188	94	2.01
CS14	MCC	4.5	50	0.97	72	59	1.21
CS15	MCC	6	RT	1.99	212	103	2.05
CS16	MCC	13	RT	2.59	152	85	1.79

[a] CS1-12 were prepared after 5 h sulfation and CS13, 14, 15 and 16 were prepared after sulfation for 6, 3, 3 and 2.5 h.
[b] Molar ratio in mol chlorosulfonic acid per mol AGU. CS1-12 were prepared through acetosulfation and CS13-16 were prepared through direct sulfation.
[c] The total DS_S of CS1-12 were determined via ^{13}C NMR and the total DS_S of CS13-16 via elemental analysis.

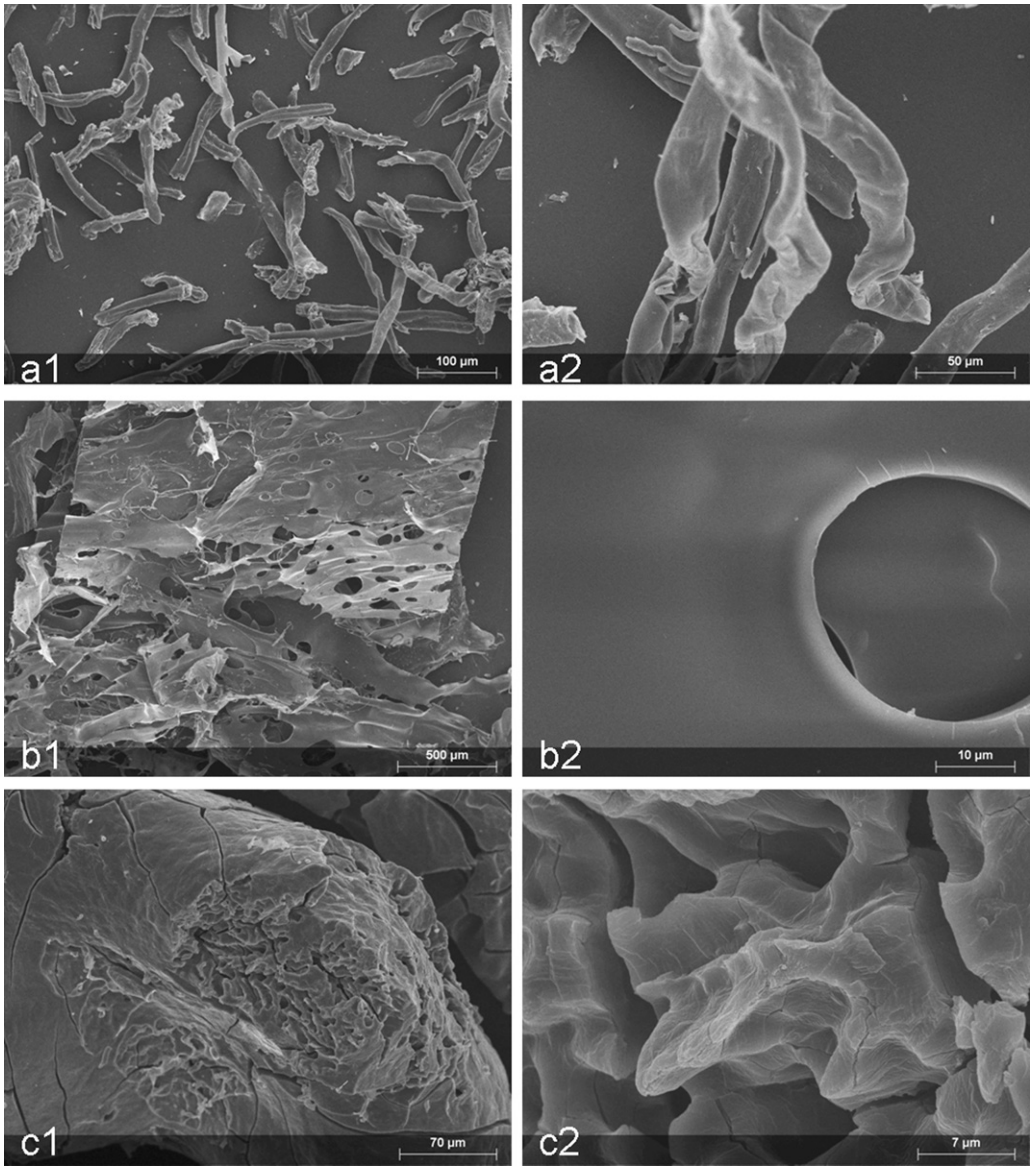

Fig. 3. SEM images of: (a) cellulose (scale bar: 100 µm in a1; 50 µm in a2), (b) CS3 (DS_S = 0.53) (scale bar: 500 µm in b1; 10 µm in b2) and (c) CS16 (DS_S = 2.59) (scale bar: 70 µm in c1; 7 µm in c2).

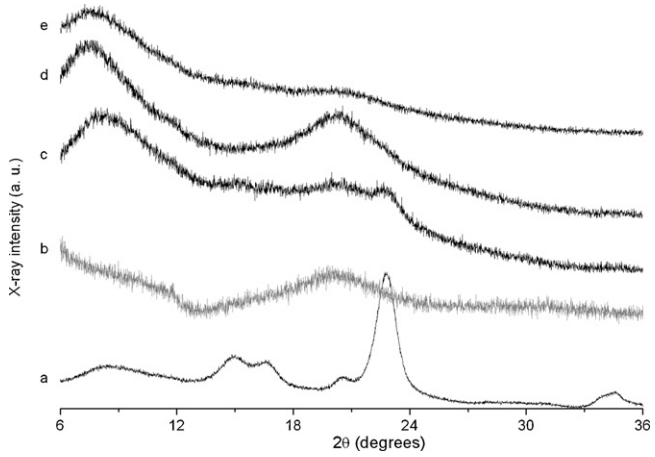

Fig. 4. WAXD diffractograms (6–36°) of: (a) MCC, (b) CS3 (DS_S = 0.53), (c) CS6 (DS_S = 0.24), (d) CS7 (DS_S = 0.43) and (e) CS12 (DS_S = 0.41) at RT.

demonstrated in Fig. 3(b and c). Cellulose sulfates have compact structures after drying and no microfibrillar structure is observable. Besides, the surface can be very smooth and porous. The pores in the films have diameters between 40 and 200 μm (Fig. 2(b)) and the cavities in the aggregates have opening diameters of around 7 μm.

As described before for bacterial cellulose, its derivatives displayed different microscopic morphologies from cellulose and they exhibited a compact structure (Barud et al., 2008; Svensson et al., 2005). Besides, microporous scaffolds have been made from bacterial cellulose with pore sizes of 300–500 μm, which can be used for bone regeneration (Zaborowska et al., 2010).

Because the compact structure of cellulose sulfate is formed after the lyophilisation of the aqueous cellulose sulfate solution, the polysaccharide chains of cellulose sulfate may bind with each other during the loss of water, forming a homogeneous product after drying.

3.4. WAXD

After the sulfation of cellulose, the crystallinity of cellulose sulfates should be different from that of cellulose. AC and MCC used in this report have crystallinities of 50.4% and 67.1% that were determined as described in (Schenzel, Fischer, & Brendler, 2005). As depicted in Fig. 4, a strong peak within the WAXD diffractogram of MCC is visible at $2\theta = 22.8°$, which is attributed to the crystalline regions of cellulose. After the sulfation, cellulose sulfates besides CS6 do not show this peak and appear to be amorphous. Within the WAXD diffractogram of CS6, a small signal at $2\theta = 22.8°$ is still visible, indicating the presence of crystalline regions within CS6.

This difference between the crystallinities of CS6 and other cellulose sulfates may lie in distinct sulfation processes. During the quasi-homogeneous sulfation, cellulose was completely dissolved in the reaction system and the intramolecular hydrogen bonds within cellulose should be destroyed, leading to decrease or disappearance of the crystalline regions. After subsequent precipitation, cellulose sulfate with an amorphous structure was obtained. It has been demonstrated that cellulose exhibited amorphous structure after the regeneration from its solution (Fischer, Voigt, & Fischer, 1999; Hameed & Guo, 2010). However, CS6 was prepared with very low amount of chlorosulfonic acid under heterogeneous conditions and a part of cellulose was possibly not dissolved during the sulfation. Subsequently, a part of crystalline regions remained and can be detected via WAXD.

4. Conclusion

Various cellulose sulfates with distinct total DS_S were synthesized from two celluloses exhibiting different *DP* values. The total DS_S were determined to be in the range of 0.21 and 2.59. The *DP* value of cellulose was strongly reduced after the sulfation and the DP_n values of cellulose sulfates were determined to be between 59 and 232. The use of higher amount of sulfating agent and/or higher sulfation temperature led to more severe decrease of the DP_n. Obtained cellulose sulfates have the polydispersities between 1.21 and 3.11.

The morphology of cellulose was changed completely after the sulfation. Lyophilized cellulose sulfates in films or aggregates demonstrated compact structures. Furthermore, the crystalline regions of cellulose were destroyed completely during the quasi-homogeneous sulfation, while part of them remained after the heterogeneous sulfation according to the WAXD analysis.

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