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Quantification of cellulose nanowhiskers sulfate esterification levels

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ABSTRACT

Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, combustion gas analysis and N_2 adsorption were used to quantify the degree of desulfation of cellulose nanowhiskers (CNWs). CNWs were produced by hydrolyzing cotton cellulose with sulfuric acid or hydrochloric acid. Hydrochloric acid treatment did not result in any cellulose chemical modification. Hydrolysis using H_2SO_4 introduced sulfate groups onto the cellulose surface. Our results indicate that commercial cotton cellulose as received contained sulfur. The sulfur content of H_2SO_4-prepared CNWs was higher than that exhibited by the original cellulose due to the esterification process. Two desulfation methods, acid-catalyzed and solvolytic desulfation, have been explored to remove the sulfate groups. Neither desulfation method examined removed the sulfate groups from H_2SO_4-prepared CNWs completely. An estimation of surface sulfate esterification levels was made based on a model of the cellulose structure and available surface area of CNWs. According to these models, more than one third of hydroxyl groups on the surface were substituted by sulfate.

1. Introduction

Acid hydrolysis of cellulose is an effective method to remove the amorphous regions of cellulose and obtain highly crystalline cellulose nanowhiskers (CNWs). Sulfuric acid and hydrochloric acid were widely used to produce CNWs from various sources as early as the late 1940s (Marchess, Morehead, & Koch, 1961; Marchessault, Morehead, & Walter, 1959; Nickerson & Habrle, 1947). These CNWs are usually elongated rods with high aspect ratio. Depending on the source of cellulose and hydrolysis conditions, they are several hundred nanometers to a few micrometers long, and 5–30 nm wide (Elazzouzi-Hafraoui et al., 2008).

CNWs purified from plant or other sources are typically cellulose I. They are outstanding fillers for nanocomposites for their renewable nature and mechanical properties (Hubbe, Rojas, Lucia, & Sain, 2006; Moon, Martini, Nairn, Simonsen, & Youngblood, 2011; Samir, Alloin, & Dufresne, 2005). They are also excellent model materials useful for studying the interaction of cellulose with other compounds such as proteins, polysaccharides and other biopolymers. Thus an understanding of the surface chemistry of the CNW resulting from their production is necessary.

Sulfuric acid prepared CNWs have negatively charged surfaces due to the esterification of hydroxyl groups by sulfate ions. The esterification levels of CNWs highly depend on hydrolysis time and acid concentrations (Dong, Revol, & Gray, 1998). It has been shown that the negative surface charge leads to more stable nanowhisker aqueous suspensions (Araki, Wada, Kuga, & Okano, 1998). Hydrochloric acid prepared CNWs have barely detectable charge and no known surface modification (Araki et al., 1998). Degradation of cellulose by sulfuric acid is more widely used due to more uniform particle dimensions (Braun, Dorgan, & Chandler, 2008) and more stable suspensions. However, H_2SO_4-prepared CNWs may exhibit negative properties for some applications. The introduction of sulfate groups results in lower degradation temperatures, which may limit implementation in thermoplastic composites (Roman & Winter, 2004). The sulfate groups on CNW surface may also affect its application as model substrates to study the interaction between cellulose and enzymes such as cellulases, and polymers including plant cell wall polysaccharides (Kittle et al., 2011; Lopez et al., 2010).

The objectives of this work were to further understand the levels of esterification on the cellulose surfaces and to develop a highly uniform sulfate free model cellulose nano-material. Both sulfuric acid and hydrochloric acid were used to produce CNWs. Two methods were used to desulfate the CNWs initially prepared using sulfuric acid. The first method was to desulfate the H_2SO_4-prepared CNWs with low concentration of acid. It has been reported that a low concentration of hydrochloric acid was able to catalyze the

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desulfation of a polysaccharide (Pomin, Valente, Pereira, & Mourao, 2005). Solvolytic desulfation was used as another way to desulfate the CNWs. The pyridinium salts of the sulfated polysaccharides were heated in dimethylsulfoxide with a small amount of methanol (Lee, Hou, Hayashi, & Hayashi, 2007; Nagasawa, Inoue, & Kamata, 1977; Nagasawa, Inoue, & Tokuyasu, 1979). These two methods were found to reduce the sulfate esterification levels in softwood CNWs in a recent study (Jiang, Esker, & Roman, 2010).

Different techniques have been explored to quantify the sulfate esterification levels of cellulose. Sulfur elemental determination by combustion gas analysis (CGA) is one of the oldest techniques used to assess the amount of sulfur (Stragand & Safford, 1949). Conductometric titration has also been used to measure the sulfate content based on the negative surface charge of CNWs (Araki et al., 1998; Bondeson, Mathew, & Oksman, 2006; Dong et al., 1998; Jiang et al., 2010). It is not, however, a direct measurement of the sulfur content. The results of elemental analysis are often higher than that from conductometric titration (Araki et al., 1998; Dong et al., 1998). Araki, Wada, Kuga, and Okano (1999) pointed out that there may be sulfate groups inaccessible to titrant.

In this report we used three techniques, Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and combustion gas elemental analysis (CGA) to determine the sulfate content of CNWs. The surface sulfate esterification levels of CNWs have been estimated based on N_2 adsorption measurements of the surface area, and a model of the cellulose crystal based on X-ray diffraction studies.

2. Materials and methods

2.1. Materials

The sulfur content of three raw cellulose materials was evaluated. Only cellulose from cotton (Whatman™ CF11) was used to prepare cellulose nanowhiskers. Avicel PH101 microcrystalline cellulose, extracted from wood pulp, was purchased from Sigma–Aldrich. Bacterial cellulose was from *Gluconacetobacter xylinus* JCM 9730 (ATCC 700178, provided by the Bioresource Center of American Type Culture Collection). It was cultured in a static HS medium (Hestrin & Schramm, 1954) (pH = 5, 30 °C) in 250 mL Erlenmeyer glass flask containing 100 mL medium. In order to remove the bacterial cells, the bacterial cellulose pellicles were treated with 0.1 M NaOH at 80 °C for 1 h and repeated once. Subsequently, the pellicles were rinsed with deionized (DI) water until the pH reached neutral and freeze dried. All the reagents were A.C.S. grades.

2.2. Preparation of CNWs

The method described by Bondeson et al. (2006) was used to prepare H_2SO_4 treated CNWs with some minor modifications. Generally, 55 g of cotton cellulose was hydrolyzed with 500 mL 63.5% (w/w) sulfuric acid (Mallinckrodt Baker) at a temperature of 45 °C for 90 min. The suspension was centrifuged (10 min, 8000 × *g*) and the supernatant was replaced with DI water to remove excess acid. Centrifugation and washing steps were repeated until the pH of the solution reached 3. The pH of the suspension was brought up to 7 using 0.1 M potassium hydroxide (EMD Chemicals Inc.) and then the suspension was dialyzed against DI water. After that, a sonication step was applied to disperse the CNWs (Branson Model 5510, Danbury, 10 min). The suspension formed two layers after sedimentation over a few days. The top layer was used for our study.

The parameters for preparing hydrochloride acid hydrolyzed cellulose were taken from Araki et al. (1998) with some minor modifications. 10 g cotton cellulose was treated with 300 mL of 4 N hydrochloric acid (EMD Chemicals Inc.) at 80 °C for 225 min. The samples were centrifuged, dialyzed and sonicated as mentioned before to obtain a well-suspended CNW solution.

2.3. Field emission scanning electron microscopy (FE-SEM)

CNW suspensions were diluted to 0.001 wt% and air dried on a gold SEM substrate. The samples were coated with a thin gold film (around 5 nm). Field emission scanning electron microscope (LEO 1530, Leo Co., Oberkochen, Germany) operating at 5 kV was used for examination of the samples.

2.4. Desulfation procedures

Desulfation of H_2SO_4-prepared CNWs was performed using two methods. For acid-catalyzed desulfation, 1 g of lyophilized CNWs was treated with 200 mL 0.025 N HCl at 80 °C for 150 min. The samples were again repeatedly centrifuged, dialyzed and sonicated.

CNWs with solvolytic desulfation were prepared according to Nagasawa et al. (1979) with some modifications. Briefly, 500 mg acidic CNWs were neutralized with roughly 3 mL pyridine and lyophilized. 100 mL 10% (v/v) methanol/dimethylsulfoxide was added into the pyridinium salt of cellulose. The suspension was stirred at 80 °C for 120 min. The reaction was stopped by cooling in an ice-water bath and the suspension was diluted with 50 mL DI water. The pH was brought up to 9 with 0.1 M NaOH. The suspension was then dialyzed against DI water until pH reached neutral.

2.5. Infrared spectroscopy (IR)

CNWs were lyophilized and grinded to fine powder thoroughly using a grinder (2000 Geno Grinder, 1200 strokes/min, 90 s). A high pressure clamp was used to press the samples on the ATR sensor of the FTIR spectrometer (Bruker Tensor 27). The reading with FTIR instrument was from 7000 to 300 cm^{-1} using 32 scans with a resolution of 4 cm^{-1}. The normalization and baseline correction of FTIR spectra was performed with OPUS software (Bruker Optics).

2.6. Combustion gas analysis (CGA)

The total sulfur content of cellulose samples was analyzed with a LECO SC-144DR sulfur and carbon analyzer. A cellulose sample of 0.1–0.4 g was loaded into the analyzer where it was combusted in pure oxygen at 1623 K. An infrared detector was equipped to detect the amount of SO_2 in the effluent.

2.7. X-ray photoelectron spectroscopy (XPS)

A droplet of 0.1 wt% CNWs suspension was air-dried on a silicon surface. XPS measurements were conducted using Al Kα excitation (Axis Ultra XPS, Kratos Analytical) for analyzing the elemental composition. A survey spectrum and high-resolution C 1s, O 1s, Cl 2p and S 2p spectra were obtained for each CNWs sample.

2.8. X-ray diffraction (XRD)

X-ray diffraction spectroscopy (PANalytical Empyrean) with Cu Kα radiation generated at 45 kV and 40 mA was used to study the crystal structure of CF11 cellulose and CNWs. XRD spectra were obtained at a rate of 2°/min from 5 to 40° 2θ. The instrumental broadening was determined from the full width at half maximum (FWHM) of four reflections of a silicon (NIST Si 640) standard. Five major crystalline peaks and one amorphous peak were deconvoluted following Park, Baker, Himmel, Parilla, and Johnson (2010) using a program named PeakFit (www.systat.com). Pseudo-Voigt function was used to fit the peaks and the broad peak at around 21.5° was assigned to be the amorphous contribution. The fitting

results were used to calculate the crystal size using the Scherrer equation:

$$B_{hkl} = \frac{K\lambda}{\cos\theta\sqrt{(\Delta 2\theta)^2 - (\Delta 2\theta_{inst})^2}} \quad (1)$$

where B_{hkl} is the average crystalline width of a specific plane (hkl); K is a constant depending on the method of taking the breadth ($K = 0.9$ is used); λ is the wavelength of incident X-rays ($\lambda = 0.15418$ nm); θ is the center of the peak; $\Delta 2\theta$ (in radius) is the FWHM of the reflection peak and $\Delta 2\theta_{inst}$ is the instrumental broadening ($\Delta 2\theta_{inst} = 0.0018$ radius).

2.9. Statistical analysis

The significant difference of the data was evaluated using student test (Minitab Statistical Software; Release 15.1; Penn State University, University Park, PA). Each data point was obtained at least from duplicates ($2 \le n \le 6$).

3. Results

3.1. Morphology of CNWs

Fig. 1 shows the morphology of Whatman™ CF11 cellulose and CNWs obtained from CF11's hydrolysate. Acid hydrolysis reduced the size of cellulose particles from hundreds of micrometers to less than a micrometer. Stable colloidal suspensions were obtained by both H_2SO_4 and HCl hydrolyzed cellulose samples. The cellulose suspension formed two layers after sedimentation over a few days. Large particles of cellulose over several micrometers that were not hydrolyzed as extensively were found at the bottom layer and were then removed. The top layer of the suspension was also not very homogenous. CNWs close to the very top part of the suspension contained fine particles with lengths of approximately 200–300 nm and widths of 15–30 nm. The appearance of both HCl and H_2SO_4-hydrolyzed cellulose samples was very similar as determined by SEM (Fig. 1b and c). At the middle of the suspensions, a mixture of particles with large and small sizes was observed in both H_2SO_4 and HCl prepared CNWs (S-CNW and H-CNW) (Fig. 1d). Most of the large particles were 200–300 nm wide and 1–3 μm long. Particles with even larger sizes were also present. This indicates that the higher order of cellulose organization may form assemblies of different sizes which are resistant to acid hydrolysis.

3.2. Infrared spectroscopy

The IR spectra of sulfated carbohydrates including cellulose were studied by other groups (Cabassi, Casu, & Perlin, 1978; Mahner, Lechner, & Nordmeier, 2001; Wang, Xie, Zheng, & Yao, 2009). Two IR absorbance bands have been used for the characterization of sulfate on carbohydrate polymers. They are the asymmetrical S=O vibration at about 1250 cm^{-1} and the symmetrical C—O—S vibration at about 833 cm^{-1} associated with the C—O—SO_3 group.

The spectra of four CNWs are shown in Fig. 2. In our previous study (Gu, Catchmark, Archibald, & Kaiser, 2010), an attempt had been made to quantify the sulfur content using the area under the 833 cm^{-1} sulfate band. However, it was too low to be accurately quantified as shown in Fig. 2. Compared to the S-CNW, H-CNW had no absorbance peak in the S=O vibration region. At C—O—S vibration region, H-CNW showed a broad valley compared to other CNWs. Two sulfate bands are still visible in acid-catalyzed desulfated S-CNW (AS-CNW) as well as solvolytic desulfated S-CNW (SS-CNW) IR spectra, indicating that neither desulfation process was able to remove the sulfate groups completely.

Table 1
Sulfur content of cellulose and CNWs samples determined by CGA.

Samples	Origin	Treatments	Sulfur content (wt%)
H-CNW	CF11	Hydrochloric acid	0.13 ± 0.03
S-CNW	CF11	Sulfuric acid	0.35 ± 0.01
AS-CNW	S-CNW	Acid-catalyzed desulfation	0.29 ± 0.03
SS-CNW	S-CNW	Solvolytic desulfation	0.24 ± 0.07
CF11	Cotton	–	0.13 ± 0.01
Avicel	Wood	–	0.10 ± 0.05
BC	*G. xylinus*	Static culture	0.12 ± 0.00
Control	Na_2CO_3	Negative control	<0.01

For other IR bands, no obvious difference was observed among samples. The broad bands in the 3650–3000 cm^{-1} region correspond to O—H stretching vibrations and the peaks near 2900 cm^{-1} are assigned to C—H stretching vibrations. For native cellulose, the crystal structure can be further defined as cellulose Iα or Iβ. These two crystalline forms exhibit different hydrogen-bonding patterns. In the IR spectrum, the O—H stretching and out-of-plane bending bands are used to distinguish these two crystal structures (Yamamoto, Horii, & Hirai, 1996). O—H stretching at 3240 cm^{-1} is for Iα and 3270 cm^{-1} for Iβ. Out of plane bending at 750 cm^{-1} is for Iα and 710 cm^{-1} for Iβ. The spectra of four CNWs demonstrate that they were all cellulose Iβ. The spectrum of cotton cellulose fiber CF11 is very similar to H-CNW (data not shown). It indicates that after the acid hydrolysis process, the cellulose retained the Iβ crystalline structure. This result is consistent with previous results (Lu & Hsieh, 2010).

3.3. Combustion gas analysis

Table 1 shows the sulfur content of different cellulose samples determined by CGA. It is not expected that sulfur would be detected in H-CNWs. To determine the source of sulfur, the sulfur content of the CF11 starting material was also measured. It exhibited comparable sulfur content as compared to H-CNWs. Since the IR spectrum of CF11 or H-CNWs did not show any sulfate band, the sulfur may be present in other chemical forms in these materials. Alternatively, the content may be too low to be detected by IR. The sulfur content of other cellulose sources including Avicel and bacterial cellulose (BC) has also been measured for comparison. It seems that all these cellulose samples contain roughly 0.1% sulfur. Since sulfur is an essential element for plants and bacteria, the sulfur may originate from other parts of the organism and may be difficult to remove.

The sulfur content of CNWs with desulfation was also measured (Table 1). Acid-catalyzed and solvolytic desulfation decreased the sulfur content of S-CNWs to 0.29% ($p = 0.07$) and 0.24% ($p = 0.05$), respectively. The solvolytic desulfation method seems to be more efficient than acid-catalyzed desulfation. However, the sulfur content of two desulfated CNWs was higher than CF11, indicating that sulfate was still present. These results are consistent with the IR results.

3.4. X-ray photoelectron spectroscopy

C 1s and O 1s peaks were very clear in survey spectrum of each CNWs sample (data not shown). However, the S 2p peak was barely detected above the noise in a survey spectrum. High resolution C 1s, O 1s, S 2p and Cl 2p spectra of four CNWs were also obtained. In H-CNW, no chloride or sulfur was detected. The sulfur content of H-CNW must be below the detection limit. In the three CNW samples prepared with sulfuric acid with or without desulfation, the S 2p peak was detected (Fig. 3). The shape of the peak did not change after desulfation, indicating the chemical state of the sulfur was the same for S-CNW and its derivations. They are likely from

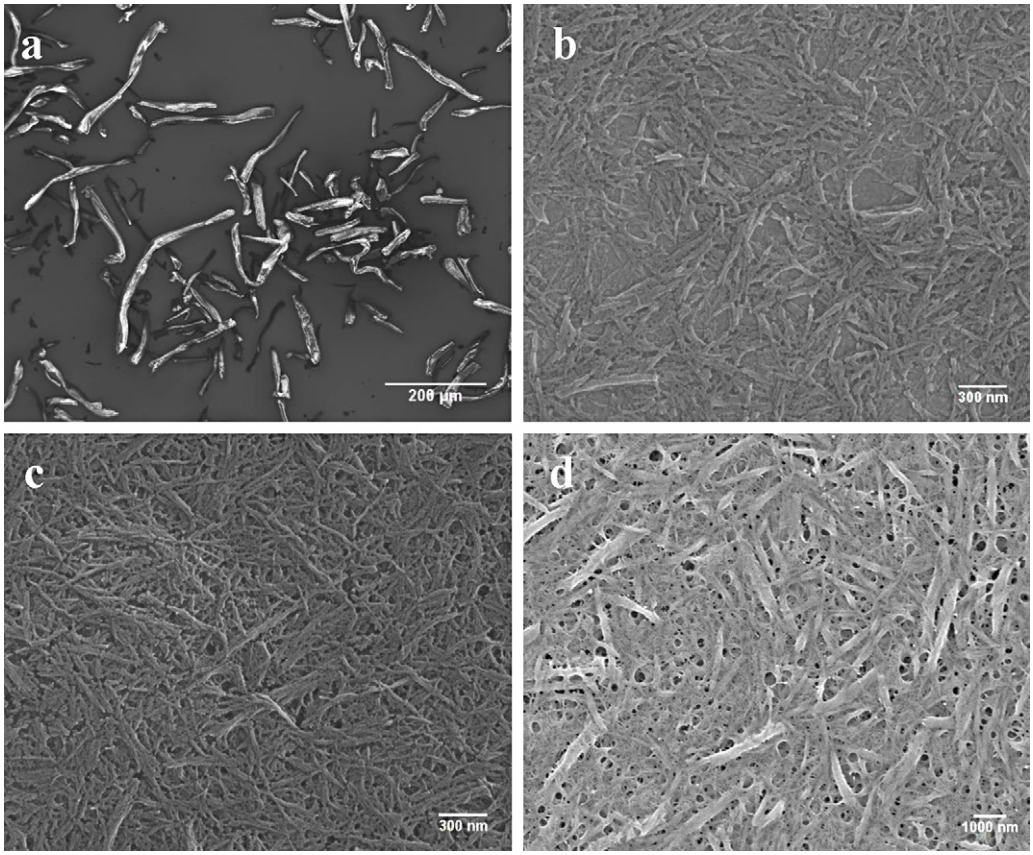

Fig. 1. SEM images of CF11 and CNWs: (a) Whatman™ CF11, (b) H-CNWs from the very top part of suspension, (c) S-CNWs from the very top part of suspension and (d) S-CNWs from the middle part of suspension.

sulfate groups. However, the peak is too low to perform any accurate peak deconvolution. Thus it is not possible to accurately study the chemical state of the sulfur using this method.

The mass concentrations and atomic C/O ratios of four CNWs sample were calculated from the integrated areas under the peaks of high resolution spectra (Table 2). The atomic C/O ratio of all the CNWs except the one with acid-catalyzed desulfation agree well with the theoretical value of cellulose (1.20). Acid-catalyzed desulfation caused a slight increase of C/O ratio which could result from the presence of adventitious carbon possibly due to contaminations during the sample preparation. It may result from random contamination rather than the sample preparation method. Hydrogen is unable to be detected using XPS, but the mass concentration of hydrogen is low in cellulose. The sulfur mass concentration

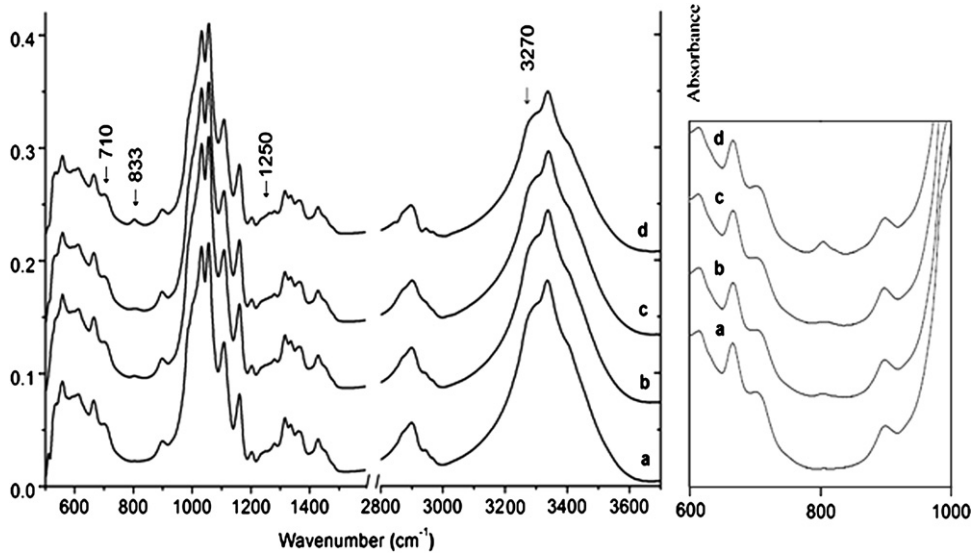

Fig. 2. IR spectra of four CNWs: (a) H-CNW, (b) S-CNW, (c) AS-CNW and (d) SS-CNW. Left: the peaks of interest are marked with the wavenumber. In order to optimize the representation, the region of 1600–2800 cm^{-1} is omitted. Right: the 600–1000 cm^{-1} region of the IR spectra.

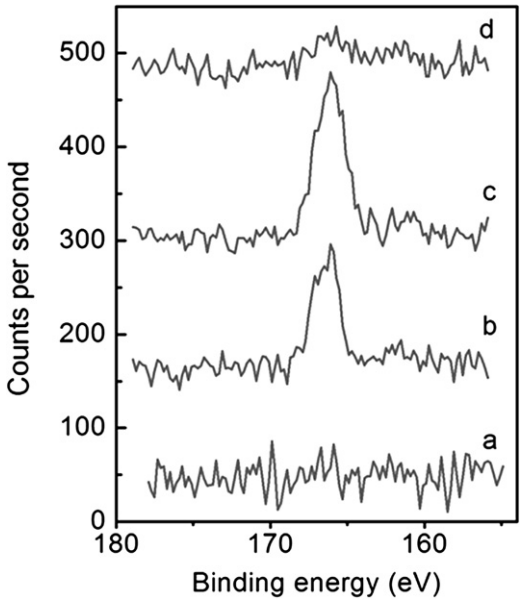

Fig. 3. S 2p peak of four CNWs: (a) H-CNWs, (b) S-CNWs, (c) AS-CNWs and (d) SS-CNWs. *Y* axis is in offset scale.

Table 2
Mass concentration of C, O, and S and atomic C/O ratio for CNW samples.

Sample	Mass concentration (%)			Atomic C/O ratio
	C	O	S	
H-CNW	47.83	52.17	–	1.22
S-CNW	48.50	51.34	0.16	1.26
AS-CNW	49.66	50.11	0.22	1.34
SS-CNW	47.67	52.29	0.03	1.21

of AS-CNWs agreed well with CGA results. Also, the sulfur content of AS-CNW was close to S-CNW while the SS-CNW had the lowest sulfur content. These results were consistent with CGA analysis. Generally, the sulfur contents of samples measured by XPS were lower than that measured by CGA. Since XPS is only a semi-quantitative tool for elemental analysis and the overall sulfur content was low, quantitative analysis of the spectrum may not be accurate compared to CGA.

3.5. X-ray diffraction

In order to study the dimensions of CNW crystals, the XRD spectra of CF11, H-CNWs and S-CNWs were obtained (Fig. 4). They all show five cellulose I characteristic peaks at around $2\theta = 14.5^\circ$, 16.6°, 20.4°, 22.7° and 34.4° (Wada, Sugiyama, & Okano, 1993). The width of crystallite determined from three main peaks, 2 0 0, 1 $\bar{1}$ 0, and 1 1 0 are shown in Table 3. No significant difference is observed between CF11 and CNWs. Since CF11 itself is a highly crystalline material, only a small portion of less ordered region is present. Roughly 10% increase in crystallinity was observed in a previous study (Guo & Catchmark, 2012). Neither sulfuric acid nor

Table 3
The crystallinity and dimensions of B_{200}, $B_{1\,\bar{1}\,0}$, and B_{110} of cellulose samples. See Table 1 for abbreviations.

Sample	Crystallinity[a]	B_{200}	$B_{1\,\bar{1}\,0}$	B_{110}
CF11	78.2 ± 0.4	7.2 ± 0.0	5.0 ± 0.0	5.8 ± 0.1
H-CNW	89.3 ± 1.6	7.2 ± 0.0	5.1 ± 0.1	5.9 ± 0.1
S-CNW	90.5 ± 1.4	7.0 ± 0.2	5.3 ± 0.3	6.0 ± 0.2

[a] Crystallinity results taken from Guo and Catchmark (2012).

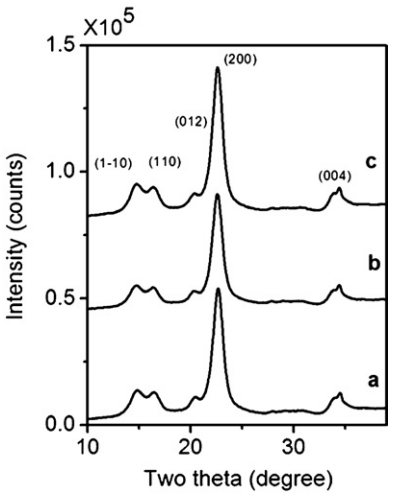

Fig. 4. XRD patterns of cellulose samples: (a) CF11, (b) S-CNWs and (c) H-CNWs.

hydrochloric acid seemed to change the shape of the crystalline part. The XRD results together with the FTIR data confirmed that the CF11 and CNWs produced from CF11 were cellulose Iβ.

4. Discussion

4.1. Sulfur in CNWs and efficiency of desulfation

The chemical state of sulfur in the original cellulose sample is unclear. However, sulfur is essential for all living cells. Production of proteins, enzymes, vitamins and certain carbohydrates all requires sulfur. Sulfur also helps chlorophyll formation in plants. Depending on plant species, the sulfur content varies from 0.1% to 6% in dry plant material. Values between 0.1% and 1% are usually observed for common species including cotton (Ensminger, 1954; Hern, 1984; Jackson, Engleman, & Peard, 1985; Jones & Isaac, 1972). During the preparing process, it is possible that trace amounts of sulfur from other plant tissues remained. The sulfur content of Avicel and bacterial cellulose was measured for comparison since sulfur is required for cell metabolism. Since conductometric titration can only measure the charge of the CNWs, other forms of sulfur are not detectable using this method. From our results, a small amount of sulfur was present in all cellulose materials examined. This explains why elemental analysis detected slightly higher sulfur contents than that assessed using conductometric titration in other studies (Araki et al., 1999; Dong et al., 1998).

During the acid hydrolysis, it is generally believed that the amorphous regions are more susceptible. For sulfuric acid hydrolysis, esterification of the hydroxyl groups occurs. Hydrochloric acid hydrolysis results in no modification of the hydroxyls on the surface (Araki et al., 1999). Our results indicate a 0.22% increase in sulfur content after sulfuric acid hydrolysis of cellulose, while no change in sulfur level using hydrochloric acid hydrolysis was observed. Different starting cellulose, hydrolysis time, temperature, acid concentrations, pretreatment conditions and post sonication time could all affect the sulfate content (Bondeson et al., 2006). Our hydrolysis conditions followed Bodenson et al., which is a common method in the literature. Whatman™ CF11 cellulose is usually used for chromatography. It is a very stable and highly crystalline material. The crystalline component of cellulose has a much lower hydrolytic susceptibility than the noncrystalline component, which may explain lower sulfur content in our CNWs relative to that published by others (Araki et al., 1999; Dong et al., 1998).

As shown in our results, neither desulfation method was able to remove the sulfate group completely. Mild acid catalyzed

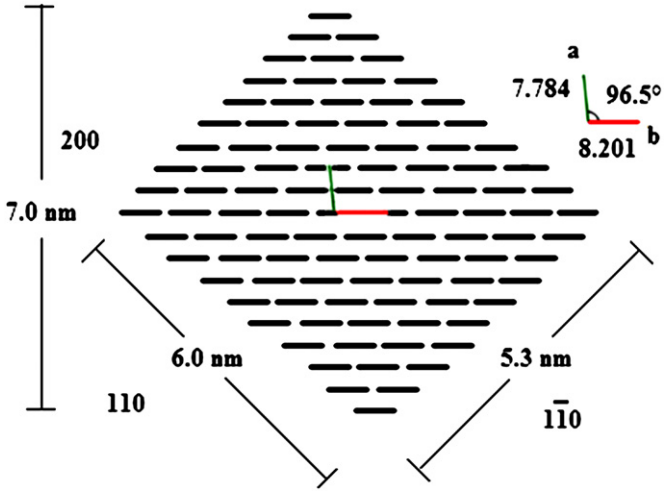

Fig. 5. Schematic of an idealized cross section of a crystalline region within a cotton CNW according to XRD analysis. One black bar represents a glucan chain.

desulfation has been reported by Pomin et al. (2005). In their study, desulfation of sulfated fucans occurred only at specific sites. Jiang et al. (2010) observed a gradual decrease of sulfur content after several rounds of acid catalyzed desulfation. Since we only performed one round of desulfation, the effect of desulfation was not as good as their final result, but it was comparable to the first round. In their study, even after seven rounds of acid catalyzed desulfation, sulfate was still detected in the resulting CNWs.

Solvolytic desulfation was shown to be acid catalyzed and the SO_3 formed is transferred to an acceptor molecule (Miller & Blunt, 1998). Different acids, acceptors and even various ammonium salts other than pyridinium salts could affect the efficiency and selectivity of desulfation (Baumann, Scheen, Huppertz, & Keller, 1998; Miller & Furneaux, 1996). In some cases, the effect of desulfation was not obvious (Nagasawa & Inoue, 1974). In our FTIR spectra (Fig. 2), the area under the C—O—S vibration region ($\sim$833 cm^{-1}) in solvolytic desulfation CNWs was larger than that of the original CNWs and the center of the peak was slightly different between the two CNW samples. One possibility is that the presence of the pyridinium salts of cellulose caused a change in the peak. From XPS study, the sulfur level was approaching the detection limit of the instrument. Although nitrogen was not detected in XPS, it is possible that the amount of pyridinium salt could be too low to be seen in the spectrum. A decrease of sulfate content was observed by CGA, but not as obvious as in other's study (Jiang et al., 2010). This may due to the limitation of conductometric titration. The presence of pyridinium salts may mask the charge of the CNWs.

From the study above, IR spectroscopy could be a rapid tool to qualitatively evaluate different prospective desulfation conditions. Although elemental analysis by CGA is very accurate, it is not able to determine the chemical state of sulfur. XPS is only a semi-quantitative tool and the detection limit is higher than the other techniques. The drawback of conductometric titration is that it is only able to detect the charge of the particle, but not a direct measurement of sulfur or sulfate.

4.2. Estimation of surface sulfate esterification levels in CNWs

According to our XRD results, the representative cross-section of the crystalline regions of the cotton cellulose is proposed (Fig. 5). It is roughly 5.3 nm × 6.0 nm. The cross-section is close to a square with 10 × 10 glucose units based on the *d*-spacing of (1 1 0), (1 $\bar{1}$ 0), and (2 0 0) plane. The crystal and molecular structure of cellulose Iβ was studied by Nishiyama, Langan, and Chanzy (2002). Their results showed that the unit-cell of monoclinic $P2_1$ crystal had dimensions of a = 7.784 Å, b = 8.201 Å, and c = 10.380 Å, and γ = 96.5°. Computer simulation studies of microfibrils revealed similar results (Matthews et al., 2006). Our XRD result is consistent with theirs based on the *d*-spacing value. Both Elazzouzi-Hafraoui et al. (2008) and Leppanen et al. (2009) proposed models for the cross-section of the crystalline region of cotton cellulose based on wide angle and small angle X-ray scattering (WAXS and SAXS). Their results were not consistent with each other. The estimated diameter of fiber width from SAXS was smaller than the smaller limit of crystallite size estimated from WAXS. Thus Nishiyama (2009) raised doubts about the validity of using SAXS to study microfibril width. Estimation of the dimensions of the cross-section (especially 1 $\bar{1}$ 0 and 1 1 0 face) requires several assumptions on the peak profile, the function to fit the amorphous background and peak, and how to deconvolute close peaks. There are few rules to justify the preferred fitting. The shape of the cross section is also debatable. With harsh acid hydrolysis, delamination may occur at the end external surface of the CNWs and rounded corners may exist even in highly crystalline cellulose (Guo & Catchmark, 2012; Nishiyama, 2009). It is still unclear whether the real shape of the crystallite is elliptical, rectangular, or a combination of these throughout the material. This may explain why the standard deviation of crystalline dimension is high in S-CNW. The delamination of surface glucan chains may be different for different crystallite parts. The model proposed in Fig. 5 is hypothetical.

To estimate how many free hydroxyls are on the surface of a crystal, we used the crystal structure parameters shown in Fig. 5. Hydroxyl groups available to be substituted are located on the 4 surfaces (1 $\bar{1}$ 0) and (1 1 0). The (2 0 0) face and the face perpendicular to the c axis are hydrophobic and do not have any hydroxyl groups. Three hydroxyl groups are available to be substituted per cellobiose on the (1 1 0) or (1 $\bar{1}$ 0) crystalline surface if any possible intra-chain hydrogen bonding is ignored. 36 glucan chains are located on the surface of the crystal and thus 120 hydroxyls are available on 36 cellobiose units. Just considering a thin section of a glucan chain with a cellobiose unit, the length is equal to the value of c in a unit cell. The surface area of this thin section of cellulose crystal is (5.3 nm + 6.0 nm) × 2 × 10.38 Å. The number of hydroxyls per unit surface area would be 5.1/nm^2 which equals to 8.5×10^{-6} mol/m^2.

From CGA results, in 1 g S-CNWs, at least 0.22% sulfur is in the form of sulfate which equals to 6.9×10^{-5} mol/g_{CNWs}. If the surface area of CNWs is known, the number of sulfate per unit surface area can be calculated. In this study, two methods have been used to estimate the actual surface area of CNWs. The first method is based on nitrogen adsorption measurements (Guo & Catchmark, 2012). The specific surface areas for S-CNW and H-CNWs are 5.1 m^2/g and 16.8 m^2/g, respectively. H-CNWs exhibit a larger surface area than S-CNWs. This result seems to be in contrast to the hypothesis that sulfuric acid hydrolyzes cellulose more completely than HCl and results in smaller CNWs. Since no micropores were found in S-CNWs, the presence of sulfate group (roughly 0.3 nm in diameter) on the S-CNW surface may occupy the micropore of CNWs preventing N_2 molecules from entering some pores. Accordingly, the surface area of H-CNW may be more representative of the true surface area of CNWs, and is used in subsequent calculations.

The second method is based on the actual dimensions of the CNWs. The width of a cotton microfibril is 3–5 nm as observed by transmission electron microscopy (Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006). However, depending on the hydrolysis conditions, the width and length of CNWs can be distributed over a wide range. Elazzouzi-Hafraoui et al. (2008) have studied the width and length of CNWs made from various sources using TEM, and a log-normal size distribution was observed. In our study, the width and length of fine and larger particles of S-CNWs as shown in Fig. 1c and d have been estimated. The width is measured

Table 4
Estimated surface sulfate esterification level from two methods.

Method estimated the surface area	Surface area (m^2/g)	Surface sulfate density[a] (mol/m^2)	Surface hydroxyls substituted by sulfate[b] (100%)
N_2 sorption	16.8	4.1×10^{-6}	48%
Theoretical calculation	22.9	3.0×10^{-6}	35%

[a] Surface sulfate density = molar of sulfate per gram CNW/Surface area of CNW.
[b] Surface hydroxyls substituted by sulfate = surface sulfate density/surface hydroxyl density.

from the middle of the rod. The width is 31.6 ± 14.0 nm and the length is 338.8 ± 143.1 nm ($n = 50$) for fine CNWs, while the width is 265.0 ± 102.9 nm and the length is 2307.1 ± 747.7 nm ($n = 23$) for larger CNWs. The ratio of the fine to larger particles on the SEM substrate was estimated from homogenized top suspensions. There are roughly 200–300 fine CNWs per μm^2 and 20–25 larger particles per 10 μm^2. The ratio of 100:1 (fine CNWs: large CNWs) should be a reasonable estimation for the whole top suspension. The density of cellulose is about 1.5 g/cm^3 (Eichhorn et al., 2010). If the cross-section of CNWs is a square, the surface area (the area from two ends of the CNWs is ignored) of 1 g CNWs is:

$$A_{CNW} = \frac{m(100A_{SCNW} + A_{LCNW})}{\rho(100V_{SCNW} + V_{LCNW})} \quad (2)$$

where A_{CNW} is the surface area per gram CNWs. A_{SCNW} and A_{LCNW} are the surface area of one small and large CNW particle, respectively ($A_{SCNW} = 31.6 \times 4 \times 338.8\ nm^2$; $A_{LCNW} = 265.0 \times 4 \times 2307.1\ nm^2$). V_{SCNW} and V_{LCNW} are the volume of one small and large CNW particle, respectively ($V_{SCNW} = 31.6^2 \times 338.8\ nm^3$; $V_{LCNW} = 265.0^2 \times 2307.1\ nm^3$). ρ is the density of cellulose ($\rho = 1.5\ g/m^3$). $m = 1$ g. The calculated surface area $A_{CNW} = 22.9\ m^2$. This number is higher than the number from nitrogen adsorption. Using microscopy techniques to quantify the size distribution of samples can be challenging due to the small amount of sample probed and the effects of drying (Braun et al., 2008). For the N_2 adsorption method, the surface area may be underestimated due to a small amount of frozen residual water occluded surface available at low liquid nitrogen temperature (Zografi, Kontny, Yang, & Brenner, 1984). In addition, compaction of the CNWs during N_2 adsorption may also reduce the surface area. Considering these factors, the difference of surface areas between the two methods was not significant.

From the surface area estimated by the two methods, the surface sulfate esterification level of CNWs has been calculated as follows. The density of free hydroxyls on the CNWs surface is assumed to be the same as on an ideal cotton cellulose crystal. The dimensions of CNWs are much larger than a single crystal from the results of our study and other studies (Elazzouzi-Hafraoui et al., 2008). One CNW consists of more than one single crystal. Depending on the hydrolysis conditions and the origin of the cellulose materials, the number of single crystals in one CNW varies. Even in the same batch of CNWs, the dimension of CNWs is a distribution. The structure of CNWs is still not well known. One popular model shows that cellulose consists of single crystal domains along the fiber direction with few, if any, amorphous domains (Nishiyama et al., 2003). The dimension estimated by XRD should be the size of one single crystalline domain. However, the number of the less ordered surface chains is not known. It is possible that sulfuric acid could penetrate the less ordered surface glucan chains of CNWs and the hydroxyls in the core of crystallite are also substituted by sulfate. Thus the density of available hydroxyls may be underestimated. The calculated surface sulfate esterification level of CNWs is listed in Table 4.

Based on the surface area obtained by N_2 adsorption, about half of the surface hydroxyls are substituted by sulfate. From theoretical calculation, roughly one third of hydroxyls are replaced by sulfate. Since hydroxyls at C-6 position are the most reactive, all of them may be substituted. From these calculations, H_2SO_4-prepared CNWs may have highly modified surface chemistry which may limit their application as a model cellulose substrate for cellulose interaction studies. Since no effective methods could remove the sulfate group completely, HCl-prepared CNWs may be a better alternative model material.

5. Conclusion

In this study, IR spectroscopy was used as a quick and sensitive method to determine the presence of sulfate ester in CNWs prepared by different protocols. The quantitative results of sulfur level by CGA and XPS were compared. The detection limit of XPS was higher than the other methods. Our results indicate that neither acid-catalyzed nor solvolytic desulfation was effective at removing all sulfates in H_2SO_4-prepared CNWs. Roughly one third to half of free hydroxyls on H_2SO_4-prepared CNWs were substituted by sulfate. HCl-prepared CNWs retained the most natural cellulose surface and should be considered as an improved material for studies of cellulose-biopolymer and other interactions.

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