The Influence of Pre-treatment Time and Sulfuric Acid on Cellulose Nanocrystals

Ya-Yu Li, Bin Wang, Ming-Guo Ma, and Bo Wang

Cellulose nanocrystals (CNCs) were produced with different pre-mixing times between the cotton fiber and the sulfuric acid at room temperature, prior to the reaction at 45 °C. The CNC0 and CNC60 films were prepared using vacuum filtration methods. Based on transmission electron microscopy observations, the dimension and yield of CNCs gradually decreased with increasing pre-mixing time. Considering the balance of yield and quality of CNCs, CNC0 was chosen as the optimal product. The synthetic process played an important role in the production of CNCs. Various CNCs had similar crystallinity index values with the increased pre-mixing time. The decreased contact angle was the result of decreased dimensions of CNCs or the additional sulfate group at the surface of the CNCs. Both thermogravimetric and contact angle analysis are sensitive for the constituents of CNCs.

Keywords: Cellulose nanocrystals; Sulfuric acid; Pre-mixing time; Characterization

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INTRODUCTION

Cellulose nanocrystals (CNCs) have been developed for their distinct properties, e.g., biocompatibility, high elastic modulus (Wang et al. 2012; Zhang et al. 2016), high specific surface area (Huang et al. 2016; Li et al. 2017), distinct optical properties (Csiszar and Nagy 2017; Zhang et al. 2017), and high aspect ratio (Chen et al. 2015). CNCs have been added into matrix to prepare mechanically enhanced materials (Gawryla et al. 2009; Pei et al. 2011; Meesorn et al. 2017; Sapkota et al. 2017; Nicharat et al. 2017), mechanically-adaptive materials (Mendez et al. 2011; Way et al. 2012; Biyani et al. 2013; Biyani et al. 2014), self-healing materials (Imato et al. 2017), synthesis templates (Padalkar et al. 2010), biosensors (Schyrr et al. 2014), and so on. Sobolciak et al. (2017) modified co-polyamide nanofibers using CNCs and a one-step method from date palm leaves, which showcased the increased mechanical properties and hydrophilicity. Yan et al. (2017) prepared bacterial cellulose nanocrystals and verified their high thermal stability and good emulsifying performance compared with bacterial cellulose. Multifunctional composites with enhanced mechanical strength and antibacterial properties were prepared by melt extrusion of three components including poly(lactic acid), CNC, and silver nanoparticles in food packages application (Fortunati et al. 2012). Shanmuganathan et al. (2010) introduced percolating networks of CNCs into polymer matrix as adaptive substrates for intracortical electrodes. When this nanocomposite was immersed into emulated physiological solution, its tensile storage moduli can be reduced significantly from 40 MPa to 5 MPa with only about 28% w/w swelling. Yu et al. (2017) obtained electrospun
composite nanofibers by adding CNCs/polyethylene glycol (PEG) to poly(lactic acid) (PLA). In comparison to neat PLA, the addition of CNCs/PEG reduced fiber diameters, enhanced fiber uniformity, and decreased the water contact angle (CA) of composite nanofibers with 10 wt.% CNCs/PEG from 117.3° to 98.0°.

There are various strategies to produce CNCs. Diluted sulfuric acid was firstly used for CNCs preparation in 1951 by Rånby. Concentrated sulfuric acid was determined to be a better option in CNC production by Mukherjee and Woods (1953), and actually their procedure is the precursor for the ubiquitous sulfuric acid method still in use today. Chieng et al. (2017) produced CNCs by H$_2$SO$_4$ acid hydrolysis of oil palm mesocarp fiber after the removal of hemicellulose and lignin. Sampath et al. (2017) prepared CNCs of approximately 200 nm to 300 nm in length and 40 nm to 50 nm in width from microcrystalline cellulose (MCC) via sulfuric acid hydrolysis. This process was used to fabricate CNCs-chitosan hydrogels with improved mechanical properties and pH sensitivity. The shape and size distribution of CNCs extracted from cotton, Avicel, and tunicate have been investigated comprehensively using transmission electron microscopy, atomic force microscopy, and X-ray scattering (Elazzouzi-Hafraoui et al. 2008). In addition to sulfuric acid hydrolysis method, Kontturi’s group developed a hydrogen chloride acid vapor hydrolysis method followed by TEMPO oxidation towards more effortless isolation of cellulose nanocrystals (Kontturi et al. 2016; Lorenz et al. 2017; Lee 2018). Besides acid hydrolysis, Trache et al. (2017) summarized six categories of methods including mechanical treatment, oxidation method, enzymatic hydrolysis, ionic liquid treatment, subcritical water hydrolysis, and combined process.

There are several strategies to optimize the synthetic condition of CNCs using the sulfuric acid hydrolysis method. Beck-Candanedo et al. (2005) examined the effects of reaction time and acid-to-pulp ratio on nanocrystal and suspension properties for hydrolyzed black spruce acid sulfite pulp. They found that longer hydrolysis times produced shorter and less polydisperse black spruce CNCs. Bondeson et al. (2006) optimized the production of CNCs from MCC with a sulfuric acid concentration of 63.5% (w/w) and a reaction time of 2 h using response surface methodology. Ilovolich (2012a, b) reported the optimal conditions for obtaining CNCs and studied carefully the dissolution process of cellulose in various concentrations of sulfuric acid and temperatures. The study found that the optimal conditions of the acidic treatment for the isolation of CNCs were the sulfuric acid concentration of 57 wt.% to 60 wt.%, acid to cellulose ratio of 8 to 10, and temperature of 45 °C to 55 °C for 40 to 60 min.

The mechanism of sulfuric acid hydrolysis of cellulose is usually explained by selective acid hydrolysis of disordered (amorphous) domains of cellulose nano-fibrils; the more resistant nanocristallites remain intact and can be isolated in a form of rod-like particles (Habibi et al. 2010). Camacho et al. (1996) analyzed the effects of temperature (25 °C to 40 °C), H$_2$SO$_4$ concentration (31% to 70% (w/v)), and the acid/substrate relationship (1 cm$^3$ to 5 cm$^3$ of H$_2$SO$_4$ per g of cellulose) on the solubilization rate of MCC and glucose production. They found that the solubilization followed zero-order kinetics and the production of glucose was a two part consecutive first-order pseudo-homogeneous reaction. Wang et al. (2014) used three phenomenological reactions to express the solubilization of cellulose fibers, which could be described by pseudo-homogenous first-order kinetics.

Nevertheless, the interaction between cellulose and sulfuric acid is not completely understood (Moon et al. 2011). For example, the process of hydrolysis of cellulose can be divided into two sequential processes, specifically of the solubilization and the
depolymerization of cellulose. The solubilization process of cellulose in sulfuric acid includes the swelling and dissolving of cellulose, meaning that the acid penetrates the noncrystalline and crystalline domains of cellulose and forms complexes (Ioelovich 2016). The depolymerization process means the decrease of degree of polymerization (DP) of cellulose macromolecular, particularly the decomposition of noncrystalline domains and finally, CNC is produced. Similar to the results of Camacho et al. (1996), these two processes may have different kinetics too. So at room temperatures and 45 °C, the relative rate of the two processes may vary. In order to verify the effect of this temperature assembly on yield and uniformity of CNC, the different pre-mixing times at room temperature prior to acid hydrolysis at 45 °C were implemented. This approach has not been reported yet. The purpose of this paper is to investigate the influences of pre-treatment with sulfuric acid prior to the hydrolysis. Herein, two levels of temperature were chosen, room temperature (~25 °C) and 45 °C, to investigate the effects of pre-mixing between cotton and sulfuric acid on the yield and properties of CNCs.

**EXPERIMENTAL**

**Materials**

Cotton was harvested from Xinjiang province, PR China. The cotton fiber was separated from the seed by rolling mill and was used as the raw cotton material. Analytical grade sulfuric acid was purchased from Beijing Chemical Factory (Beijing, China). The content of sulfuric acid was 95% to 98%. Deionized water (3 μS cm⁻¹) was prepared with a Heal force Water Purification System (Shanghai, China).

**Sulfuric Acid Hydrolysis of Cellulose**

First, 10 g raw cotton fiber was added to a 500 mL beaker containing 200 mL of 64.1% (w/w) sulfuric acid. To investigate the effects of pre-mixing time at room temperature on the yield and properties of CNCs, the mixture was stirred at 100 rpm with a magnetic rotor for 0, 30, 60, 120, and 180 min. The beaker was transferred to a water bath at 45 °C for 60 min. Immediately following the acid hydrolysis, the suspension was diluted with 10-fold deionized water to quench the reaction. To remove the unreacted cotton fiber and some leaves, the solution was passed through nylon filters of 100-mesh and 500 mesh. The suspension was centrifuged at 10,000 rpm for 10 min to concentrate the cellulose and remove excess aqueous acid. The resultant CNCs precipitate was rinsed, re-centrifuged three times, and dialyzed against water for one week until the electrical conductivity outside the dialysis bag was below 10 μS cm⁻¹. The resultant CNCs aqueous suspension was approximately 3% to 5% by weight. The produced CNCs with pre-mixing times of 0, 30, 60, 120, and 180 min were coded as CNC0, CNC30, CNC60, CNC120, CNC180, respectively. A 15 mL CNCs aqueous suspension was freeze dried for analysis.

**Film Preparation**

Approximately 10 mL of a CNCs aqueous suspension (~3 wt%) was added to the vacuum filtration apparatus mounted with a 0.22 μm PTFE membrane. After pumping for 8 h, the CNCs film had formed on the PTFE membrane and had a smooth surface. A micrometer was used to measure the thickness of the film. The yield of CNCs was obtained by weight from both the lyophilized sample and the film. The production process is illustrated in Fig. 1.
Fig. 1. The production process of CNC suspension and films with different pre-mixing times
CNCs Characterizations

*Transmission electron microscopy*

Drops of 0.001 wt.% CNC suspensions were deposited on glow-discharged carbon-coated copper grids (300 mesh). The specimens were then negatively stained with 1% phosphotungstic acid and observed using a Philips CM200 transmission electron microscope (TEM; Philips, Netherlands) operating at 80 kV. For each CNCs sample, the width and length of about 100 particles were measured from the TEM images using the Image J 1.46r software (https://imagej.nih.gov/ij/index.html).

*X-ray diffraction (XRD) analysis*

X-Ray diffraction measurements were conducted using a Panalytical X'Pert PRO MPD (Karlsruhe, Germany). The diffracted intensity of the Cu Kα radiation (0.154 nm, 40 kV, and 40 mA) was measured in a 2θ range between 10° and 50°. The CNCs films samples were characterized, and the crystallinity index (CrI) was determined by an empirical method using the following equation (Segal et al. 1959),

\[
CrI(\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100
\]

where \(I_{002}\) is the maximum intensity of the (002) lattice diffraction peak and \(I_{am}\) is the intensity scattered by the amorphous part of the sample. The diffraction peak for the plane (002) is located at a diffraction angle of around \(2\theta = 22.7°\), and the intensity scattered by the amorphous part was measured as the lowest intensity at a diffraction angle of around \(2\theta = 18.7°\).

*Fourier transform infrared (FT-IR) spectroscopy*

Fourier transform infrared spectroscopy was carried out on an FT-IR spectrophotometer (TENSOR II, Bruker Optics, Karlsruhe, Germany) using the KBr disk method. Before testing, the raw cotton fiber or CNCs was dried at 60 °C for 24 h and then ground in an agate mortar with KBr. The mass ratio between the sample and the dried KBr was 1:300. Thirty-two scans were carried out of each sample recorded from a range of 4000 cm\(^{-1}\) to 400 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\) in the transmission mode.

*Thermogravimetric analysis (TGA)*

The thermal stability of the CNCs was characterized using thermogravimetric analysis on a Shimadzu DTG-60 instrument (Kyoto, Japan). The samples (~ 5 mg) were heated from room temperature to 800 °C at a rate of 10 °C min\(^{-1}\) under a nitrogen flow rate of 50 mL min\(^{-1}\).

*Measurement of contact angle*

The contact angle (CA) of the CNCs films with deionized water was measured using the dynamic contact angle meter (KINO SL200KS, KINO Industrial company Ltd., City, America) mounted with CAST2.0 software (KINO Industrial Co., Ltd., America).

*Birefringence of CNCs suspension*

Approximately 0.2 mL of the CNCs suspension was injected into a rectangular vessel with a length of 40 mm, width of 10 mm, and a thickness of 1 mm. This vessel was ultra-sounded for 20 min at room temperature. Then this vessel was observed by a crossed polarizing microscope (model 58XC, Shanghai Optical Instrument Factory, Shanghai, China).
RESULTS AND DISCUSSION

Particle Morphology and Yield Analysis

Transmission electron microscopy (TEM) images of CNCs prepared by pre-mixing for 0 min and 60 min at room temperature prior to the reaction at 45 °C are shown in Fig. 2. The CNCs had a needle-like shape. The lengths and widths were obtained by measuring more than 100 individual CNCs rods in several images, as listed in the statistic results in Table 1 and Fig. 3. The length and width of the CNC60 was lower than those of CNC0. This result suggests that longer time spent by the mixed cotton with sulfuric acid resulted in smaller CNCs (Jiang and Hsieh 2013; Chen et al. 2015). In the case of CNCs produced from Whatman filter paper, Dong et al. (1998) showed that the length of the nanocrystals steadily decreased from 390 nm to 177 nm as the hydrolysis time increased from 10 min to 240 min (Dong et al. 1998). The standard deviation of distributions of length and width of CNC60 also decreased. Similar results were reported by Beck-Candanedo et al. (2005). The CNC60 had a more uniform dimension than CNC0. In CNC180, no needle-like shape was found.

Table 1. Statistics of Dimensions of CNC0 and CNC60

<table>
<thead>
<tr>
<th>Sample Codes</th>
<th>Length</th>
<th>Width</th>
<th>Aspect ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>RSD</td>
</tr>
<tr>
<td>CNC0</td>
<td>145</td>
<td>76</td>
<td>52.2%</td>
</tr>
<tr>
<td>CNC60</td>
<td>137</td>
<td>58</td>
<td>42.8%</td>
</tr>
</tbody>
</table>

Note: Error of measurement of length and width in TEM images is 7 nm and 0.6 nm, respectively
The yield values of various CNCs are shown in Table 2 and Fig. 4. The yield of CNC dropped drastically from CNC0 to CNC60, by 17.0% to 4.2%. This trend is consistent with previously reported results (Bondeson et al. 2006; Kargarzadeh et al. 2012; Wang et al. 2012; Jiang and Hsieh 2013). The yields of CNCs and preparation methods in the literature are summarized in Table 3. The CNCs yield of this work is close to the value of Jiang and Hsieh (2013) and Salminen et al. (2017). This may be due to the difference of condition during the preparation of CNCs. Considering the balance of yield and quality of CNCs, CNC0 was chosen as the optimum product in this paper.

![Graph](image-url)

**Fig. 3.** Distribution of length and width of CNC0 (a1 and a2) and CNC60 (b1 and b2).

**Fig. 4.** The relationship between yield and pre-mixing time at room temperature.
Table 2. Yield of CNCs using Different Pre-mixing Time at Room Temperature

<table>
<thead>
<tr>
<th>Sample Codes</th>
<th>CNC0</th>
<th>CNC30</th>
<th>CNC60</th>
<th>CNC120</th>
<th>CNC180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (%)</td>
<td>17.0</td>
<td>4.2</td>
<td>3.5</td>
<td>2.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 3. Yield of CNCs and Preparation Conditions in the Literature and This Work

<table>
<thead>
<tr>
<th>Raw material</th>
<th>method</th>
<th>Hydrolysis temperature/°C</th>
<th>Hydrolysis time/min</th>
<th>Solid:liquid ratio</th>
<th>CNC yield/%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleached kraft wood pulp</td>
<td>Sulfuric acid, 65%</td>
<td>70</td>
<td>30</td>
<td>1:10</td>
<td>50-60</td>
<td>Revol et al. 1992</td>
</tr>
<tr>
<td>Cotton-based filter paper powder</td>
<td>Sulfuric acid, 64%</td>
<td>26-65</td>
<td>10-240</td>
<td>1:8.75</td>
<td>34-48</td>
<td>Dong et al. 1998</td>
</tr>
<tr>
<td>MCC</td>
<td>Sulfuric acid, 63.5%</td>
<td>44.0</td>
<td>130.3</td>
<td>1:10.2</td>
<td>30</td>
<td>Bondeson et al. 2006</td>
</tr>
<tr>
<td>Cellulose extracted from kenaf bast fiber</td>
<td>Sulfuric acid, 65%</td>
<td>45</td>
<td>40</td>
<td>Not Mentioned</td>
<td>41</td>
<td>Kargarzadeh et al. 2012</td>
</tr>
<tr>
<td>Bleached Kraft eucalyptus dry lap pulp</td>
<td>Sulfuric acid, 58%</td>
<td>56</td>
<td>100</td>
<td>1:8, Stirring, 200 rpm</td>
<td>55.7</td>
<td>Wang et al. 2012</td>
</tr>
<tr>
<td>MCC</td>
<td>Sulfuric acid, 57-60%</td>
<td>45-55</td>
<td>40-60</td>
<td>1:8-10</td>
<td>70-75</td>
<td>Ioelovich 2012a</td>
</tr>
<tr>
<td>MCC</td>
<td>Sulfuric acid, 57-60%</td>
<td>45</td>
<td>60</td>
<td>1:10</td>
<td>65-70</td>
<td>Ioelovich 2012b</td>
</tr>
<tr>
<td>Pure cellulose isolated from rice straw</td>
<td>Sulfuric acid, 64%</td>
<td>45</td>
<td>60</td>
<td>1:8.75</td>
<td>16.9</td>
<td>Jiang and Hsieh 2013</td>
</tr>
<tr>
<td>MCC, cotton and pulp</td>
<td>6 mol/L HCl aqueous solutions under hydrothermal conditions</td>
<td>110</td>
<td>180</td>
<td>1:16667(60mg/L)</td>
<td>93.7</td>
<td>Yu et al. 2013</td>
</tr>
<tr>
<td>Bleached kraft eucalyptus pulp</td>
<td>Sulfuric acid, 58%</td>
<td>55</td>
<td>180</td>
<td>Not Mentioned</td>
<td>70</td>
<td>Wang et al. 2014</td>
</tr>
<tr>
<td>Bleached kraft eucalyptus pulp</td>
<td>Sulfuric acid, 58%</td>
<td>35-80</td>
<td>15-240</td>
<td>1:10</td>
<td>70</td>
<td>Chen et al. 2015</td>
</tr>
<tr>
<td>Softwood sulfite pulp</td>
<td>Sulfuric acid, 64%, and ultrasound</td>
<td>45</td>
<td>120</td>
<td>1:15</td>
<td>71.0</td>
<td>Guo et al. 2016</td>
</tr>
<tr>
<td>Cotton-based filter paper</td>
<td>Hydrogen Chloride Vapor hydrolysis</td>
<td>Room temperature</td>
<td>60</td>
<td>none</td>
<td>97.4</td>
<td>Kontturi et al. 2016</td>
</tr>
<tr>
<td>MCC</td>
<td>TEMPO-mediated oxidation</td>
<td>Room temperature</td>
<td>~30</td>
<td>Not Mentioned</td>
<td>17–20</td>
<td>Salminen et al. 2017</td>
</tr>
<tr>
<td>Cotton-based filter paper</td>
<td>TEMPO-mediated oxidation after hydrolysis by HCl vapour</td>
<td>Room temperature</td>
<td>hydrolysis for 12 h</td>
<td>none</td>
<td>50</td>
<td>Lorenz et al. 2017</td>
</tr>
<tr>
<td>Raw cotton</td>
<td>Sulfuric acid, 64.1%</td>
<td>45</td>
<td>60</td>
<td>1:20</td>
<td>17.0</td>
<td>This work</td>
</tr>
</tbody>
</table>
The interaction between cotton fibers and sulfuric acid can be divided into two sequential processes, specifically that of the solubilization and the depolymerization of cellulose. As reported by Camacho et al. (1996) and Wang et al. (2014), these two processes have different kinetics. At 45 °C, the kinetic constant, \( k \), in the solubilization depolymerization process would be about ten times that of \( k \) at 25 °C (Camacho et al. 1996). The reaction rate at 45 °C would be about ten times that of the rate at 25 °C. However, in this paper, along with the increased pre-mixing time at 25 °C, the yield decreased remarkably. This means that the process at 25 °C played an important role in the production of CNCs, which could be comparable with that at 45 °C. Therefore, the two processes should be more complicated than the results of Camacho et al. (1996) and Wang et al. (2014).

**X-ray Diffraction Analysis**

XRD experiments were conducted to analyze the crystallinity of various CNCs. The XRD patterns for CNC0 and CNC60 films are shown in Fig. 5. All diffractograms were typical of semi-crystalline materials, displaying an amorphous broad hump and crystalline peaks. As shown in Table 4, four well-defined crystalline peaks of cellulose I were present at \( 2\theta = 14.8^\circ, 16.8^\circ, 22.8^\circ, \) and \( 34.3^\circ \) (Kargarzadeh et al. 2017).

![X-ray diffraction patterns of CNC0 and CNC60 films prepared by vacuum filtration](image)

**Fig. 5.** X-ray diffraction patterns of CNC0 and CNC60 films prepared by vacuum filtration

**Table 4.** Diffraction Peak, Crystallinity Index, and Thickness of CNC0 and CNC60 Films

<table>
<thead>
<tr>
<th>Sample Codes</th>
<th>Peak 1 Position</th>
<th>Peak 2 Position</th>
<th>Peak 3 Position</th>
<th>Peak 4 Position</th>
<th>Peak 5 Position</th>
<th>CrI (%)</th>
<th>Film Thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC0</td>
<td>14.8</td>
<td>16.8</td>
<td>22.9</td>
<td>34.3</td>
<td>46.9</td>
<td>92.5</td>
<td>173</td>
</tr>
<tr>
<td>CNC60</td>
<td>14.8</td>
<td>16.8</td>
<td>22.8</td>
<td>34.3</td>
<td>46.9</td>
<td>92.6</td>
<td>137μm</td>
</tr>
</tbody>
</table>

The crystallinity indexes (CrI) determined from these diffractograms are shown in Table 4. The CrI was 92.5% for CNC0. The CrI value of raw cotton was approximately 79.2% (Segal et al. 1959). Thus, after the acid hydrolysis treatment, the CrI value increased. This finding confirms that the cellulosic amorphous polysaccharides were efficiently removed using the acid treatment. The CrI value increased little upon the increase of pre-mixing time at room temperature from 0 min to 60 min. This result indicated...
that the amorphous region of cotton fiber had been removed completely. It should be noted that the peak intensities of CNC60 were lower than that of the corresponding peaks of CNC0, indicating the decreased crystallinity (Liu et al. 2008).

FT-IR Analysis

Figure 6 displays the FT-IR spectra of raw cotton and five kinds of CNCs. The band at 3350 cm\(^{-1}\) is the O-H stretching vibration peak. The bands at 2918 cm\(^{-1}\) and 2851 cm\(^{-1}\) are the C-H peaks. The band at 1636 cm\(^{-1}\) is the bending vibration peak of water. The band at 1372 cm\(^{-1}\) is the bending vibration peak of C-H. The bands at 1162, 1112, 1060, and 1034 cm\(^{-1}\) are the peaks of C-O-C-O-C (Suflet et al. 2006). The band at 898 cm\(^{-1}\) is related to the C–H rocking vibration (Alemdar and Sain 2008), which is the characteristic absorption of cellulose. The band at 614 cm\(^{-1}\) is the fingerprint absorption of a hexatomic ring in carbohydrate. Hence, there is little difference among these FT-IR spectra, which is the consequence of the similar chemical structure of various CNCs.

![FT-IR spectra](image)

**Fig. 6.** FT-IR spectra of raw cotton (a0) and five kinds of CNCs under different pre-mixing time: (a) 0 min; (b) 30 min; (c) 60 min; (d) 120 min; (e) 180 min

Thermogravimetric Analysis

The TG and DTG curves of the raw cotton materials and the three kinds of CNCs produced under different pre-mixing times are shown in Fig. 7. There are several overlapping peaks in the curves. Peakfit 4.0 software was used to deconvolute the DTG curves (Roman and Winter 2004). The CNCs codes, center values, temperature ranges, and areas of deconvoluted peaks are listed in Tables 5 through 7, respectively. The raw cotton has three peak codes at 1, 4, and 5. Peak 1 centered at 66.4 °C corresponded to the evaporation of water. Peak 4 centered at 340.7 °C corresponded to the pyrolysis of cellulose and has a similar position and temperature range (Broido 1969; Yang et al. 2007). Peak 5 centered at 411.6 °C had the largest temperature range of the three peaks. This maybe
corresponded to charring process of solid residues reported by Wang et al. (2007). Cotton fibers usually have a cellulose content of more than 80 wt.% (Zhang et al. 2012). In these three peaks, peak 4 had the maximum area ratio of 95%, indicating that raw cotton contained mostly cellulose.

These three peaks also appeared in three kinds of CNCs around a similar position and would represent similar chemical components as seen in raw cotton. CNC0 had two more peaks than raw cotton, and both the CNC60 and CNC180 also had some changes in their peaks in comparison to the CNC0. These changes of peaks can be observed in Table 5. Because peaks 3, 4, and 5 had higher area ratios than others, and the analysis of peaks 2 and 6 was difficult due to its low content. Only the changes of peaks 3, 4, and 5 are discussed in relation to these samples. Peak 3 should be ascribed to the pyrolysis of cellulose catalyzed by sulfated groups at the surface of the CNCs (Roman and Winter 2004; Man et al. 2011). By increasing the pre-mixing time at room temperature, the area ratio of peak 3 increased gradually from 15% to 30%, and the area ratio of peak 4 decreased from 75% to 56%. In considering the results of XRD, CNCs had similar CrI values for various CNCs. After conductometric titration were carried out on CNC0 and CNC60, the contents of sulfate groups were 89 and 214 mmol/kg, respectively. One can see the increased sulfate group along with the increase of pretreatment time. Further, this caused the changes of the area ratio of peaks 3 and 4. We proposed the possible mechanism to explain the increase thermal degradation with acid treatment. The degradation of cellulose catalyzed by sulfuric

![TG and DTG curves of raw cotton (a) and three kinds of CNCs: (b) CNC0; (c) CNC60; (d) CNC180](image-url)
acid would cause more char residues (Kim et al. 2001; Roman and Winter 2004; Wang et al. 2007). These char residues covered the surface of CNC and slowed the heat transfer of CNC, delaying the pyrolysis of CNC (Simmons and Gentry 1986).

The color of CNC0 and CNC60 was claybank, and the color of CNC60 was deeper than that of CNC0 (Fig. 1). The commercial CNCs have a white color and by tedious sediment separation steps, white CNCs were obtained from this experiment also. The claybank color may be due to the existence of impurities in the products. These impurities might be the small molecules of xylan, lactic acid, and levulinic acid, etc, during acid hydrolysis procedure (Labet and Thielemans 2011). In combination with the gradually decrease yield of CNCs, one can understand the increase of the relative content of impurities.

### Table 5. Centered Value of Peaks in Deconvoluted DTG Curves of Raw Cotton and CNCs

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak 1</td>
</tr>
<tr>
<td>raw cotton</td>
<td>66.4</td>
</tr>
<tr>
<td>CNC0</td>
<td>68.5</td>
</tr>
<tr>
<td>CNC60</td>
<td>61.0</td>
</tr>
<tr>
<td>CNC180</td>
<td>73.1</td>
</tr>
</tbody>
</table>

### Table 6. Temperature Range of Peaks in Deconvoluted DTG Curves of Raw Cotton and CNCs

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Temperature Range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak 1</td>
</tr>
<tr>
<td>raw cotton</td>
<td>26.0-106.8</td>
</tr>
<tr>
<td>CNC0</td>
<td>28.3-108.7</td>
</tr>
<tr>
<td>CNC60</td>
<td>2.1-119.9</td>
</tr>
<tr>
<td>CNC180</td>
<td>26.3-119.9</td>
</tr>
</tbody>
</table>

### Table 7. Area Ratio of Peaks in Deconvoluted DTG Curves of Raw Cotton and CNCs

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Area Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak 1</td>
</tr>
<tr>
<td>raw cotton</td>
<td>2</td>
</tr>
<tr>
<td>CNC0</td>
<td>3</td>
</tr>
<tr>
<td>CNC60</td>
<td>6</td>
</tr>
<tr>
<td>CNC180</td>
<td>3</td>
</tr>
</tbody>
</table>

### Contact Angle Analysis

The images of contact angles (CA) of CNCs films are shown in Fig. 8. The CA values of CNCs films are listed in Table 8. By increasing the pre-mixing time, the CA decreased from 56.19° to 46.65°. This result suggested that the hydrophilicity of CNCs increased. The increased CA could be the result of a decrease in dimension of CNCs or the increase of sulfate groups at the surface of CNCs.
Fig. 8. Images of CA of CNC0 and CNC60 films

Table 8. Contact Angle of CNCs Films

<table>
<thead>
<tr>
<th>Film name</th>
<th>Contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC0</td>
<td>56.19</td>
</tr>
<tr>
<td>CNC60</td>
<td>46.46</td>
</tr>
</tbody>
</table>

**Birefringence of CNCs Suspension**

The fingerprint-like textures of CNCs suspensions were observed by a polarizing microscope, as shown in Fig. 9. The micrograph shows that the half pitch of chiral nematic ordered phase was 7 μm to 18 μm. This observation, in addition to the reported results (Revol et al. 1992), verified the successful production of CNCs.

Fig. 9. The fingerprint-like multi-domains formed in the CNC’s suspension shows a half pitch of 7 μm to 18 μm

**CONCLUSIONS**

1. Cellulose nanocrystals (CNCs) were produced using different pre-mixing times between the cotton fiber and the sulfuric acid at room temperature prior to a reaction at 45 °C. It was concluded that the dimension of CNCs was gradually decreased, in a uniform manner, with the increasing pre-mixing time. The yield of CNCs decreased, too.

2. The process at 25 °C played an important role in the production of CNCs, indicating that the dependence of the two processes on temperature should be more complex.
3. The CNC0 and CNC60 films were prepared using a vacuum filtration method. When XRD was carried out on these films, it was demonstrated that the crystalline polymorph of cellulose I was maintained.

4. From the result of the FT-IR and TG, the main constituents of various CNCs were verified to be cellulose. Besides this, the quantities of sulfate groups in the surface of CNCs increased gradually from CNC0 to CNC60.

5. The decrease of contact angle (CA) of water was the result of the decreased dimension of CNCs or the increase of sulfate groups on the surface of the CNCs. CA is an indirect method used to indicate the relative dimensions of CNCs.

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