

EFFECTS OF REACTION CONDITIONS ON THE SHAPE AND CRYSTALLINE STRUCTURE OF CELLULOSE NANOCRYSTALS

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The properties and possible applications of cellulose nanocrystals (CNCs) are directly associated with their shape and crystalline structure. In this context, the objective of the present work was to evaluate the influence of acid hydrolysis conditions on the morphology and crystalline structure of CNCs. The variables for hydrolysis conditions were the following: acid concentration (50 and 55% v/v), time (20 and 30 min) and reaction temperature (35 °C and 45 °C). The materials obtained were characterized and their yields were determined. Investigations on the shape and dimensions of the CNCs were carried out using transmission electron microscopy (TEM). In addition, X-ray diffraction analyses were performed to obtain information on the crystallinity and the polymorphic state of nanocrystals. Regardless of acid concentration and reaction time, the formation of spherical nanocrystals was observed with a mixture of the cellulose crystal structures I and II in different proportions in the reactions conducted at 35 °C. For the reactions conducted at 45 °C, predominantly rod-shaped nanocrystals with a cellulose I crystal structure were obtained.

Keywords: cellulose, acid hydrolysis, crystallinity, cellulose nanocrystals, cellulose polymorphs

INTRODUCTION

Cellulose is the most abundant biopolymer on earth and it is the main structural component of plant cells.^{1,2} The chemical structure of cellulose is composed of a linear homopolysaccharide composed of D-glucopyranose units, joined by β -(1 \rightarrow 4)-linkages. The repeating unit is a glucose residue, known as cellobiose.³ Each glucose residue has three hydroxyl groups that play an important role in the organization of the cellulose molecules into crystals that can have long-term stability.⁴

Cellulose I is a native cellulose found in nature and occurs in two allomorphic forms (I α and I β). Native cellulose can be converted into cellulose II by regeneration or mercerization. Regeneration involves the preparation of a cellulose solution in an appropriate solvent or an intermediate derivative followed by coagulation and recrystallization. Mercerization involves intracrystalline cellulose swelling in a concentrated NaOH solution by washing and recrystallization. Since the transition of cellulose I to II is not reversible, the structure of cellulose II is a stable form when compared to the metastable structure of cellulose I.^{5,6,7}

Cellulose II has antiparallel chains, while cellulose I has parallel chains.^{6,8,9} Both structures exhibit dominant intra-chain hydrogen bonds at the O3-H \cdots O5 positions, these bonds provide chain stiffness and linearity. The difference between cellulose I and II is that structure I predominantly has hydrogen bonds at position O6-H \cdots O3, whereas in structure II they are at position O6-H \cdots O2.¹⁰ Cellulose I has better mechanical properties, while cellulose II has benefits in terms of functionality.¹¹⁻¹⁴

Recently, considerable interest has been directed towards obtaining CNCs due to their improved properties when compared with those of cellulosic fibers. These properties include low thermal expansion, high aspect ratio, high surface area per unit volume, high strength and rigidity.¹⁵⁻¹⁷ They may confer useful characteristics in reinforcing agents, coating additives, food packaging, gas barriers, optical and electronic devices for the application of CNCs in nanocomposites.^{16,18,19}

Cellulose nanocrystals with cellulose I or II crystalline structures can be obtained by different techniques. The morphology of CNCs, size, yield and degree of crystallinity depends on the source of the cellulosic material, the preparation conditions (hydrolysis time, temperature and acid concentration) as well as on the technique used. However, since the cellulose cleavage occurs randomly during the acid hydrolysis process, the CNC dimensions are not uniform.²⁰ Several works report that longer hydrolysis times reduce the diameter of nanocrystals.^{16,21-25} Fewer studies report on obtaining different morphologies and crystalline structures through variations in the hydrolysis conditions. In their work, Lu and Hsieh²⁶ obtained cellulose I nanocrystals with a mixture of rod-, sphere- and net-shaped morphologies when hydrolyzing cotton cellulose with sulfuric acid (64-65% w/w) at 45 °C for 60 min. For the same hydrolysis conditions, Kargarzadeh *et al.*²¹ obtained stem-shaped nanocrystals through acid hydrolysis of kenaf cellulose. Although the authors used the same hydrolysis conditions, different morphologies were obtained, in this case showing the influence of the raw material on the morphology of the final product.

Cellulose nanocrystals composed of cellulose I have been extensively studied.^{14,27-29} However, there are few published studies on obtaining CNCs II, and in most of them the conversion of cellulose I into II occurs through alkaline treatment (mercerization). For example, Jin *et al.*¹³ obtained CNCs II by mercerizing CNCs I with NaOH for 30 min at room temperature. Concentrations of 0, 2.0, 5.0, 8.0, 10, 12.5, 15 and 17.5% w/w of NaOH were tested, however, cellulose II nanocrystals were obtained only for NaOH concentrations of 15 and 17%. For alkaline treatment at room temperature, the polymorphic transformation of cellulose I into II begins at an alkali concentration of 12.5% and is complete at a concentration of 17.5%.^{30,31} However, if the temperature is changed, polymorphic transformation can occur at different alkali concentrations.¹³ Cellulose II nanofibers were obtained by Abe and Yano³² by mercerizing wood cellulose nanofibers in 15% w/w NaOH solution. CNCs II were obtained by Yue *et al.*³³ by pre-treatment of cotton fibers with NaOH at room temperature for 4 h, and subsequent hydrolysis with sulfuric acid. According to the authors, only the fibers pretreated with 15 and 20% NaOH resulted in CNC II after the acid hydrolysis.

Another way of obtaining CNCs II reported in the literature is the hydrolysis with sulfuric acid. Sèbe *et al.*³⁴ obtained CNCs II from cotton microcrystalline cellulose, which was directly submitted to hydrolysis with 66% sulfuric acid (44 °C, 60 min). CNCs II from Mombasa grass were obtained by Martins *et al.*³⁵ by hydrolysis with 11.22 M sulfuric acid at 40 °C and reaction times of 10, 20, 30 and 40 min. The crystallinity of CNCs can be affected during the conversion of cellulose I to cellulose II. For example, Martins *et al.*,³⁵ Yue *et al.*³³ and Jin *et al.*¹³ reported having obtained CNCs II with lower crystallinity than the CNCs I, and even inferior to the raw materials used to obtain nanocrystals. Since the high crystalline structure is responsible for several CNC characteristics,¹⁷ and directly interferes with the mechanical properties,^{7,17} the low crystallinity of CNCs II can restrict its applications.

The shape, size and degree of crystallinity of CNCs depends on the source of the cellulosic material and the conditions of the extraction process.²⁶ The objective of the present work was to understand how the acid hydrolysis conditions can influence the morphological characteristics, the crystalline structure and the crystallinity of the pine cellulose CNCs. Understanding the influence of the hydrolysis conditions on obtaining CNCs is indispensable for obtaining a high-quality material, of high performance and at the lowest possible cost.

EXPERIMENTAL

Raw material

Bleached kraft pulp (BKP) provided by a regional company with 99% holocellulose content and $67.80 \pm 0.32\%$ crystallinity was used to obtain the cellulose nanocrystals. 98% Sulfuric acid (Sigma Aldrich, Brazil) was used without further purification.

Obtaining CNCs

The cellulose fibers were hydrolyzed using 50% and 55% v/v sulfuric acid solutions. Reaction temperatures of $35 \text{ °C} \pm 1 \text{ °C}$ and $45 \text{ °C} \pm 1 \text{ °C}$ and reaction times of 20 and 30 minutes were evaluated. In all the reactions, an acid/cellulose ratio of 25:1 (mL/g cellulose) was maintained. After the hydrolysis, the reaction was stopped by the addition of deionized ice water. Subsequently, the resulting suspension was centrifuged at 9000 rpm for 30 min to remove the nanocrystals from the acid solution. With the deposited nanocrystals, the supernatant (acid solution) was discarded. The nanocrystals were then dispersed in deionized water and the resulting suspension was dialyzed for 5 days to neutral pH. Finally, a few drops of chloroform were added to prevent bacterial growth

and the suspensions were stored in a refrigerator. The resulting nanocrystalline suspensions were denoted as CNC20_35_50, CNC30_35_50, CNC20_45_50, CNC30_45_50, CNC20_35_55, CNC30_35_55, CNC20_45_55 and CNC30_45_55. The hydrolysis conditions of 45 °C and 55% v/v of sulfuric acid, used in this work, were severe, causing the degradation of the CNCs and consequently resulting in a low yield (<2%), thus the CNCs obtained under these conditions were not used.

Yield

The yield of the CNCs was calculated by Equation 1:^{28,36}

$$\text{Yield (\%)} = \left(\frac{m_f}{m_i} \right) \times 100 \quad (1)$$

where yield (%) corresponds to the yield of CNCs (%); m_f is the mass of dry CNCs (g) and m_i corresponds to cellulose mass (g). The results were presented as mean and standard deviation of triplicates. For the calculation of yield, three samples were prepared under the same experimental conditions and analyzed statistically.

Crystallinity

To obtain information regarding the crystallinity of the samples and their polymorphic state, the X-ray diffraction (XRD) technique was used. The analyses were conducted in an X-ray diffractometer (Philips X'Pert, CuK α radiation), operated at 40 kV and 30 mA, over a scan range of 5-30° 2 θ . The step size was 0.02° and the scan rate of 0.5°/min.

Since overlapping of the amorphous area peak can occur, the use of crystalline and amorphous area regions in the XRD spectra is more suitable for calculating the crystallinity. OriginPro software (<http://www.originlab.com/>) was used to apply the Gaussian peak fitting method to adjust crystalline and amorphous peaks. The Miller indexes employed for the location of the peaks are in accordance with French.³⁷ Crystallinity was calculated by dividing the total area of all the crystalline peaks by the total area of the crystalline peaks plus the area of the amorphous peak according to Equation (2):³⁸⁻⁴¹

$$I_c (\%) = \frac{\sum A_{cryl}}{\sum A_{cryl} + \sum A_{amph}} \quad (2)$$

The crystal size, τ (nm), perpendicular to the lattice plane (the (200) plane for cellulose I $_{\beta}$ and the (020) plane for cellulose II) was calculated by the Scherrer equation (Eq. 3):

$$\tau = \frac{K\lambda}{\beta \cos \theta} \quad (3)$$

where K is the correction factor (0.9); λ is the wavelength of the X-ray radiation (1.54 Å); β is the corrected angular width at half maximum intensity of the diffraction peak in radians; and θ is half of the 2 θ angle where the highest diffraction peak occurred.

The spacing between the planes (the (200) plane for cellulose I $_{\beta}$ and the (020) plane for cellulose II), d (nm), is calculated using the Bragg equation (Eq. 4):

$$n\lambda = 2d \sin \theta \quad (4)$$

where n is an integer; λ is the wavelength of incident radiation; and θ is the angle between the incident ray and the scattering plane.

The percentage of cellulose I and II present in the CNCs was determined by the area of the peaks corresponding to each type of cellulose.

CNC morphology

Information on the shape and size of the CNCs was obtained by transmission electron microscopy (TEM) (Jeol, JEM-2100, Japan) at 80 kV. Initially, dilute suspensions of the suspended CNC samples were prepared with the addition of distilled water and then dispersed through an ultrasonic probe (Unique Sonicator, 19 kHz, 500 W, DES500, São Paulo, Brazil) for 10 min with a 70% output control. A small amount of the suspensions was then deposited on formvar-carbon (300 mesh) copper grids and dried at room temperature for 24 h. The samples were then stained negatively with 2% uranyl acetate solution, washed three times and dried under ambient conditions for a further 24 h. Next, the grids were submitted to TEM analysis. For the determination of the mean diameter of the CNCs, 10 images were randomly selected among the characterizations performed in triplicate and the size of at least 1000 nanocrystals was analyzed with the help of ImageJ software.

To better analyze the size distribution of the CNCs, histograms were plotted with the help of OriginPro software (<http://www.originlab.com/>). Through the histograms, it is possible to observe the different sizes obtained for the same sample and the diameter range of most CNCs.

RESULTS AND DISCUSSION

Yield

The hydrolysis of kraft pulp fibers with sulfuric acid is a heterogeneous process involving diffusion of the acid in the cellulose fibers, resulting in cleavage of the glycosidic bonds and possible sulfation of the hydroxyl groups of cellulose (*i.e.*, the conversion of cellulose-OH to cellulose-OSO₃H); other secondary reactions, such as cellulose dehydration or oxidation, may also occur. Hemicelluloses, if present in cellulose fibers, will undergo similar reactions, but at a higher rate because of their higher reactivity.¹⁹ Table 1 shows the average yield of CNCs obtained under each evaluated condition.

The yield is a key factor in determining the economics of the hydrolysis process and is directly related to the reaction conditions used: time, temperature and acid concentration.^{19,42} As can be seen in Table 1, the yield follows a downward trend with increasing reaction time. With longer reaction time, decreasing yields are expected because the cellulose chains in the less ordered regions are hydrolyzed to separate the crystalline domains that reduce in size as the surface chains are further hydrolyzed, while cellulose fragments are broken into soluble oligo- and monomers.^{19,28} The reduction of the yield with the increase of the reaction time was also reported in another study.²⁸ Higher yields were obtained in reactions at temperatures of 35 °C and shorter hydrolysis time. It is observed that the increase of the reaction temperature accelerated the hydrolysis process, since the yields obtained at 45 °C are lower than those obtained at lower temperatures. This lower yield is justified by the fact that the mass transfer in intra-fiber pores is intensified with increasing temperature due to the increase in the diffusion coefficient of H⁺ in aqueous solution.⁴²

Some studies have reported that CNC yields increase with increasing reaction time until an optimal reaction time is reached, after which a maximum yield of nanocrystals is obtained.^{13,19} From this optimum point onwards, the yields would then decrease with increasing reaction time. This behavior was not observed in this work, probably owing to the hydrolysis conditions used. It should also be considered that the determination of the yield of CNCs takes into account the dry mass resulting from the hydrolysis reaction and the dry cellulose mass used in the reaction. Thus, the yield presented herein is composed of both the crystalline material and the amorphous material not yet solubilized. Thus, it is expected that under milder reaction conditions, higher yields will be obtained due to non-solubilization of all amorphous regions. As the reaction time increases, the yields are expected to be lower, since greater solubilization of the amorphous regions occurs, even the solubilization of the crystalline region.

Crystallinity

In the XRD characterizations, a peak at approximately $2\theta = 23.0^\circ$ (plane 200) and weaker peaks observed at $2\theta = 14.9^\circ$ (plane 1-10), $2\theta = 16.7^\circ$ (plane 110) and $2\theta = 20.5^\circ$ (plane 012) are characteristic of cellulose I. For cellulose II, these peaks are analyzed at approximately $2\theta = 22.2^\circ$ (plane 020), $2\theta = 20.0^\circ$ (plane 110) and 12.2° (plane 1-10).³⁷ The X-ray diffractograms of the CNCs in the different conditions studied in this work are presented in Figure 1 (a), (b) and (c). The formation of materials with typical cellulose I and cellulose II crystalline structure is observed. Figure 2 shows the peak deconvolution method used to locate the cellulose I and/or II peaks, as well as the amorphous region peak. The areas of the peaks were used to calculate the percentages of cellulose I and II (Table 2), crystallinity, crystal size and d-spacing (Table 3).

Table 1
CNC yield for different acid concentrations, temperature and reaction times

Reaction time	H ₂ SO ₄ 50% v/v		H ₂ SO ₄ 55% v/v
	35 °C	45 °C	35 °C
20 min	78.1 ± 1.1 ^a	32.9 ± 0.8 ^b	42.6 ± 1.1 ^c
30 min	72.1 ± 0.4 ^d	21.0 ± 1.9 ^e	25.3 ± 1.1 ^f

Different overlapping letters indicate significant differences ($p \leq 0.05$) between the yields of CNCs. The results are presented as mean and standard deviation of triplicate measurements for each condition

Table 2
Percentage of cellulose I and II of CNCs

Sample	Percentage of cellulose (%)	
	Cellulose I	Cellulose II
BKP	100.00 ± 0.00	-
CNC20_35_50	33.87 ± 0.08	66.13 ± 0.08
CNC30_35_50	26.31 ± 0.34	73.69 ± 0.34
CNC20_45_50	95.23 ± 0.87	4.73 ± 0.87
CNC30_45_50	100.00 ± 0.00	-
CNC20_35_55	21.14 ± 0.58	78.86 ± 0.58
CNC30_35_55	6.12 ± 2.84	93.82 ± 2.77

Table 3
CNCs: crystallinity, crystal size and spacing between planes (200) and (020)

Sample	Crystallinity (%)	Crystal size (nm)		d-spacing (nm)	
	Gaussian peak fitting	(200) plane	(020) plane	(200) plane	(020) plane
BKP	67.80 ± 0.32	4.28 ± 0.14	-	0.40 ± 0.00	-
CNC20_35_50	70.90 ± 1.12	-	4.34 ± 0.25	-	0.41 ± 0.00
CNC30_35_50	75.67 ± 0.24	-	5.33 ± 0.11	-	0.41 ± 0.00
CNC20_45_50	76.33 ± 1.17	4.56 ± 0.21	-	0.40 ± 0.00	-
CNC30_45_50	83.92 ± 2.06	4.04 ± 0.22	-	0.40 ± 0.00	-
CNC20_35_55	76.56 ± 1.91	-	6.32 ± 0.06	-	0.41 ± 0.00
CNC30_35_55	85.26 ± 1.93	-	5.61 ± 0.40	-	0.41 ± 0.00

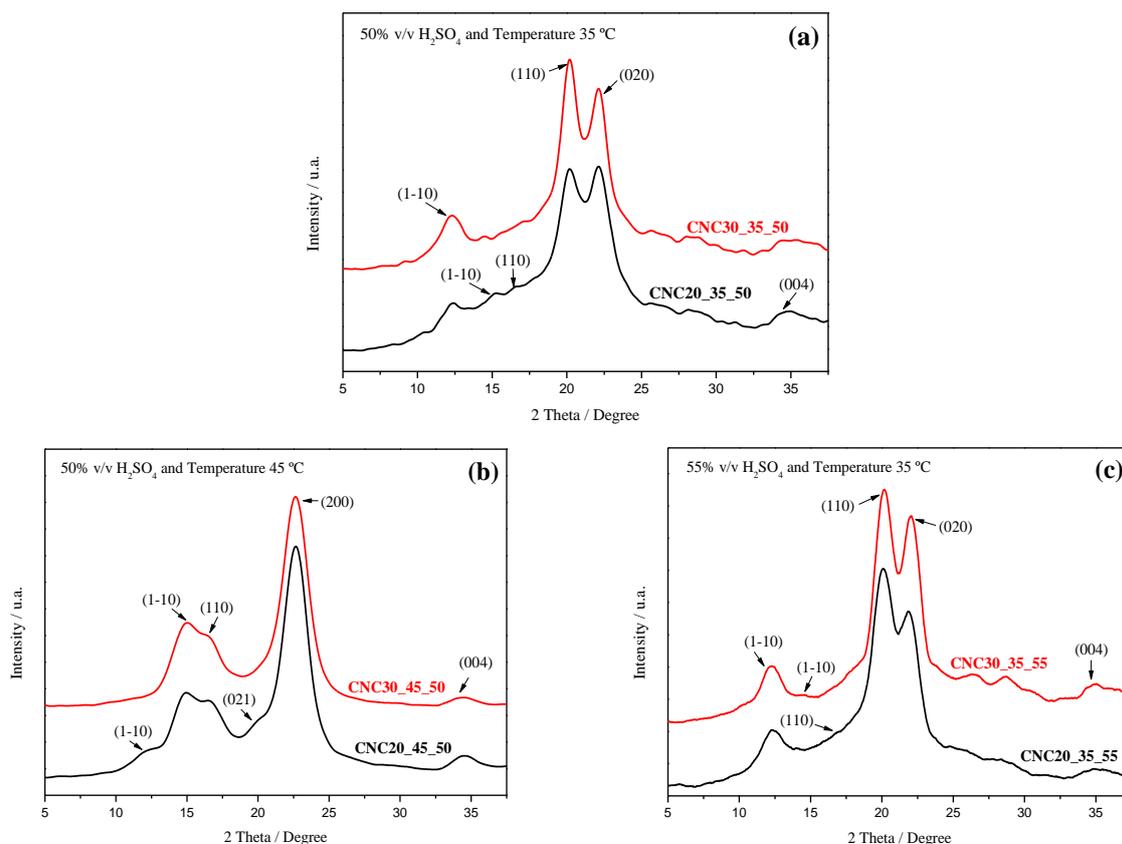


Figure 1: X-ray diffractograms for: (a) CNCs II prepared with 50% v/v H₂SO₄, temperature of 35 °C and reaction times of 20 and 30 min; (b) CNCs I with 50% v/v H₂SO₄, temperature of 45 °C and times of 20 and 30 min and (c) CNCs II with 55% v/v H₂SO₄, temperature of 35 °C and reaction times of 20 and 30 min

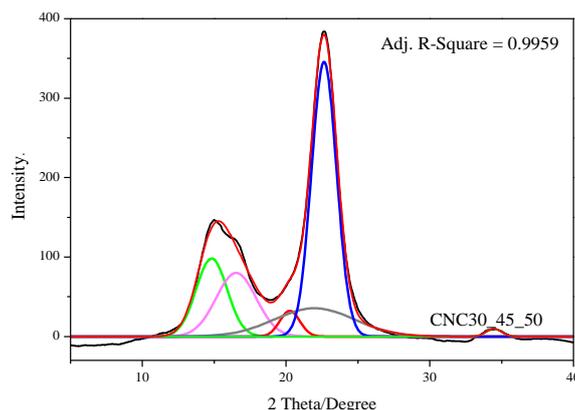


Figure 2: XRD diffraction spectra of a cellulose I sample showing the peak deconvolution method

As can be seen in Table 2, the obtained CNCs are formed by cellulose I or a mixture of cellulose I and II in different proportions. The CNCs obtained with 50 or 55% v/v of H₂SO₄ and reaction temperature of 35 °C have a crystalline structure of cellulose I and II, with cellulose II being predominant in all the samples. An increase in the percentage of cellulose II is observed with more severe hydrolysis conditions (longer reaction time and higher acid concentration), sample CNC30_35_55 (the most severe reaction conditions) presenting the highest percentage of cellulose II (93.82 ± 2.77%). For the nanocrystals obtained with 50% v/v of H₂SO₄ and a temperature of 45 °C, type I cellulose is predominantly observed. Under these hydrolysis conditions, the increase of the reaction time from 20 to 30 min allowed obtaining CNCs with 100% cellulose I.

The crystal size (Table 3) for cellulose I (plane 200), between 4.0 and 4.6 nm, is in agreement with those reported in other studies.^{13,34} It is observed that the crystal size for cellulose I decreased with increasing reaction time. For cellulose II (plane 020), it is observed that in the reactions with 55% v/v H₂SO₄, the crystal size also decreased with increasing reaction time. For the CNCs obtained with 50% v/v H₂SO₄, the crystal size increased with increasing reaction time. This characteristic can be related to the fact that sample CNC20_35_50 presents a great percentage of cellulose I in its composition, interfering in the definition of cellulose II peaks; and with the increase of the reaction time and consequent reduction of the percentage of cellulose I (sample CNC30_35_50), the peak for cellulose II became more defined. Larger crystal sizes for cellulose II compared to cellulose I are also reported in other studies.³³ For the d-spacing (nm) of planes 200 (cellulose I) and 020 (cellulose II), calculated by the Bragg equation, crystal sizes of 0.40 and 0.41 nm, were observed, respectively.

The presence of cellulose II is associated with reprecipitation of cellulose after hydrolysis, since the sulfuric acid solution may act as a solvent for this.^{35,43} The cellulose nanocrystals obtained at 35 °C, regardless of acid concentration (50 or 55% v/v) and reaction time (20 or 30 min) showed cellulose I and II characteristic peaks (Fig. 1 (a) and (c)). In these samples, the diffraction peaks corresponding to cellulose II in $2\theta = 12.2^\circ$ and $2\theta = 20.1^\circ$, planes 1-10 and 110 respectively, and the peak $2\theta = 22.1^\circ$ (plane 020), are observed. The peaks corresponding to cellulose I are observed at $2\theta = 16.7^\circ$ (plane 110) and $2\theta = 15.0^\circ$ (plane 1-10).

As shown in Figure 1 (a), the hydrolysis conducted with lower acid concentration and lower temperature (50% v/v acid and 35 °C) reduced the amorphous component from the starting material ($I_c = 67.80 \pm 0.32\%$), however, the small increase observed in crystallinity and the high yield (between 70 and 75% approximately) makes one believe that this hydrolysis condition is not adequate for the complete solubilization of the amorphous regions of the cellulose and the high yield observed is mainly associated with the hydrolysis and dissolution of the hemicelluloses present in the fibers.¹⁹

In the reactions conducted with the highest acid concentration, represented in Figure 1 (c) (55% v/v acid and 35 °C), the sample obtained with 30 min of reaction showed high crystallinity ($I_c = 85.26 \pm 1.93$), thus indicating that this hydrolysis condition was adequate to obtain CNCs II with high crystallinity. The yield of 25.3% indicates that despite high crystallinity under these hydrolysis conditions, a part of the cellulose crystals suffered degradation during the hydrolysis process.

For the reactions conducted at 45 °C, 50% v/v sulfuric acid and 30 min of reaction, the formation of CNCs with crystalline structure of cellulose I is observed (Fig. 1 (b)). The peaks corresponding to cellulose I are observed at $2\theta = 22.7^\circ$ (plane 200), $2\theta = 16.7^\circ$ (plane 110) and $2\theta = 15.0^\circ$ (plane 1-10).

However, for the sample prepared with a reaction time of 20 min (CNC20_45_50), a small peak is observed at $2\theta = 12.2^\circ$, corresponding to cellulose II, indicating that this sample also presents a small proportion of cellulose II in its composition. High crystallinity was observed in the sample prepared with 30 min of reaction where $I_c = 83.92 \pm 2.06$. Statistically, samples CNC30_45_50 and CNC30_35_55 did not show significant difference between the means, *i.e.*, they were statistically the same at a 95% confidence level ($p \geq 0.05$).

When comparing the yield results (Table 1) with those of crystallinity (Table 3), it can be observed that the CNCs obtained with 50% v/v H₂SO₄ at a temperature of 35 °C show the highest yields, but they also show the least crystallinity indices when compared to the other conditions evaluated. These results may indicate that in these samples, the nanocrystals are still formed by large amounts of amorphous regions not yet solubilized by sulfuric acid. If the reaction times of 20 and 30 min in these hydrolysis conditions are compared, it is observed that the reduction of the yield with the increase of the reaction time from 20 to 30 min caused an increase of the CNC crystallinity from 70.90 ± 1.12 to $75.67 \pm 0.24\%$. When analyzing the other reaction conditions, the same behavior is observed: decrease of the yield with the increase of the reaction time and increase of crystallinity. For the samples prepared with 50% v/v H₂SO₄ and reaction temperatures of 35 and 45 °C, it is observed that the increase in temperature also influences the characteristics of the materials obtained. In addition to reducing the CNC yield, the increase of the reaction temperature caused increased crystallinity due to a higher solubilization of the amorphous regions. It can be suggested that the increase of crystallinity by the solubilization of the amorphous regions would occur until an optimal point of crystallinity (maximum crystallinity) was reached, from which one would obtain materials with decreasing crystallinity due to solubilization of the crystalline regions with increasing reactions times.

As the regenerated cellulose (cellulose II) is more susceptible to hydrolysis than native cellulose (cellulose I),⁴³ it can be stated that the reactions conducted at 45 °C are more aggressive than those conducted at 35 °C, and that the hydrolysis temperature has a greater influence on the hydrolysis process of the amorphous regions than the increase of the sulfuric acid concentration from 50 to 55% v/v. It is observed that a different degree of cleavage of the crystalline cellulose chains occurs during hydrolysis with lower acid concentration.¹⁹ Thus, the type II cellulose recrystallization may have occurred simultaneously with chain cleavage during the hydrolysis process, which leads to the appearance of cellulose II nanocrystals.^{34,44,45} The treatment of cellulose with concentrated acids at room temperature may have an effect on cellulose, which is similar to alkaline treatment,⁴⁶ thus, after an intermediate swell with sulfuric acid and subsequent removal of the swelling agent at room temperature, the native cellulose can be transformed into cellulose II.⁴⁷

Martins *et al.*,³⁵ Sèbe *et al.*,³⁴ Jin *et al.*¹³ and Yue *et al.*³³ have also reported obtaining cellulose II nanocrystals through acid hydrolysis. However, these studies report having obtained cellulose II nanocrystals with much lower crystallinity than cellulose I. Martins *et al.*³⁵ obtained CNCs from Mombasa grass with a crystalline structure of cellulose II with maximum crystallinity of 69.5%, while those of cellulose I showed maximum crystallinity of 80%. Jin *et al.*¹³ obtained CNCs II by mercerizing CNCs I with 15 and 17 wt% NaOH. The authors report CNCs II with maximum crystallinity of 68.2%. Yue *et al.*³³ obtained CNCs II by hydrolyzing pretreated cotton fibers with 15 and 20% wt NaOH, and CNCs with structure I for cotton fibers pretreated with 10 wt% NaOH and not treated with NaOH. The obtained cellulose II nanocrystals presented maximum crystallinity of 63% while the cellulose I nanocrystals presented maximum crystallinity of 79.2%. Sèbe *et al.*³⁴ obtained cellulose II nanocrystals through the acid hydrolysis of cotton microcrystalline cellulose with sulfuric acid controlling both the amount of H₂SO₄ introduced as well as the time of addition. These authors do not report the crystallinity of the nanocrystals obtained. Although the raw materials used are different, it is observed that these studies report having obtained nanocrystals with crystallinity that is much lower than that obtained in this study. To our knowledge, it is the first time that CNCs II of high crystallinity and equal to the crystallinity of cellulose I are obtained.

Morphology

CNC morphology was evaluated by transmission electron microscopy. TEM is considered an efficient technique in the characterization of CNCs.⁴⁸ Under controlled conditions, the acid hydrolysis of the cellulose fibers dissolves the amorphous regions maintaining the crystalline domains. The treatment should also reduce the size of fibers from micron to nanometer scale.⁴⁹

Through TEM analysis, it was observed that the CNCs that presented a cellulose II crystalline structure, *i.e.* the samples prepared at 35 °C, presented predominantly particles in the form of spheres, as represented in Figure 3 (a). For the samples obtained at 45 °C and composed of cellulose I, predominantly rod-shaped particles were observed, as shown in Figure 3 (b). It is assumed that different reaction temperatures promote the dissolution of the amorphous part, breaking and organizing the crystalline part in a differentiated way, thus obtaining materials with different morphological characteristics (Fig. 4).

To the best of our knowledge, there are no reports on obtaining sphere-shaped CNCs II through hydrolysis with sulfuric acid. Jin *et al.*¹³ report on the formation of spherical CNC II through CNC I mercerization with 17 wt% NaOH and CNC I in the form of rods. According to the authors, the alkaline treatment resulted in the agglomeration of the spherical particles due to the reduction of the surface charge. Therefore, it would not be the correct route for obtaining spherical CNC II. In the works published by Sèbe *et al.*,³⁴ Yue *et al.*³³ and Martins *et al.*,³⁵ obtaining rod-shaped CNCs II with smaller diameters than those of cellulose I was verified.

In the samples prepared with 20 min reaction times, some particle agglomerates were observed, such as those observed in Figure 5 (a) and (b). This characteristic may be due to the drying process of the suspension on the carbon film,⁵⁰ or due to the ionic charge on the surface of these particles that tend to agglomerate larger particles due to the electrostatic attraction.⁵¹ According to Shi *et al.*,⁵² smaller particles have a higher surface charge, and, consequently, greater repulsion between them. Nanoparticle agglomerates have also been reported in previous work.^{50,53,54}

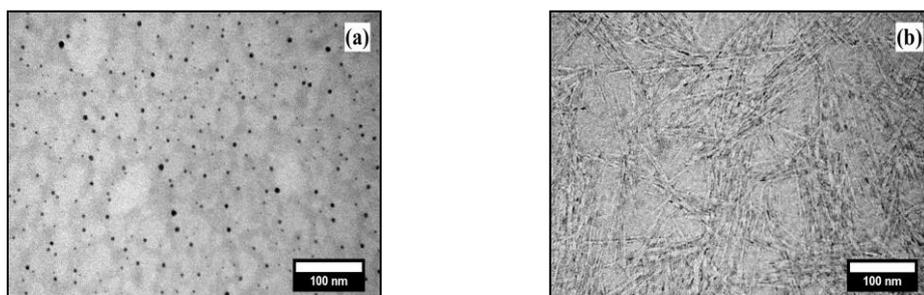


Figure 3: Morphology of sphere- (a) and rod-shaped (b) cellulose nanocrystals

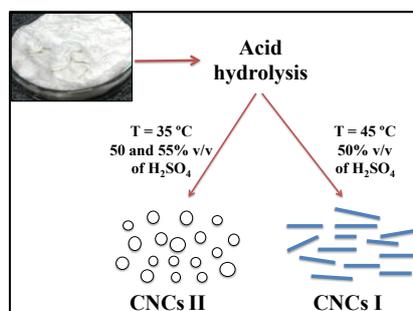


Figure 4: Reaction conditions for obtaining CNCs with cellulose I and II crystalline structures

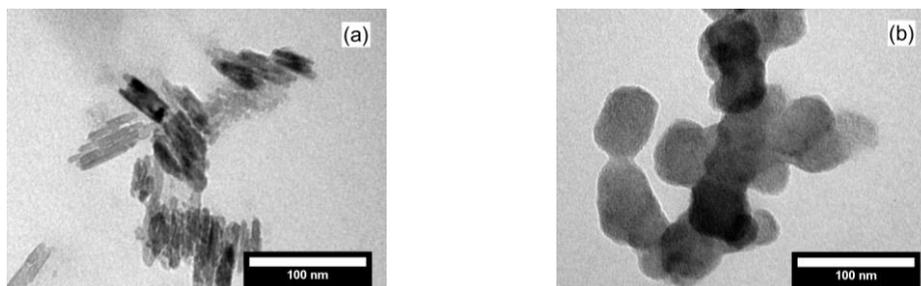


Figure 5: CNCs agglomerates observed in the samples prepared with 20 min hydrolysis: CNC30_45_50 (a) and CNC30_35_55 (b)

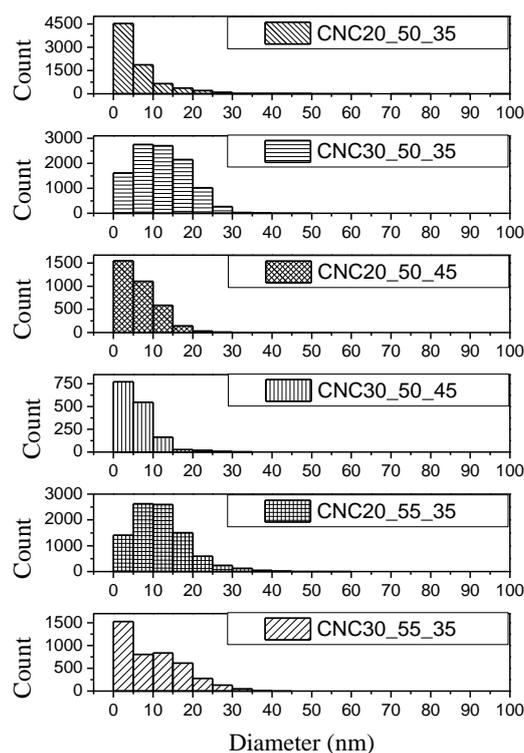


Figure 6: Particle size distribution

The diameter distribution of at least 1000 particles for each sample is shown in the histograms of Figure 6. Analyzing the histograms shown in Figure 6, all the samples have particles in the nanometric scale. However, as the cellulose cleavage occurs randomly during the acid hydrolysis process, the CNC dimensions are not uniform.⁵⁵

Evaluating the three conditions, it was observed that the acid concentration, hydrolysis time and temperature directly influenced the diameter (Fig. 6) and the shape of the CNCs (Fig. 2). Studying the acid hydrolysis of a commercial kraft pulp, Xu *et al.*²⁹ demonstrated that when the hydrolysis time increased from 25 to 30 min, the mean particle diameter decreased from 22.85 to 19.43 nm. The authors also observed that the increase in hydrolysis temperature and higher acid concentration also lead to a reduction in mean diameter. Jin *et al.*¹³ obtained spherical-shaped nanocrystals of cellulose II with diameters between 20 and 40 nm. Sèbe *et al.*³⁴ observed rod-shaped CNCs with a mean diameter of 6.3 ± 1.7 nm, while Yue *et al.*³³ reported diameters of 15.0 ± 4.5 nm and 14.2 ± 3.0 nm. The diameters observed here are larger than those observed by the cited authors. However, it is worth mentioning that most of the studies evaluate the size (diameter/length) and the shape of the CNCs through an average of approximately 100 particles. The analysis of few particles or images may not represent the reality of the sample as a whole.

According to a study by Yue *et al.*,³³ the use of CNCs II in the formation of nanocomposites with poly(ethylene oxide) (PEO) provided higher tensile strength gains than the nanocomposites prepared with CNCs I. These results were obtained even though CNCs II had lower crystallinity than CNCs I, 61.5% and 79.2%, respectively. These results make us optimistic, since the use of CNCs II with a high crystallinity as that obtained in this work could provide greater gains than those already reported by Yue *et al.*³³ The sphere shape of the CNCs II could also interfere in a positive way due to the greater surface area.

CONCLUSION

In this study, it was observed that the type of raw material does not determine the morphological characteristics and the crystalline structure of CNCs. Samples with different crystal structures and different morphologies can be obtained by varying only the hydrolysis conditions. Irrespective of the acid concentration, reactions conducted at 35 °C resulted in CNCs with a mixture of the cellulose crystal structures I and II, and with spherical morphology. The variations carried out under the

hydrolysis conditions resulted in CNCs with high percentages of cellulose II and high crystallinity. For the samples prepared at 45 °C, it was possible to obtain CNCs with high crystallinity, cellulose crystal structure I and predominant rod-shaped morphology. It is observed that, among the hydrolysis conditions, temperature has a greater influence on the characteristics of the final product obtained. The obtaining of CNCs II with high crystallinity can provide excellent gains as a reinforcing agent in several applications. This work demonstrates the possibility of obtaining CNCs with different characteristics using different acid hydrolysis conditions for a single source of raw material.

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REFERENCES

- ¹ M. Murali, S. V. Reddy, M. Manjusri, K. B. Sujata and A. Mohanty, *Prog. Polym. Sci.*, **38**, 1653 (2013).
- ² S. Hansson, E. Östmark, A. Carlmark and E. Malmström, *ACS Appl. Mater. Interfaces*, **1**, 2651 (2009).
- ³ H. P. S. A. Khalil, A. H. Bhat and A. F. I. Yusra, *Carbohydr. Polym.*, **87**, 963 (2012).
- ⁴ M. J. John and S. Thomas, *Carbohydr. Polym.*, **71**, 343 (2008).
- ⁵ S. Dumitriu, "Polysaccharides: Structural, Diversity and Functional Versatility", Marcel Dekker Inc., 2004, pp. 41-68.
- ⁶ C. Aulin, Ph. D. Thesis, Royal Institute of Technology, Stockholm, 2009.
- ⁷ G. Siqueira, J. Bras and A. Dufresne, *Polymer*, **2**, 728 (2010).
- ⁸ C. M. Lee, A. Mittal, A. L. Barnette, K. Kafle, Y. B. Park *et al.*, *Cellulose*, **20**, 991 (2013).
- ⁹ K. H. Gardner and J. Blackwell, *Biopolymers*, **13**, 1975 (1974).
- ¹⁰ Y. Nishiyama, G. P. Johnson, A. D. French, V. T. Forsyth and P. Langan, *Biomacromolecules*, **9**, 3133 (2008).
- ¹¹ I. Diddens, B. Murphy, M. Krisch and M. Muller, *Macromolecules*, **41**, 9755 (2008).
- ¹² H. Y. Wang, D. G. Li, H. Yano and K. Abe, *Cellulose*, **21**, 1505 (2014).
- ¹³ E. Jin, J. Guo, F. Yang, Y. Zhu, J. Song *et al.*, *Carbohydr. Polym.*, **143**, 327 (2016).
- ¹⁴ R. Li, J. Fei, Y. Cai, Y. Li, J. Feng *et al.*, *Carbohydr. Polym.*, **76**, 94 (2009).
- ¹⁵ H. Fukuzumi, T. Saito, T. Iwata, Y. Kumamoto and A. Isogai, *Biomacromolecules*, **10**, 162 (2009).
- ¹⁶ Y. Chen, C. Liu, P. R. Chang, X. Cao and D. P. Anderson, *Carbohydr. Polym.*, **76**, 607 (2009).
- ¹⁷ R. J. Moon, A. Martini, J. Nairn, J. Simonsen and J. Yougblood, *Chem. Soc. Rev.*, **40**, 3941 (2011).
- ¹⁸ S. Belbekhouche, J. Bras, G. Siqueira, C. Chappey, L. Lebrun *et al.*, *Carbohydr. Polym.*, **83**, 1740 (2011).
- ¹⁹ Z. Lu, L. Fan, H. Zheng, Q. Lu, Y. Liao *et al.*, *Bioresour. Technol.*, **146**, 82 (2013).
- ²⁰ Y. Habibi, L. A. Lucia and O. J. Rojas, *Chem. Rev.*, **110**, 3479 (2010).
- ²¹ H. Kargarzadeh, I. Ahmad, I. Abdullah, A. Dufresne, S. Y. Zainudin *et al.*, *Cellulose*, **19**, 855 (2012).
- ²² X. M. Dong, T. Kimura, J. F. Revol and D. G. Gray, *Langmuir*, **12**, 2076 (1996).
- ²³ S. Beck-Candanedo, M. Roman and D. Gray, *Biomacromolecules*, **6**, 1048 (2005).
- ²⁴ D. Bondeson, A. Mathew and K. Oksman, *Cellulose*, **13**, 171 (2006).
- ²⁵ M. F. Rosa, E. S. Medeiros, J. A. Malmonge, K. S. Gregorski, D. F. Wood *et al.*, *Carbohydr. Polym.*, **81**, 83 (2010).
- ²⁶ P. Lu and Y. L. Hsieh, *Carbohydr. Polym.*, **82**, 329 (2010).
- ²⁷ N. Johar, I. Ahmad and A. Dufresne, *Ind. Crop. Prod.*, **37**, 93 (2012).
- ²⁸ F. Jiang and Y. L. Hsieh, *Carbohydr. Polym.*, **95**, 32 (2013).
- ²⁹ Q. Xu, Y. Gao, M. Qin, K. Wu, Y. Fu *et al.*, *Int. J. Biol. Macrom.*, **60**, 241 (2013).
- ³⁰ H. Halonen, P. T. Larsson and T. Iversen, *Cellulose*, **20**, 57 (2013).
- ³¹ J. F. Revol, A. Dietrich and D. A. I. Goring, *Can. J. Chem.*, **6**, 1724 (2011).
- ³² K. Abe and H. Yano, *Carbohydr. Polym.*, **85**, 733 (2011).
- ³³ Y. Yue, C. Zhou, A. D. French, G. Xia, G. Han *et al.*, *Cellulose*, **19**, 1173 (2012).
- ³⁴ G. Sèbe, F. Ham-Pichavant, E. Ibarboure, A. L. C. Koff and P. Tingaut, *Biomacromolecules*, **13**, 570 (2012).
- ³⁵ D. F. Martins, A. B. De Souza, M. A. Henrique, H. A. Silverio, W. P. F. Neto *et al.*, *Ind. Crop. Prod.*, **65**, 496 (2015).
- ³⁶ H. A. Silvério, W. P. F. Neto, N. O. Dantas and D. Pasquini, *Ind. Crop. Prod.*, **44**, 427 (2013).
- ³⁷ A. D. French, *Cellulose*, **21**, 885 (2014).
- ³⁸ P. Ahvenainen, I. Kontro and K. Svedstro, *Cellulose*, **23**, 1073 (2016).
- ³⁹ Y. Hu and N. Abidi, *Langmuir*, **32**, 9863 (2016).
- ⁴⁰ C. Ruan, Y. Zhu, X. Zhou, N. Abidi, Y. Hu *et al.*, *Cellulose*, **23**, 3417 (2016).
- ⁴¹ S. Park, J. O. Baker, M. E. Himmel, P. A. Parilla and D. K. Johnson, *Biotechnol. Biofuels.*, **3**, 10 (2010).
- ⁴² W. Y. Hamad and Q. T. Hu, *Chinese J. Chem. Eng.*, **88**, 392 (2010).

- ⁴³ Q. Xiang, Y. Y. Lee, P. O. Pettersson and R. W. Torget, *Appl. Biochem. Biotechnol.*, **107**, 505 (2003).
- ⁴⁴ R. N. Ibbett, D. Domvoglou and D. A. S. Phillips, *Cellulose*, **15**, 241 (2008).
- ⁴⁵ L. G. Tang, D. N. S. Hon, S. H. Pan, Y. Q. Zhu, Z. Wang *et al.*, *J. Appl. Polym. Sci.*, **59**, 483 (1996).
- ⁴⁶ T. Heuzer, "The Chemistry of Cellulose", John Wiley and Sons Inc., 1994, pp. 133-135.
- ⁴⁷ B. G. Rånby, *Acta Chem. Scand.*, **6**, 101 (1952).
- ⁴⁸ D. Maria, S. Garcia and J. M. Lagaron, *Cellulose*, **17**, 987 (2010).
- ⁴⁹ M. A. S. Azizi Samir, F. Alloin and A. Dufresne, *Biomacromolecules*, **6**, 612 (2005).
- ⁵⁰ M. K. M. Haafiz, A. Hassan, Z. Zakaria and I. M. Inuwa, *Carbohydr. Polym.*, **103**, 119 (2014).
- ⁵¹ H. Liu, D. Liu, F. Yao and Q. Wu, *Bioresour. Technol.*, **101**, 5685 (2012).
- ⁵² J. Shi, S. Q. Shi, H. M. Barnes and J. C. U. Pittman, *BioResources*, **6**, 879 (2011).
- ⁵³ M. Jonoobi, A. Khazaeian, P. Md. Tahir, S. S. Azry and K. Oksman, *Cellulose*, **18**, 1085 (2011).
- ⁵⁴ N. Wang, E. Ding and R. Cheng, *Polymer*, **48**, 3486 (2007).
- ⁵⁵ M. Wada and T. Okano, *Cellulose*, **8**, 183 (2001).