

# CELLULOSE NANOCRYSTAL PRODUCTION FOCUSING ON CELLULOSIC MATERIAL PRE-TREATMENT AND ACID HYDROLYSIS TIME

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## ABSTRACT

Cellulose nanocrystals have been applied as reinforcing material in polymer matrices due to their excellent mechanical properties and the fact that they are derived from natural fibers, which are biodegradable in the environment and come from renewable sources. This paper studies the morphological, dimensional, chemical and surface characteristics of cellulose nanocrystals (CNC) isolated from cotton fibers pre-treated with sodium hydroxide (NaOH) 2% m/v focusing on hydrolysis time. Such characterization used transmission electron microscopy (TEM), X-ray diffractometry (XRD), Fourier-transform infrared spectroscopy (FTIR), thermogravimetry (TGA), zeta potential, dynamic light scattering (DLS) and energy-dispersive X-ray spectroscopy (EDS). The isolated cellulose nanocrystal yield was evaluated based on hydrolysis of the material with 65% sulfuric acid (m/m), at a temperature of 50 °C, acid:biomass ratio of 20 mL g<sup>-1</sup> and hydrolysis times of 50, 70, 90, 110 and 130 min. It was found that treatment with 2% NaOH provided satisfactory accessibility to the fibers and the effect of hydrolysis time on CNC production showed a peak yield at 110 min, 33%. The CNC had negative surface charges, -30.0 to -53.3 mV, giving stability to the suspensions. CNC obtained under the conditions evaluated can be used in the composition of composites in various hydrophilic matrices because maximum degradation temperatures are above 200 °C and because they are stable and of uniform size.

**Keywords:** Cellulose nanocrystals, nanomaterials, pre-treatment, acid hydrolysis, yield.

## 1. INTRODUCTION

Research into the development of biodegradable materials from renewable sources is growing. Some of the main reasons for this include: (1) damage to the environment when using polymer materials from fossil sources, which are unsatisfactory in terms of biodegradability for the planet's current conditions; (2) the lack of sustainability of

this source leading to uncertainty in its supply considering medium and long-term scenarios; and (3) the availability of biopolymers, at relatively low cost when compared to synthetic nanoparticles.

Several raw materials have been used to obtain cellulose nanocrystals, such as eucalyptus, tunicates, algae, sisal, bamboo, cotton, etc., as well as agricultural waste such as corn husk, soybean hulls and sugarcane bagasse, among others (SILVA *et al.*, 2009 and PEREIRA *et al.*, 2014). However, as the high content of cellulose results in higher yield and high quality products (MERLINI, 2016), cotton fiber was chosen for this study as one of the raw materials with the highest cellulose content in the biosphere, 88-96% according to Martins *et al.*, 2011.

Since processing conditions may influence the characteristics of these nanomaterials, it becomes important to be aware of several aspects related to the production process, such as the conditions of the isolation process: hydrolysis time and temperature; type and concentration of the acid used, as well as the ratio between it and the biomass, and the methodology for purifying and drying these nanostructures. All these aspects may influence, to a greater or lesser degree, the morphological and surface characteristics of cellulose nanocrystals and, as a result, the characteristics of the materials when they are used, in addition to affecting production yield. Thus, the main aim of this study was to examine the influence of hydrolysis time on the yield and on the morphological, dimensional and surface charge characteristics of cellulose nanocrystals (CNC) in order to produce uniform, high quality material with reproducible characteristics. In this way, understanding this influence is important to take full advantage of the potential of these nanoparticles for later use.

## 2. METHODS

The experiments were conducted at the Industrial Processes and Materials Laboratory (LABPIM) of the Department of Chemistry, and characterizations at the Packaging Laboratory (LABEM) of the Department of Food Technology, the X-ray Diffraction at the

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This study was conducted using Brazilian cotton fibers supplied by Companhia Industrial de Cataguases, located in the city of Cataguases (MG), as the source of cellulose nanocrystals. Sigma Aldrich concentrated sulfuric acid, 95-99%, was used to prepare the solution with a concentration of 65% m/m. Sodium hydroxide (NaOH), Prochemicals brand, 97%. Alphatec brand Acetone, 99.8%. Deionized water was used in all experiments, either in the preparation of the reagent or in the CNC production process.

## 2.1 The experiment

### 2.1.1 Pre-treatment

The cotton fiber was manually cleaned to remove visible solid impurities, such as bits of stalks and leaves. The sample was stored in a polyethylene bag and its dry content was determined using adaptations of the TAPPI T 550-om-98 standard (TAPPI, 2000). The masses of the empty containers were determined after being dried in the oven at the same temperature, and with the cotton samples, and then dried in an oven at  $105 \pm 3$  °C until constant mass. Results are in triplicate. The o.d. (oven-dried) content can be calculated using Equation 1.

$$To.d. = \frac{C - A}{B - A} \times 100 \quad (1)$$

In which: To.d. content: absolutely dried content (%); A: container mass (g); B: sample mass + container (g); C: dry sample mass + container (g).

Initially, the cotton fiber was pre-treated using extraction following the methodology adapted from TAPPI T 264-om-97 (TAPPI, 2000). Extraction with acetone was carried out in a Soxhlet extractor for 6h, 6 cycles/h, in order to promote removal of waxes and fats contained in the cell wall of the fiber to purify it. To evaluate this treatment, the hydrodynamic diameter of the particles of the suspensions obtained was determined.

In order to increase acid accessibility to the cotton fibers so as to achieve greater uniformity of the hydrolysis reaction, pre-treatment was carried out with NaOH at different concentration levels, namely 2%, 4%, 8% and 17.5% (m/v), dry basis starting material under conditions adapted from Roohani *et al.* (2008), namely: 60 minutes, fiber ratio alkaline solution 1g: 50 mL, 70 °C. Halfway, it was shaken in order to guarantee homogeneous pre-treatment action in all the biomass used. After the alkaline treatment time had elapsed, the samples were washed with deionized water until filtrate pH values between 6 and 7 were reached. The material was washed in a fine mesh fabric bag to prevent loss of fibrous material. After washing, the assembly (bag and fibers) was centrifuged for 3 cycles of 6 min each at 750 rpm. Afterwards, the material was transferred quantitatively

to a beaker and left in desiccator until constant mass was reached. 2 o.d. cotton fiber was treated for each hydrolysis performed. The choice of alkaline treatment concentration was made according to the crystallinity index determined by X-ray diffraction. In order to determine the alkaline pre-treatment yield, the cotton samples were dried at a temperature of 60 °C until constant mass was obtained and then the samples were placed in desiccator for 72 hours, which was determined according to Equation 2.

$$R(\%) = \left( \frac{m_f}{m_i} \right) \times 100 \quad (2)$$

In which: R: yield (%);  $m_f$ : mass obtained after drying (g);  $m_i$ : initial mass of biomass (g) (dry basis).

### 2.1.2 Isolation of nanocrystals *via* acid hydrolysis

#### 2.1.2.1 Effect of centrifugation stage on CNC yield

Before evaluating the effect of hydrolysis time on yield and CNC characteristics, an experiment was carried out to evaluate which centrifugation cycle would be adopted in order to achieve the most stable and uniform material possible. CNC were isolated under the following process conditions: 20 mL of H<sub>2</sub>SO<sub>4</sub> 65% m/m; 50 min; 50 °C per gram of dry cotton pre-treated with NaOH 2% m/v, and carried out in triplicate. These suspensions were generated in 6 cycles of centrifugation. The pH and turbidity of the suspensions were evaluated prior to the dialysis step (pH adjustment).

Aliquots of 10 mL of each suspension of cellulose nanocrystals were dried at 60 °C in an oven with air circulation until constant mass, following dialysis (adapted from MARTINS *et al.*, 2011). To estimate the yield (R %) in CNC, Equation 3 was used. The results were presented as mean and standard deviation of the triplicates. For calculation purposes, yields were calculated considering the total volume generated from each suspension. After the centrifugation stage, the residue was dried until constant mass in an oven at  $105 \pm 3$  °C, with air circulation and then quantified.

$$R(\%) = \left( \left( \frac{MRo.d. - MR}{Ma} \right) \times \frac{Va}{10} \right) \times 100 \quad (3)$$

In which: R: yield (%); MRo.d.: mass of the container with the dry sample (g); MR: container mass (g); Ma: pre-treated cotton initial mass (dry basis) (g); Va: total volume of analyzed sample (mL).

#### 2.1.2.2 Effect of hydrolysis time

The hydrolysis conditions for studying time at five levels, 50, 70, 90, 110 and 130 min were as described in item 2.1.2.1. The pre-treated cotton fiber was subject to hydrolysis in a beaker, while stirring with a magnetic stirrer, according to the conditions described previously, being performed in triplicate. Next, the hydrolysis product was vacuum-filtered in a sintered glass crucible and received in kitasate containing 150 mL deionized water 0° C in order to stop the hydrolysis reaction. After this stage, the suspensions were centrifuged in a Hermle Z 326K centrifuge at

12,000 rpm, force equivalent to 16,580 g, in 15 min cycles and temperature of 23 °C. After each centrifuge cycle, the supernatant was collected. The precipitate was retained in the tube, to which deionized water was added, stirred, and subject to another cycle of centrifugation. This procedure was performed until the third cycle of centrifugation. At this stage, using a laser, the presence of suspended nanocrystals was observed. In this case, the presence of nanoparticles in the suspension would form a light beam due to light scattering. In addition, the pH and turbidity of the suspension obtained in each cycle was evaluated. The residues obtained after the centrifugation cycles were conditioned for further quantification. Subsequently, the nanocrystalline suspensions were dialyzed against deionized water on cellulose membrane SERVA® dialysis tubing, MWCO 3500, for a time such that the last dialysis water had a pH between 6 and 7. The dialyzed suspensions were sonicated for 20 min at 25 °C. Cellulose nanocrystals, products of acid hydrolysis, were named H50, H70, H90, H110 and H130, for hydrolysis time of 50, 70, 90, 110 and 130 min, respectively.

## 2.2 Characterization of cellulose nanocrystals

### 2.2.1 Zeta potential ( $\xi$ ) and Light scattering (DLS)

Measuring zeta potential is a way of evaluating the surface charge in mV and discussing the stability of suspensions, thus obtaining information about their behavior over time. In addition to this measure, the average size (hydrodynamic diameter) of particles was analyzed. This was determined through Dynamic Light Scattering – DLS and from measuring electrophoretic mobility using Zetasizer equipment, Nano Series by Malvern Instruments. The analyses were performed at room temperature using a fixed detection angle of 173°, operating at a wavelength of 633 nm. The data was obtained in triplicate.

### 2.2.2 X-ray diffractometry (XRD)

The relative crystallinity index of the starting materials and nanocrystals studied were determined by X-ray diffraction as described by Segal *et al.* (1959), in accordance with Equation 4.

$$I_c = \left( \frac{I_{002} - I_{am}}{I_{002}} \right) \times 100 \quad (4)$$

In which:  $I_c$  = crystallinity index (%);  $I_{am}$  = intensity of amorphous diffraction;  $I_{002}$  = maximum diffraction intensity.

The X-ray diffraction analyses were performed at room temperature in a Bruker D8-Discover diffractometer using Ni filter and Cu- $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ), angular variation of 10 to 40° ( $2\theta$ ), 3°/min speed, 40kV voltage and 40 mA current.

To calculate  $I_c$ , in accordance with Equation 4, by means of the relation between the peaks of maximum intensity for the plane (002) which is located at a diffraction angle around  $2\theta = 22.6^\circ$  and the intensity dispersed by the amorphous part,

which is measured as the minimum intensity at an angle of diffraction of about  $2\theta = 18^\circ$ , after correction of the baseline. By means of X-ray diffraction results and by comparison with results in literature, it was possible to make inferences about the type of cellulose found in the unprocessed sample and in the fiber after pre-treatment.

### 2.2.3 Thermogravimetric analysis (TGA)

Changes in the thermal behavior of the starting sample and the CNC at different hydrolysis time levels were evaluated by thermogravimetric analysis. For this analysis, a Shimadzu DTG60H apparatus was used. The analyses were carried out under a nitrogen gas atmosphere at a constant flow of 50 mL min<sup>-1</sup>, using approximately 4 mg of CNC. The thermogravimetric curves were obtained from 30 °C to the maximum temperature of 700 °C, with a heating rate of 10 °C min<sup>-1</sup> (ROMAN and WINTER, 2004). The thermogravimetric (TG) curve was obtained to evaluate the loss of mass as a function of temperature. From the first derivative of the loss of mass as a function of temperature, the second curve (DTG) was generated, which enabled the maximum thermal degradation temperatures of the samples for the five hydrolysis time levels studied to be determined. Based on the TG curves, mass loss calculations (%) were made. The initial, maximum and final thermal degradation temperatures were determined considering the wet mass of the sample at room temperature and the oven-dried mass at the temperature of 100 °C.

### 2.2.4 Transmission electron microscopy (TEM)

Transmission electron microscopy analyses were used to obtain information about the morphology and dimensions of the nanocrystals obtained. The transmission electron microscope used in these analyses was the Zeiss EM 109 to 80 kV model. To prepare samples for TEM analysis, suspensions at a concentration equal to 0.6 g L<sup>-1</sup> were sonicated in ultrasound (Cristáfoli®) for 24 min, then one drop of the suspension was placed on a nickel grid (300 Mesh) covered with a Formvar film. After 5 min, the excess water was removed with filter paper. With the nanocrystals already adsorbed to the grid, it was inverted over a drop of 0.5% (m/v) uranyl. After 15 min, the grid was removed from the uranyl and passed through a drop of distilled water to remove the excess contrast agent. The duly identified grids were dried for 24 hours, at room temperature, inside a desiccator closed with silica gel. For each suspension, of the samples referring to the times of 50, 90 and 130 min, two grids were made. After obtaining the photomicrographs, the ImageJ program was used to estimate the diameter and length of the nanocrystals.

### 2.2.5 Energy-Dispersive X-ray Spectroscopy (EDS)

Energy-dispersive X-ray spectroscopy (EDS) identifies the relative elemental composition of a given sample. EDS analyses are integrated features of the scanning electron microscope. In this study, the JEOL - JSM - 6010LA model was used, operated with a 15 kV acceleration

voltage. For analysis of the surface of the cotton fibers and the nanocrystals, the samples were fixed on a carbon tape and secured in sample holders.

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of pre-treatment

For the initial pre-treatment, with acetone extraction only, there was no isolated CNC production following the acid hydrolysis step (Table 1). In a way, these results make sense as acetone has only acted solubilizing soluble substances in this solvent that are part of the cell wall. The mean values of the hydrodynamic diameter of the nanocrystals, shown in Table 1, were based on the cumulating method for adjusting the DLS experimental data that gives a value for the diameter known as the Z-mean.

Given then results achieved, for the two hydrolysis times studied, and compared with those in literature for cotton fibers (250 and 150 nm, LIMA *et al.*, 2003, and MARTINS *et al.*, 2011, respectively) the need was found to carry out a pre-treatment able to promote accessibility of the acid in the structure of the cotton fiber. The residues were not quantified and no further analyses of the CNC suspensions obtained after the centrifugation step of the hydrolyzed material and treated only with extraction in acetone were performed.

**Table 1. Average values of hydrodynamic diameter of the CNC following extraction of the fibers with acetone**

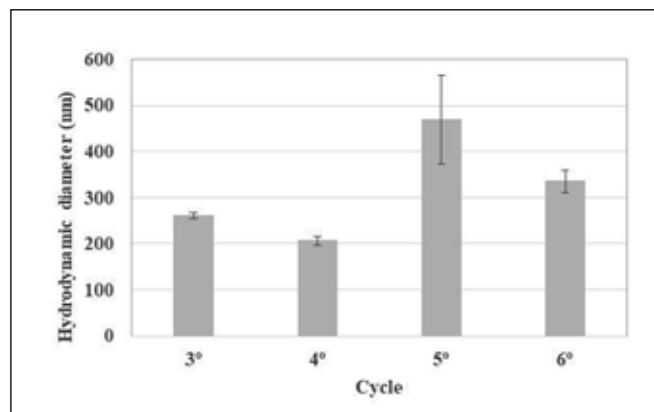
Cycle	H50 min, nm	H70 min, nm
3	1,510	878
4	1,552	985
5	2,260	1,078
6	2,542	1,492

Thus, the cotton fibers were treated with alkaline solutions, with different concentration levels, without previous extraction with acetone, and later subjected to acidic hydrolysis as described in the methodology for this study. The tests to evaluate the level of alkali concentration to be used in the pre-treatment were based on the crystallinity index obtained from X-ray diffraction analysis, the values for each level were as shown in Table 2.

**Table 2. Crystallinity index for natural cotton and pre-treated with NaOH.**

Sample	Ic (%)
Natural cotton	90.3
NaOH 2%	70.8
NaOH 4%	62.2
NaOH 8%	59.3
NaOH 17.5%	53.4

To proceed with the studies, NaOH 2% pre-treatment was chosen, because this pre-treated fiber had a higher crystallinity



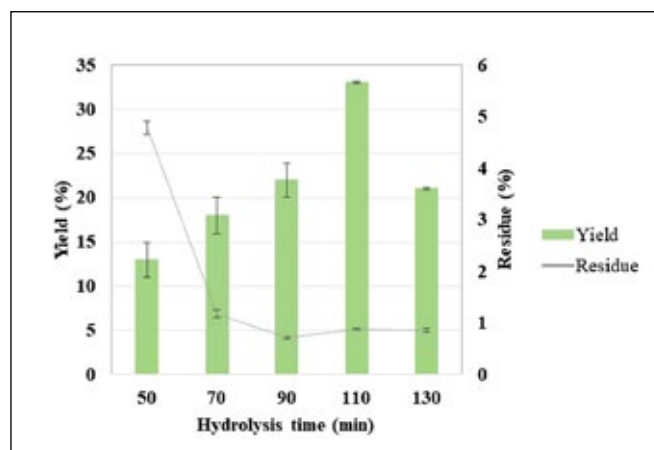
**Figure 1: Mean hydrodynamic diameter results for the different centrifuge cycles for fibers pre-treated with 2% NaOH (hydrolysis conditions: 20 mL of 65% w/w H<sub>2</sub>SO<sub>4</sub> per gram of pre-treated dry cotton 50 min 50 °C)**

index between the levels processed. The dimensions of the CNC obtained with the 2% NaOH pre-treatment were satisfactory in relation to the hydrodynamic diameter when compared with literature as can be seen in Figure 1. This may have been achieved due to the increase of accessibility of the acid to the hydroxyls of the cellulose chains, caused by the swelling of the material with the alkaline treatment and consequent improvement in the reactivity of the acid with the reactive hydroxyls more evenly in the fiber structure.

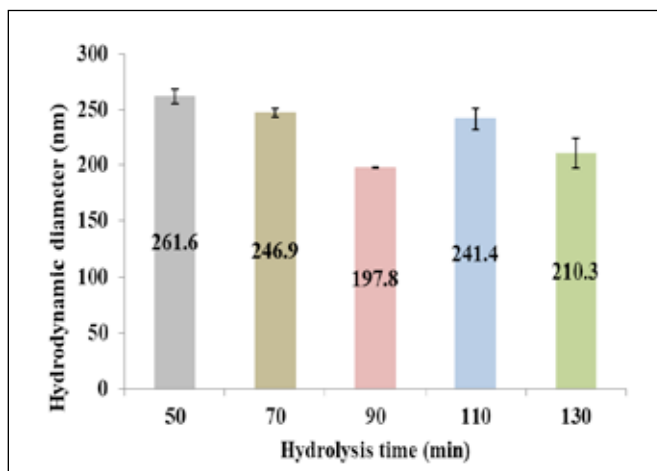
#### 3.2 Effect of hydrolysis time on CNC production

##### 3.2.1 Yield assessment

The mean yield after alkaline pre-treatment, 2% NaOH was 92.24% ±0.01%. According to the results shown in Figure 2, CNC yield increased with the hydrolysis time up to the time of 110 min, reaching the maximum value, ≈33%. For hydrolysis time studied in excess of this, the mean yield was lower. This fact may be related to the hydrolysis of accessible surface glucose units. The behavior of the residue values was consistent with yield, with reduction in hydrolysis



**Figure 2: Effect of hydrolysis time on yield and residue.**



**Figure 3:** Effect of hydrolysis time on the hydrodynamic diameter measured by light scattering.

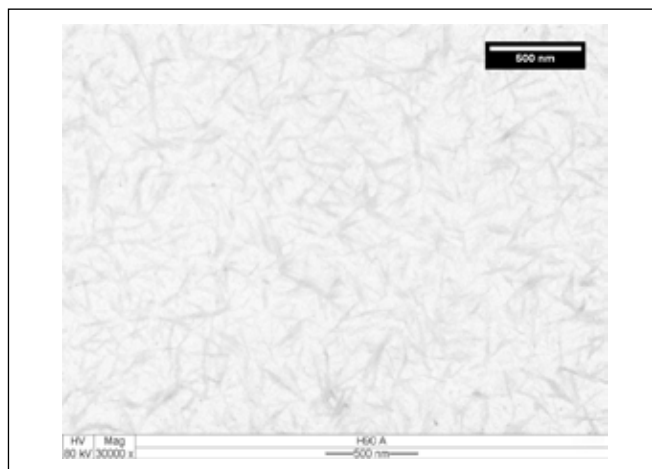
time tending to stability after 90 min. Thus, for the conditions studied, the best value corresponds to the time of 110 min. Literature depicts values of 30% to approximately 70% yield. However, the values quoted are not specified as to which spin cycle, implying that they would be all cycles together, i.e. yield of the centrifugation step as a whole. However, such comparisons should be made with caveats, since several factors influence this parameter, such as the type of cellulosic starting fiber, the acid hydrolysis conditions used, as well as the centrifugation cycles.

### 3.2.2 Dimensions and morphology

The mean results found in the CNC suspension for the hydrodynamic diameter extracted from the cotton fiber are shown in Figure 3. Through the images obtained by transmission electron microscopy for hydrolysis times of 50, 90 and 130 min, it is possible to see that the nanocrystals were individualized with elongated format of high aspect ratio (Figure 4). The results of length, estimated using the *ImageJ* program (Table 3), were smaller when compared with those achieved by DLS (Figure 3), this because the measure obtained in the DLS is relative, as the equipment takes the measurement from a fixed angle ( $173^\circ$ ) which understands that the particles are spherical because they are in Brownian motion, therefore, the values are higher than the values of the microscopy, besides the high concentration use

**Table 3. CNC dimensions for times of 50, 90 and 130 min estimated using ImageJ.**

Sample	Length (nm)	Diameter (nm)	Aspect ratio (L/D)
H50 min	161.3 ± 23	12.7 ± 1.1	12.7 ± 0.78
H90 min	175.6 ± 8.9	13.6 ± 1.9	13.4 ± 1.9
H130 min	164.6 ± 4.4	10.9 ± 0.92	15.6 ± 1.1

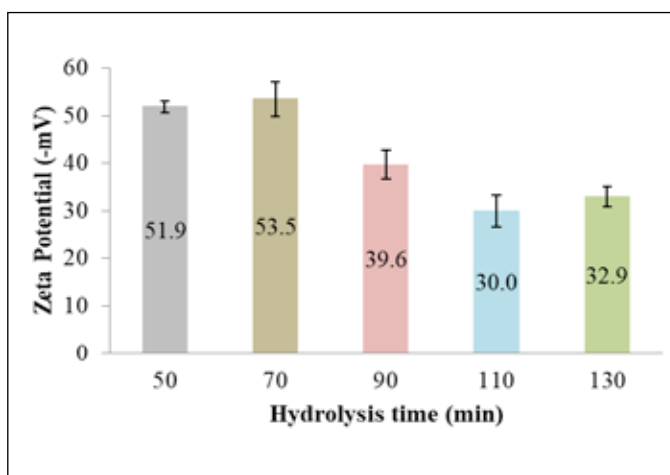


**Figure 4:** Transmission electron microscopy images for H90 min

Even the suspensions having been subject to 24 min of sonication, the images show some CNC aggregates, especially for the time of 130 min. This can be explained by the reduction in the potential zeta value, in module, shown in Figure 5 for times of 50, 90 and 130 min. This behavior may be related to the reduction of the negative group content, thus reducing the stability of the suspension and increasing the trend of aggregate formation. The reduction of the negative groups can be explained in part by the reduction of the sulfur content with hydrolysis time, as shown in Table 4 for these respective hydrolysis times.

### 3.2.3 Zeta Potential

The surface charge is an important parameter since it is related to the stability of the suspension. During the process of producing nanocrystals from hydrolysis with sulfuric acid, sulfonation reactions of the hydroxyls occur forming sulfate esters that are negatively charged (HENRIQUE *et al.*, 2015). The reduction of zeta potential over time may be due to the reversibility of the sulfonation reaction over time after 70 min.



**Figure 5:** Mean distribution of the zeta potential as a function of hydrolysis time.

### 3.2.4 Energy-Dispersive X-ray Spectroscopy (EDS)

Values achieved for the sulfur content in the EDS analysis are shown in Table 4 for the times studied. It is expected that longer hydrolysis time would generate a higher formation of sulfate groups and, consequently, a high sulfur content (DONG; REVOL; GRAY, 1998; ROMAN and WINTER, 2004). However, this was not observed in this study. This event can be justified by suspensions being slightly acidic pH ( $4 \leq \text{pH} \leq 5$ ). During the reaction of the cellulosic material with sulfuric acid, in addition to acid catalyzed hydrolysis, the sulfonation reaction of hydroxyl groups occurs (SJOSTROM, 1993). As this reaction is reversible, it is possible that, with the development of the hydrolysis reaction, the desulfonation reaction is encouraged. Another aspect that could be considered would be that, as reaction time increases, the cellulosic material, especially the amorphous region, is being degraded. These materials also compete with the same sulfonation reaction that occurs on the CNC surface.

**Table 4. Composition of carbon (C), oxygen (O) and sulfur (S) of CNC and cotton samples.**

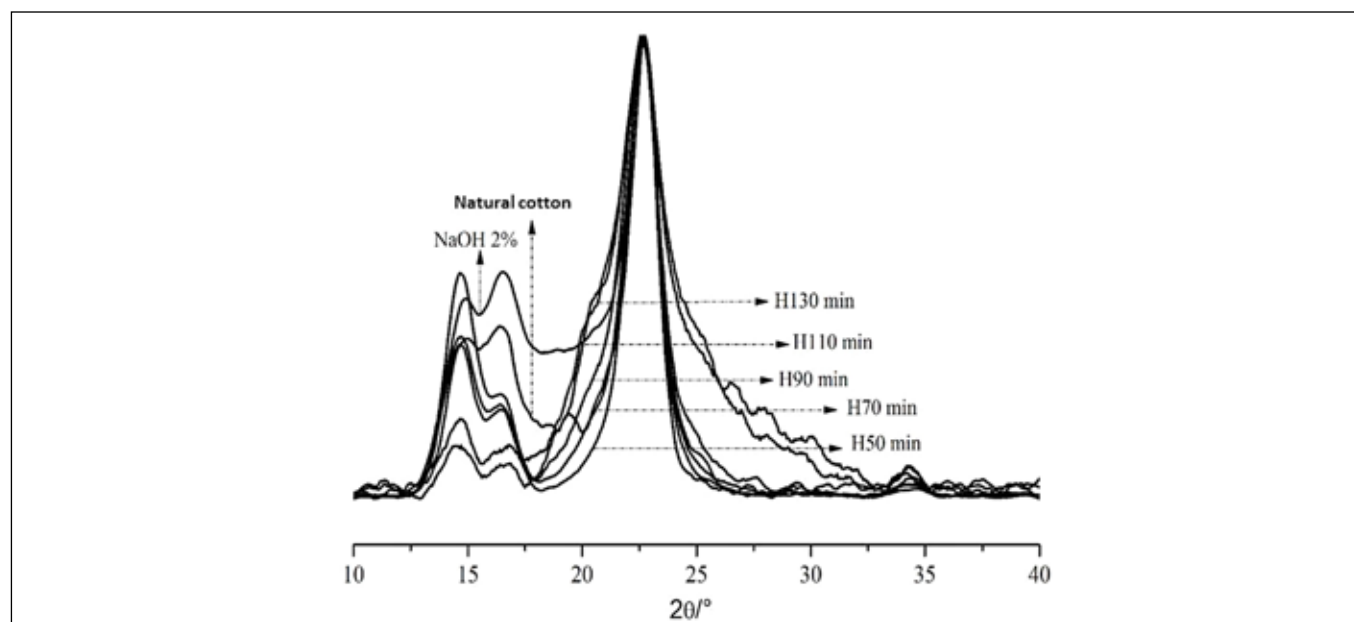
Sample	C (%)	O (%)	S (%)
Natural cotton	77.58	22.42	-
H50 min	74.21	28.75	1.04
H70 min	76.03	45.65	0.96
H90 min	70.52	28.21	0.69
H110 min	54.01	45.65	0.34
H130 min	70.52	28.75	0.52

### 3.2.5 Evaluation of crystallinity and polymorphism

The X-ray diffractograms of the natural cotton fiber, pre-treated with 2% NaOH and the cellulose nanocrystals obtained under different acid hydrolysis time conditions are shown in Figure 6. The presence of two peaks close to  $16^\circ$  was found for the natural and pre-treated fiber which, according to literature, is specific to fibers with higher cellulose content, such as cotton (MARTINS *et al.*, 2011). It can also be inferred, from literature, that nanocrystals treated with different hydrolysis times and natural cotton fibers and those treated with NaOH 2% have a predominance of cellulose I, native cellulose crystalline structure, through the characteristic peaks presented in around  $2\theta = 14.7^\circ; 16.5^\circ; 22.6^\circ$  and  $34.3^\circ$  (KRÄSSIG, 1993; FORD *et al.*, 2010; HENRIQUE *et al.*, 2015 and SUN *et al.*, 2016).

### 3.2.6 Thermogravimetric analyses (TG/DTG)

Thermal stability is an important parameter for choosing the nanoparticle to be used as reinforcement in a polymer matrix (ROMAN and WINTER, 2004). The thermogravimetric curves for the pretreated fiber with 2% NaOH and the nanocrystals are shown in Figures 7A and 7B, respectively. The TG curves correspond to mass loss as a function of temperature and the DTG curves refer to the first derivative of the TG curves and are related to the mass variation of the sample as a function of time while the sample is subject to a temperature range. The corresponding values are shown in Table 5. The curves show the thermal events occurring, the first (1) observed for both the pre-treated fiber and the CNC samples, between approximately 30 and  $160^\circ\text{C}$  and is generally attributed to evaporation of the adsorbed



**Figure 6:** X-ray diffraction of natural cotton, pre-treated with 2% NaOH and CNC.

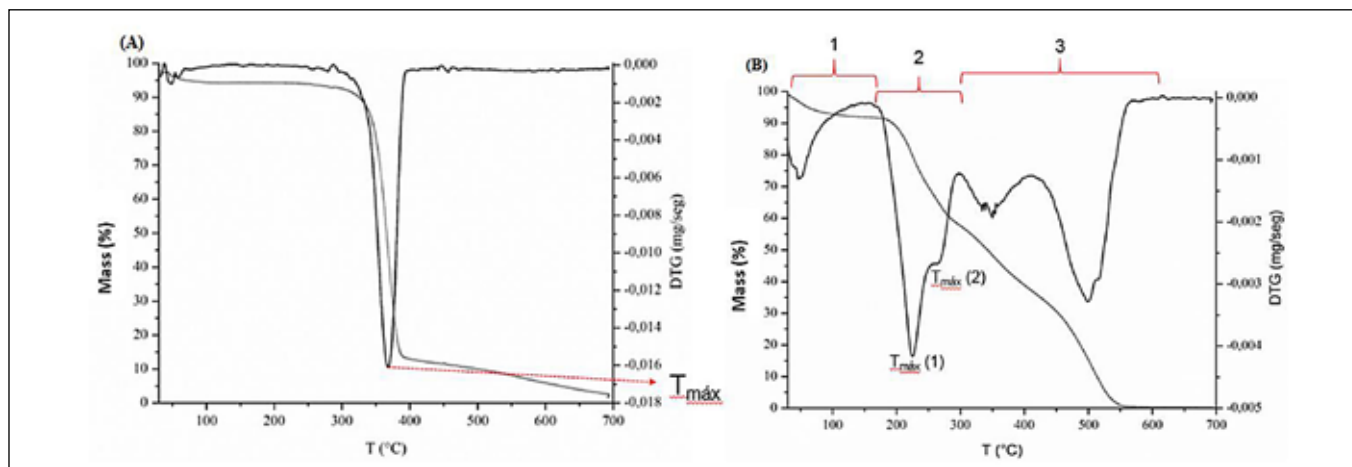


Figure 7: TG/DTG curves of the samples of (A) pre-treated cotton fiber and (B) CNC.

water. For CNC, the percentage of water released was around 6.5% and for fiber it was, on average, 4%. The second event (2) refers to the process of thermal decomposition of cellulose that consists of several processes: depolymerization, dehydration and decomposition of the glycosidic units (ROMAN and WINTER, 2004). In this event, we observed two peaks for the CNC sample, which according to literature, the first ( $T_{max}(1)$ ) is attributed to the degradation of the most accessible regions, which are highly sulfated, and the second ( $T_{max}(2)$ ) corresponds to the breaking of the crystalline fraction that was not attacked by the sulfuric acid. (FORTUNATI *et al.*, 2013). The third event (3), observed only for CNC (Figure 7B), is associated with cellulose pyrolysis (studies indicate that they occur in the 300 to 600° range).

Some studies report that peaks above 425 °C refer to the degradation of carbonized waste generating low molecular weight gaseous products. Based on Table 5, it can be seen that the CNC degradation temperatures were higher than 200 °C, which is positive because the matrices that will have the CNC incorporation have a melting point close to this temperature. Thus, the degradation temperature of the CNC should be higher, as the goal is to increase the strength of the matrix. In general, the highest percentage of mass loss occurred between the maximum degradation temperatures. Regarding residual mass, the CNC with the highest sulfur content probably degrade in a larger proportion, generating low mass at the end of the analysis, while the less sulfated ones obtained a larger residual mass.

Table 5. Thermal events and respective mass losses.

Sample	T (°C)	Tmax (°C)	Mass loss (%)	Residual mass 700 °C
Cotton (NaOH 2%)	315-400	368	78.38	0
	140-300		34.92	
H50 min	300-415	234-270	20.73	0.05
	415-580		35.68	
H70 min	160-300	225	34.23	14.70
	300-410		20.28	
H90 min	410-570	256-280	36.85	4.55
	200-320		38.3	
H110 min	320-415	261	17.64	2.60
	170-290		32.38	
H130 min	290-400	267	22.35	0.75
	400-520		36.67	
	180-295		33.56	
	295-395		21.39	
	395-520		32.64	

#### 4. CONCLUSIONS

The main objective of this study was to evaluate the effect of hydrolysis time on dimensional and surface load characteristics. According to the results achieved in this study, the following conclusions can be drawn:

The fibrous material used, natural cotton, was resistant to acid hydrolysis under conditions normally used to obtain cellulose nanocrystals, showing the importance of a chemical pre-treatment to provide uniform acid access to the structure;

The alkaline pre-treatment stage with 2% NaOH was efficient at improving fiber accessibility and homogeneity of hydrolysis to obtain cellulose nanocrystals;

pH and turbidity measurements were shown as simple and efficient techniques to control the centrifugation stage, avoiding CNC losses, being confirmed by the determination of the yield and the concentration of the suspension in subsequent analyses;

The effect of time did not obtain clear behavior in the physical, morphological, surface and surface charge characteristics;

The effect of hydrolysis time on CNC production had a maximum

yield point at 110 min, showing that adjusting conditions may improve this result. Although the maximum yield value, 33%, is not high, using the hydrolysate to produce cellulosic ethanol could be a joint alternative;

The nanocrystals had negative surface charges conferring stability to the suspensions of these materials. Although there was no clear trend in the effect of time, all suspensions were stable;

The nanoparticles obtained under the conditions evaluated can be used in the composition of composites in various hydrophilic matrices, as maximum degradation temperatures are above 200 °C because they are stable and of uniform size.

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