

Preparation and Characterization of Cellulose Nanocrystals using Soluble Grade Cellulose from Acid Hydrolysis of Huizache (*Acacia farnesiana* L. Willd.)

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High purity cellulose nanocrystals (NCCs) were obtained through the generation of various nanoparticle suspensions from different hydrolysis conditions of soluble grade cellulose (α -cellulose) prepared from huizache wood (*Acacia farnesiana* L. Willd.). These NCCs exhibited different surface morphologies and properties. Hydrolysis was carried out using both sulfuric and hydrochloric acids, while the methodology was based on 2³ factorial designs of experiments. The obtained nanocrystals were characterized for particle size distribution, morphology, size of the NCCs, residual surface charge of the nanoparticles, and chemical composition using various experimental techniques. The two acid hydrolysis procedures produced NCCs suspensions that exhibited a unimodal or bimodal pattern of the Gaussian type, but differed in their properties. Average NCCs particle size obtained by hydrolysis with different concentrations (60 to 65%), at different temperatures (45 to 55 °C) and treatment times (45 to 65 mins) with H₂SO₄ acid and HCl acid were approximately 100 to 260 nm and between 75 nm to 512 nm, respectively, indicating influence of concentration and treatment time with both acids. Also, residual load analysis of sulphate groups in the NCCs suspensions in producing NCCs by H₂SO₄ hydrolysis revealed a minimum load of 39 mmol/kg and a maximum of 505 mmol/kg of NCCs.

Keywords: *Acacia farnesiana*; Acid hydrolysis; Nanocellulose crystals; α -Cellulose; Soluble grade cellulose

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INTRODUCTION

Recently, studies on the design and development of nanostructured materials have gained both scientific and technological interest in several disciplines (Mendoza and Rodríguez-López 2007; Habibi *et al.* 2010; Abdul Khalil *et al.* 2014; Andrade *et al.* 2015; Magalhães *et al.* 2017; Satyanarayana *et al.* 2017). Due to their size, nanostructures exhibit unique properties that make their use attractive in various applications in which both synthetic and natural materials have been incorporated (Corrales 2015). Nanotechnology research has been prioritized among various research agencies in many countries (Wegner and Jones 2006). For example, in the United States, nanotechnology is widely promoted among various research groups, such as the National Nanotechnology Initiative (NNI-2001) (Gardner *et al.* 2008). In fact, reports are available

regarding possible markets for cellulose nanocrystal enabled products (Cowie *et al.* 2014). The study and development of new materials based on nanostructured biomaterials, particularly those of lignocellulosic origin, has been the subject of many research works (Ahuja and Kumar 2009; Brinchi *et al.* 2013; Rosli *et al.* 2013; Reyes *et al.* 2014; Thakur 2014; Andrade *et al.* 2015; Kumode *et al.* 2017; Satyanarayana *et al.* 2017).

Cellulose has potential as a nanomaterial because of its abundance in nature and its nanofibrillar structure. This polysaccharide has special characteristics that make it an exceptional material, such as excellent mechanical properties, high rigidity, low cost, and biodegradability. Cellulose fibers are formed by microfibrils, which have disordered (amorphous) or highly ordered (crystalline) regions (Kamide 2005; Klemm *et al.* 2005). The amorphous regions of the cellulose fibrils can be selectively hydrolyzed by acids, excluding the crystalline (less reactive) regions, to obtain nanosized regions called whiskers, nanocrystals, or cellulose nanofibers that possess exceptional mechanical and optical properties (Favier *et al.* 1995; de Souza Lima and Borsali 2004). Cellulose nanocrystals have a cylindrical shape, with dimensions between 100 nm to 300 nm in length and 3 nm to 5 nm in diameter (de Souza Lima and Borsali 2004), and a Young's modulus of up to 145 GPa, which is greater than that of Kevlar (130 GPa) or steel wires (200 GPa) (Šturcová *et al.* 2005).

Acid treatment (acid hydrolysis) is the main process used to produce nanocrystalline cellulose particles (NCCs), which are the smallest building blocks released from original cellulose fibers (Rehman *et al.* 2014). High mechanical properties are due to these block arrangements. Sulfuric acid (H₂SO₄) and hydrochloric acid (HCl) are widely used in NCCs preparation. However, the dispersibility of NCCs derived from these two types of acids is different (Zeni *et al.* 2016). Cellulose chain ruptures occur randomly during the acid hydrolysis process, and the dimensions of NCCs are not uniform.

Different acids also affect the properties of the suspension: hydrolysis with hydrochloric acid produces cellulose rods with minimal surface loading, while the use of sulfuric acid provides highly stable aqueous suspensions, due to the esterification of the surface of hydroxyl groups under load of sulfate groups (Zeni *et al.* 2016). Under controlled conditions, the nanocrystals that are obtained using sulfuric acid allow to be grafted randomly by sulphate groups on the surface of the cellulose microfibrils inducing an electrostatic layer that covers them (de Souza Lima and Borsali 2004). The hydrolysis conditions are known to affect the properties of the resulting nanocrystals, and these must be sufficiently mild to avoid complete hydrolysis to glucose (Casillas *et al.* 2018).

Due to the abundance of sulphate groups charged on their surface, nanocrystals obtained from H₂SO₄ hydrolysis are easily dispersed in water, while those produced from HCl hydrolysis do not disperse as easily. In fact, they tend to flocculate in aqueous suspensions (Casillas *et al.* 2018). When the concentration of NCCs exceeds a critical value, the suspensions form a typical chiral nematic ordered (cholesteric) phase. This phenomenon of phase separation for colloidal particles shows the optical characteristics of a liquid crystal characteristic of cellulose nanocrystals (Majoinen *et al.* 2012). Compared with cellulose fibers, NCCs possess many advantages, such as the nanoscale dimension, high strength and specific modulus, high surface area, unique optical properties, *etc.* (Zeni *et al.* 2016). The properties of NCCs depend on several factors, such as sources of cellulose, reaction time, temperature, and the acids used for hydrolysis (Abe *et al.* 2007; Ahuja and Kumar 2009; Beck-Candanedo and Gray 2005; Elazzouzi-

Hafraoui *et al.* 2008; Brinchi *et al.* 2013; Rosli *et al.* 2013; Reyes *et al.* 2014; Andrade *et al.* 2015; Kumode *et al.* 2017; Satyanarayana *et al.* 2017; Thakur 2014). These unique physicochemical properties and broad application perspectives have attracted substantial interest from scientific and industrial researchers (Peng *et al.* 2011).

Cellulose nanocrystals offer many advantages as reinforcement materials in the matrix of nano compounds because of their high anisotropy, high rigidity, and low density (1590 kg/m³) (Beck-Candanedo and Gray 2005). They come from a sustainable and biodegradable natural source that potentially offers low environmental and animal health risks, as well as low production costs relative to nanocrystals from other sources (de Souza Lima and Borsali 2004). Cellulose nanocrystal suspensions have been prepared from various cellulose sources, such as tunicates (Favier *et al.* 1995; Araki *et al.* 1998), bacteria (Grunert and Winter 2002), soft woods and hardwood (O'Sullivan 1997; Beck-Candanedo and Gray 2005) besides agave waste (Ponce-Reyes *et al.* 2014). However, there is no report on the preparation and characterization of nanocrystals from the huizache (*Acacia farnesiana* L. Willd) plant. Casillas *et al.* (2019) developed a method to produce soluble grade cellulose from this plant; the produced pulp could be useful for various applications including nanocrystal preparation.

With the above considerations, the authors have attempted to use a plant, which is considered to be a pest due to its ability to colonize pastures and other disturbed habitats (Parrotta 2000) besides, it is grown in large quantities, whereby value addition to a waste plant can be achieved. Accordingly, the main objective of this study was to obtain NCCs from soluble grade cellulose pulp from the huizache plant (*Acacia farnesiana*) using a controlled acid hydrolysis treatment with two different acids, *viz.*, H₂SO₄ and HCl. For this, a factorial 2³ experimental design for both H₂SO₄ and HCl hydrolysis was used with different conditions of concentration, temperature and time of treatment. The obtained NCCs were characterized for various properties with the final aim of finding uses for these nanomaterials in various sectors, particularly in the paper industry.

EXPERIMENTAL

Materials

Samples of huizache wood were collected from the spring forest region, located in the municipality of Tala, Jalisco, Mexico.

Methods

Raw material preparation

Soluble grade cellulose from huizache (*Acacia farnesiana* L. Willd.) was prepared as reported previously (Casillas *et al.* 2018). Briefly the process is as follows: The huizache wood was removed from the bark and the chips were subjected to the pulping process using alkaline sulphite, anthraquinone, methanol (ASAM) solutions prior to elemental chlorine free (ECF) bleaching (Casillas *et al.* 2018).

Pulp preparation for NCCs production

Pulp necessary to prepare the NCCs was obtained by controlled acid hydrolysis using H₂SO₄ acid. To increase the contact area between the acids and the bleached huizache pulp, it was first ground and then sieved in a mill using a 60-mesh screen.

Cellulous nanocrystal production

The nanocrystals of the huizache cellulose were produced by controlled hydrolysis of the bleached *Acacia farnesiana* pulp obtained at the University of Guadalajara, Mexico, following an earlier reported method (Majoinen *et al.* 2012).

Experimental design for NCCs production through controlled hydrolysis

The NCCs were produced from the high purity cellulose of the huizache that were subjected to a two-step hydrolysis. The acids used during the controlled hydrolysis were independent of each other and were chemically pure or laboratory-grade sulfuric and hydrochloric acids. To obtain NCCs particles of different sizes and different morphologies with or without the absence of residual charge, various experimental conditions were adopted based on 2³ factorial designs, with some central points. Two experimental designs were developed, one for HCl and the other for H₂SO₄.

Table 1(a). Factorial Design 2³ for NCCs Production by Controlled H₂SO₄ Hydrolysis

Experiment No.	Concentration (%)	Temperature (°C)	Treatment Time (min)
1	65.0	45	45
2	62.5	50	55
3	65.0	55	65
4	62.5	50	55
5*	62.5	50	55
6	65.0	45	65
7	60.0	45	45
8	60.0	55	65
9*	65.0	55	45
10	60.0	45	65
11	60.0	55	45

* Note: These experiments showed unimodal Gaussian-type pattern in Fig. 3

Table 1(b). Details of Factorial Design 2³ for NCCs Production by Controlled HCl Hydrolysis

Experiment No.	Concentration (N)	Temperature (°C)	Treatment Time (min)
1	2	70	30
2	8	70	200
3	2	50	115
4	5	70	115
5	8	70	30
6	5	90	200
7	5	50	200
8	2	70	200
9	5	90	30
10	5	70	115
11	5	50	30
12	5	70	115
13	8	50	115
14	2	90	115
15	8	90	115

The designs considered the concentration of acids, treatment time, and temperature during hydrolysis. Response variables included the average NCCs length and the residual load of strong acid groups, particularly in the case of H₂SO₄, which was expressed in mmol/kg of NCCs. Tables 1(a) and 1(b) list the conditions used for controlled hydrolysis with H₂SO₄ and HCl acids.

NCCs preparation by acid hydrolysis

For each experiment, a pre-determined amount of acid was added to 30 g of cellulose on a dry basis in a three-neck flask [Tables 1(a and b)]. Once the material was in contact with the acids, the hydrolysis was continued for a certain length of time (45 min to 65 min for H₂SO₄ and 30 min to 200 min for HCl) until the time corresponding to each experiment was reached. The reaction was stopped by adding deionized water (18 MΩ-cm purity) at 4 °C. Each experiment was carried out in a water bath in the temperature range of 5 °C to 100 °C, with the equipment having precision ± 0.005 °C.

Washing and purification of NCCs suspensions

The NCCs suspensions were centrifuged in Thermo-Orion equipment (Sorvall ST 16, Federal District, Mexico) with a capacity of 4 mL x 400 mL for 10 min at a rotation speed of 4,500 RPM, which eliminated the supernatant material after each treatment. Several successive centrifugations were carried out until it was possible to obtain a NCCs gel. This centrifuged material was transferred to dialysis membrane tubes and placed inside a container with deionized water. The material was left in continuous agitation for 4 to 6 days until the water reached a constant pH of 5 to 6. After eliminating the residual acid excess, the NCCs suspensions were subjected to an ultrasound treatment (VCX-500, Sonics, Newtown, CT, USA) for 5 min to promote better dispersion in the suspension. Finally, the obtained nanocrystal suspensions were stored in plastic containers under refrigeration.

Characterization techniques of NCCs

The NCCs suspensions were characterized for chemical composition using attenuated total reflectance Fourier transform infra-red spectroscopy (ATR FT-IR), atomic force microscopy (AFM), and dynamic laser scattering (DLS)). The morphology was characterized through atomic force microscopy (AFM), the particle size distribution was characterized by dynamic laser light scattering (DLS), and the residual load was measured by conductometric analysis.

Chemical composition of huizache-technical cellulose NCCs (ATR FT-IR)

To perform the chemical analysis of the prepared NCCs, the suspensions were sonicated for homogenization. Then, 10 mL aliquots were taken according to the concentration of each of the suspensions to form a film. The films were placed in containers with total dryness in a vacuum oven at 40 °C. In order to avoid the elimination of sulfate groups in the case of suspensions hydrolyzed with sulfuric acid and during the time when the aliquot of suspension is evaporated with nanocrystals.

Once the NCCs films were formed, they were analyzed in the Perkin Elmer FT-IR spectrophotometer (Spectrum GX, Mexico City, Mexico) using an ATR attenuated total reflectance additive provided with a diamond crystal. The spectra were obtained between 4000 cm⁻¹ to 500 cm⁻¹ with a resolution of 4.00 cm⁻¹ with 16 scans.

Atomic Force Microscopy (AFM)

An AFM microscope (Multi mode-8, Bruker, Beijing, China) was used for the morphological analysis of the NCCs. Cellulose nanocrystal samples were diluted to a concentration of 0.005 wt. % and sonicated in an ultrasonic bath. Then, 10 μL of each of the samples were deposited in mica grade V1 discs. They were allowed to dry in Petri dishes.

During each analysis, the technique was used intermittently in the air (tapping mode). The amplitude, height, and phase images of 2 μm x 2 μm size were taken from different sections of the sample, as well as 5 μm x 5 μm sized images, with a resolution of 512/512 pixels/line. Micro silicon tips coated with aluminum (AC160TS-R3, Oxford Instruments, California, USA) were used for the acquisition of the images.

Conductometric titration to analyze the charge density of NCCs suspensions

This analysis was carried out in triplicate following the Report of Scandinavian Pulp, Paper, and Board Testing Committee, 2002 (SCAN-CM 65:02) by conductometric titration to determine the total content of the acid groups in the NCCs suspensions. For this analysis, a pH/conductivity meter (Star A212, Thermo Fischer Scientific, Federal District, Mexico) coupled with a three-neck flask was used, and the conductivity and pH of the solution were measured simultaneously.

During the titrations with conductometric monitoring, aliquots of 100 mL suspension were used. These were titrated with a 0.5 N sodium hydroxide (NaOH) solution. This was added drop by drop using an electronic micro-burette (Titrette, Sigma Aldrich, Toluca, Mexico), and coupled with a three-neck flask. The initial conductivity of the suspension was determined by adding 0.20 mL of NaOH with constant agitation. Alkaline solution was added until the pH reached between 12 and 13. Finally, the conductivity *versus* the amount of added NaOH obtained was plotted for each experimental condition used in the corresponding design.

Particle size distribution analysis

The particle size distribution is one of the most important properties of nanomaterials. Accordingly, particle size analysis of the prepared NCCs was carried out in a DLS system (Malvern Zeta Sizer Nano S 90, Worcestershire, UK), which can measure particle sizes of less than one μm . This instrument is capable of improved detection of aggregates, measurement of small, diluted samples, or samples in a high concentration.

For this analysis, the NCCs suspensions were diluted to 0.2% v/v and filtered using a syringe filter of 25 mm diameter and 1.6 μm pore size. Subsequently, the samples were sonicated in an Elma ultrasound bath (Frechen, Germany) for 5 min with an operating frequency of 20 kHz.

Then, 200 μL of the NCCs suspension and 800 μL of deionized water placed in a polystyrene cell, which was placed inside the Zeta Sizer and a series of 3 scans with 10 repetitions each was made. This was repeated continuously for each sample, until at least three plots with a similar appearance were obtained.

RESULTS AND DISCUSSION

Nano Cellulose Crystals Suspension

Figure 1 (a-f) shows aqueous colloidal suspensions of NCCs obtained in this study by controlled acid hydrolysis using both H_2SO_4 and HCl under different conditions.

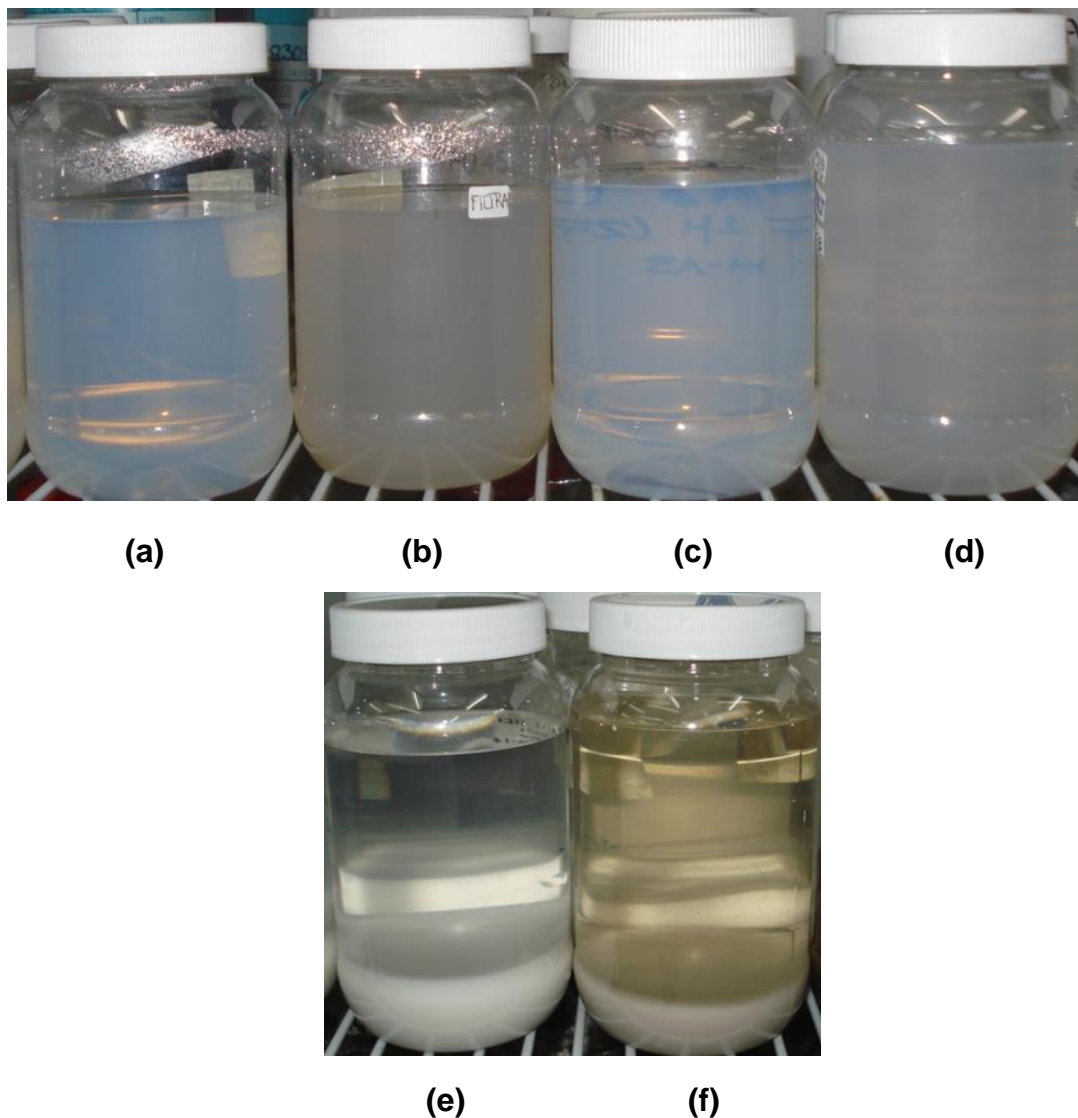


Fig. 1. Colloidal suspensions of NCCs obtained by hydrolysis with H_2SO_4 and HCl . (a-d): Hydrolysis with H_2SO_4 ; (a)- experiment No. 4; (b)-Experiment No. 1; (c)- Experiment No. 11; (d)- Experiment No. 9; (e-f)-Hydrolysis with HCl : (e)- Experiment No. 6, and (f)- Experiment No. 8

Based on Fig. 1, the NCCs extracted with H_2SO_4 appeared to be well suspended, dispersed, and stable, while those extracted with HCl tended to flocculate, leading to their precipitation.

Chemical Analysis of NCCs by ATR FT-IR

Figure 2 (a-c) shows comparative ATR FT-IR spectra of NCCs films and suspensions obtained by hydrolysis with H_2SO_4 and HCl in different experimental

conditions. Part 2 (a) shows the spectra of prepared nanocrystals under different acid treatments and conditions, while Fig. 2(b) compares the experimental conditions of the nanocrystal treatments with different types of HCl and H₂SO₄. Analysis of these figures is made below following the earlier reported analysis (Bolio-López *et al.* 2011).

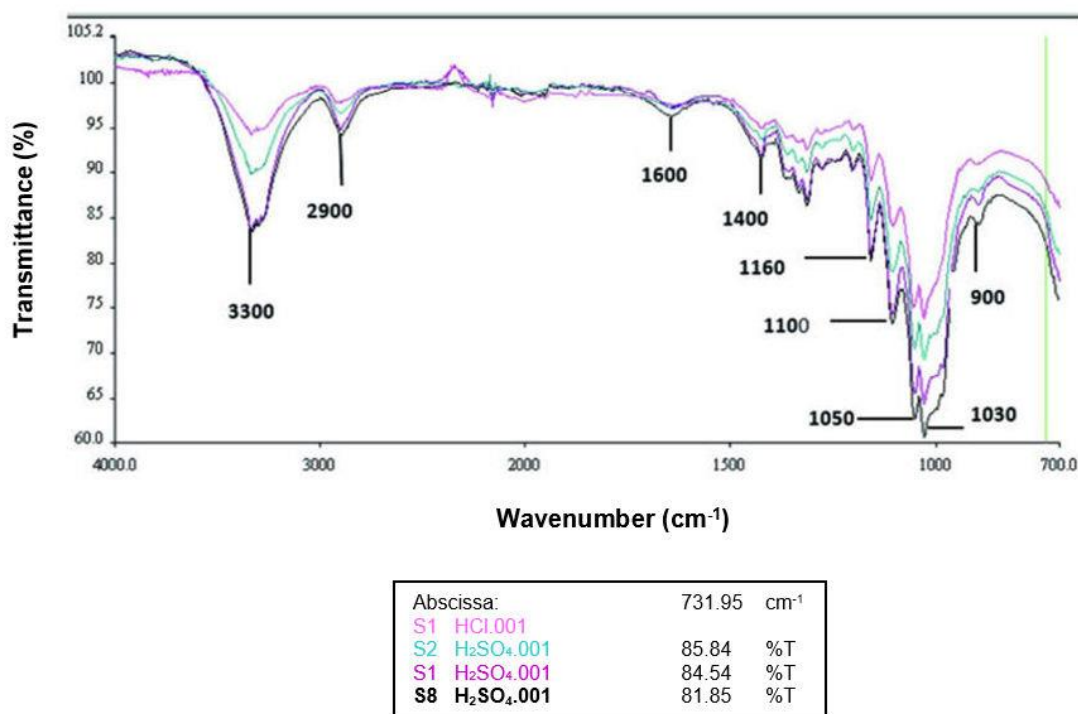


Fig. 2(a). Comparative ATR FT-IR spectra of NCCs films obtained with H₂SO₄ and HCl under different experimental conditions. [S1- H₂SO₄: Sample of treatment 1 of H₂SO₄; S1 HCl: Sample of treatment 1 of HCl; S2 H₂SO₄: Sample of treatment 2 of H₂SO₄; S8 H₂SO₄: Sample of treatment 8 of H₂SO₄]

The characteristic band at 3300 cm⁻¹ can be attributed to the OH groups belonging to the cellulose, which indicated the degree of crystallinity of the material. Further, the bands observed between 2900 cm⁻¹ and 1400 cm⁻¹ can be attributed to the stretching of the C-H groups, while the band at 1600 cm⁻¹ can be attributed to vibrations corresponding to the O-H groups present in the structure of NCCs. The trough observed at 1600 cm⁻¹ can be ascribed to the adsorbed water. On the other hand, the peak observed normally at 1731 cm⁻¹, which corresponds to hemicelluloses, is absent, suggesting either their elimination or absence of this component in the NCCs (Bolio-López *et al.* 2011).

In contrast, Fig. 2(c) shows a weak peak at 800 cm⁻¹, which corresponds to the stretching of the C-O-S (carbon-oxygen-sulfur) bonds of the NCCs. This is typical of NCCs produced by hydrolysis using H₂SO₄ acid; but this is absent in the NCCs obtained with HCl (Bolio-López *et al.* 2011). Furthermore, the peaks at 1100 cm⁻¹ and 1150 cm⁻¹ are the characteristic bands of the cellulose and can be attributed to the asymmetric stretching of C-O-C bonds of NCCs.

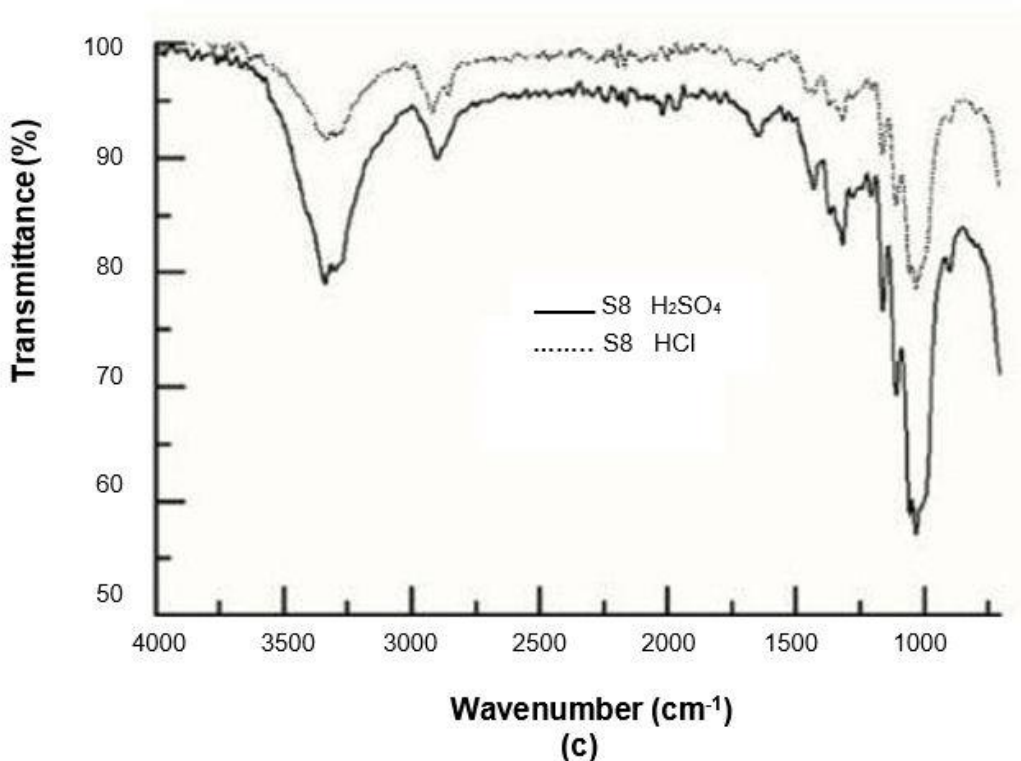
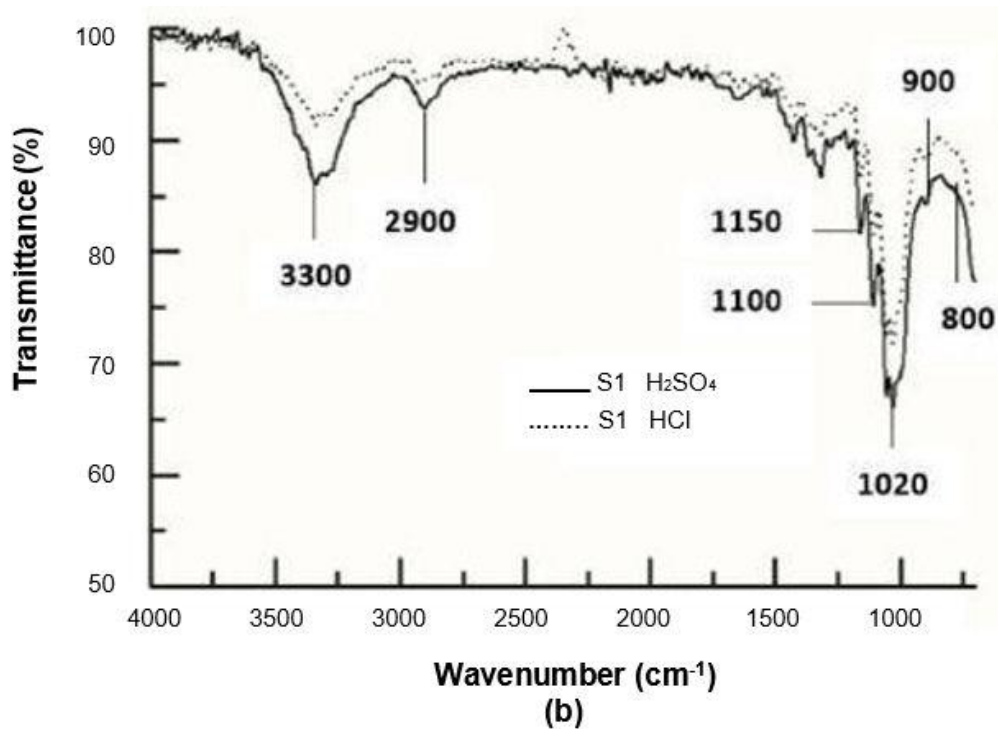


Fig. 2 (b, c). Comparative ATR FT-IR spectra of suspensions of NCCs obtained with different types of acid and different experimental conditions. [S1- H₂SO₄: Sample of treatment 1 of H₂SO₄; S1 HCl: Sample of treatment 1 of HCl; S8 H₂SO₄: Sample of treatment 8 of H₂SO₄; S8 HCl: Sample of treatment 8 of HCl.

Finally, the peak at 1020 cm^{-1} in all the samples can be attributed to the vibrations of the β -glucosidic bond of the cellulose at a wavelength of 900 cm^{-1} (Bolio-López *et al.* 2011). In addition, Fig. 2 shows that there were little differences between the various samples.

Analysis of Particle Size Distribution by DLS Using Laser

Figure 3 shows the particle distribution of NCCs obtained with H_2SO_4 in experiments 5 and 9 (Marked with * in Table 1), which were determined by DLS. The distribution was a unimodal pattern of the Gaussian type. In contrast, experiments 1, 2, 3, 4, 6, 7, and 8 showed bimodal patterns. These results suggest that under the used experimental conditions, NCCs with different morphologies can be produced. Furthermore, these results may be due to the amount of fines produced during the bleaching sequences followed in this study and also to the various experimental conditions used here.

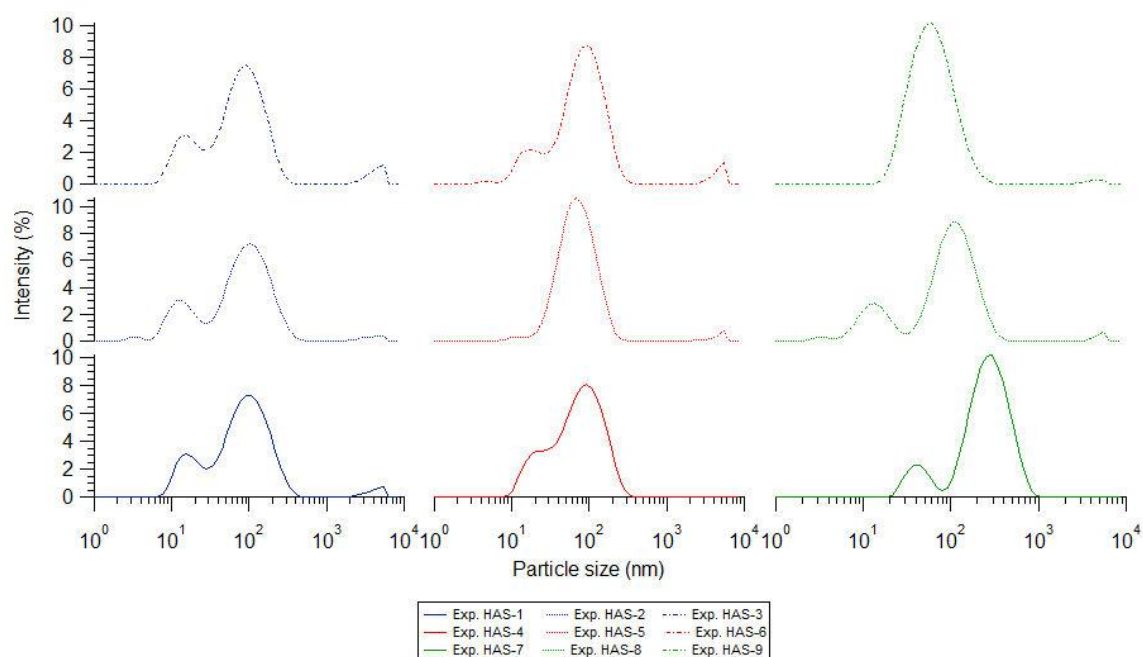


Fig. 3. Plot of particle size distribution of NCCs obtained with H_2SO_4 in laser light scattering measurements

Figure 3 also shows that a particle size of approximately 100 nm was observed in experiments 1, 2, 3, 4, 5, 6, and 8. However, under the conditions of experiment 7, the average nanocrystal particle size was greater than 100 nm, whereas those obtained in experiment 9 were less than 100 nm.

Based on the analysis of the experimental design, a three-dimensional graph was obtained to evaluate the interaction of the experimental variables on the nanocrystal sizes, which considered the concentration of acids as a statistically significant factor. As shown in Fig. 4, to achieve NCCs with large dimensions, low concentrations of H_2SO_4 and low temperature should be used. According to the size response due to the variables actions, an acid concentration of 60%, temperature of $45\text{ }^\circ\text{C}$, and treatment time of 45 min is required to obtain NCCs with the size of approximately 265 nm. However, higher

concentration and temperatures with a short treatment time are recommended to obtain NCCs with sizes less than 75 nm. It may also be noted that the yield of NCCs was from 10 to 14%.

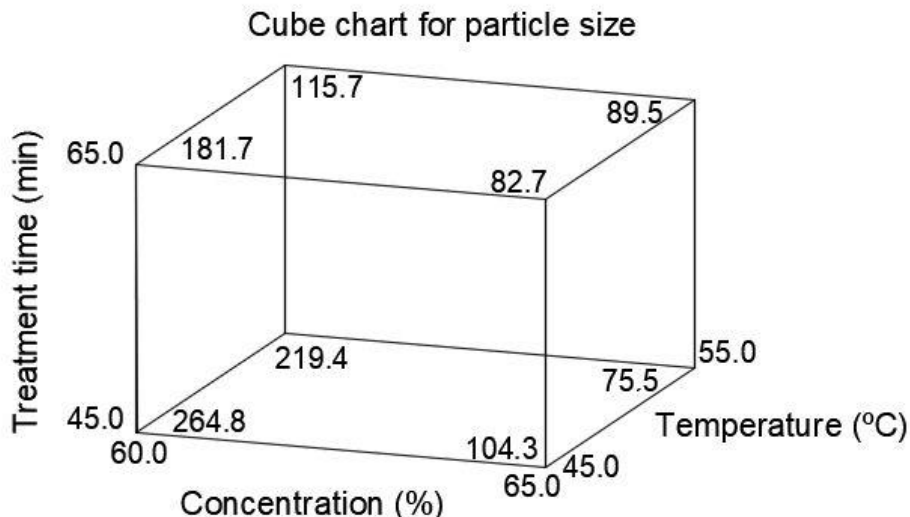


Fig. 4. Three-dimensional plot of interaction of various experimental variables with respect to the size of NCCs

Figure 5 shows the results of the particle size distribution from the measurement in the NCCs suspensions obtained by HCl hydrolysis. A unimodal pattern of particle size distribution occurred in experiments 4, 5, 8, and 15 with the NCCs particle sizes of approximately 243 nm, 512 nm, 466 nm, and 320 nm for experiments 4, 5, 8, and 15, respectively. Experiments 7 and 14 show a bimodal pattern of a particle size distribution having a wide range of NCCs sizes below 100 nm to approximately 400 nm. These results showed that the NCCs obtained with HCl would be of larger particle size (diameter) than those obtained with H₂SO₄. Furthermore, the size distribution of NCCs obtained with HCl showed a longer average length than those produce with H₂SO₄.

A response surface design was created based on the results of the particle sizes at different HCl concentrations and temperatures used. Figure 6 shows that low acid concentrations with a high temperature would produce NCCs with large dimensions, while high acid concentration with a high temperature would result in nanoparticles of smaller dimensions.

The maximum length of the average particle size of 265 nm was obtained with samples hydrolyzed by H₂SO₄ compared with 512.5 nm observed for samples hydrolyzed by HCl. This suggested that the length of NCCs depends on the hydrolyzing media. A previous study revealed that NCCs obtained from wood cellulose hydrolyzed by H₂SO₄ produce NCCs lengths of 180 nm to 200 nm (Favier *et al.* 1995). This statement agrees with an earlier reported conclusion that regardless of their origin, all NCCs exhibit a large length distribution depending on factors such as the variable percentage of non-crystalline regions, the packing structure of the cellulose chains, the acid hydrolysis conditions, and the non-selectivity of the hydrolysis reaction (de Souza Lima and Borsali 2004).

In fact, several studies have reported NCCs having different dimensions depending on the source and experimental conditions (Beck-Candanedo and Gray 2005; Abe *et al.* 2007; Elazzouzi-Hafraoui *et al.* 2008; Ahuja and Kumar 2009; Andrade *et al.* 2015).

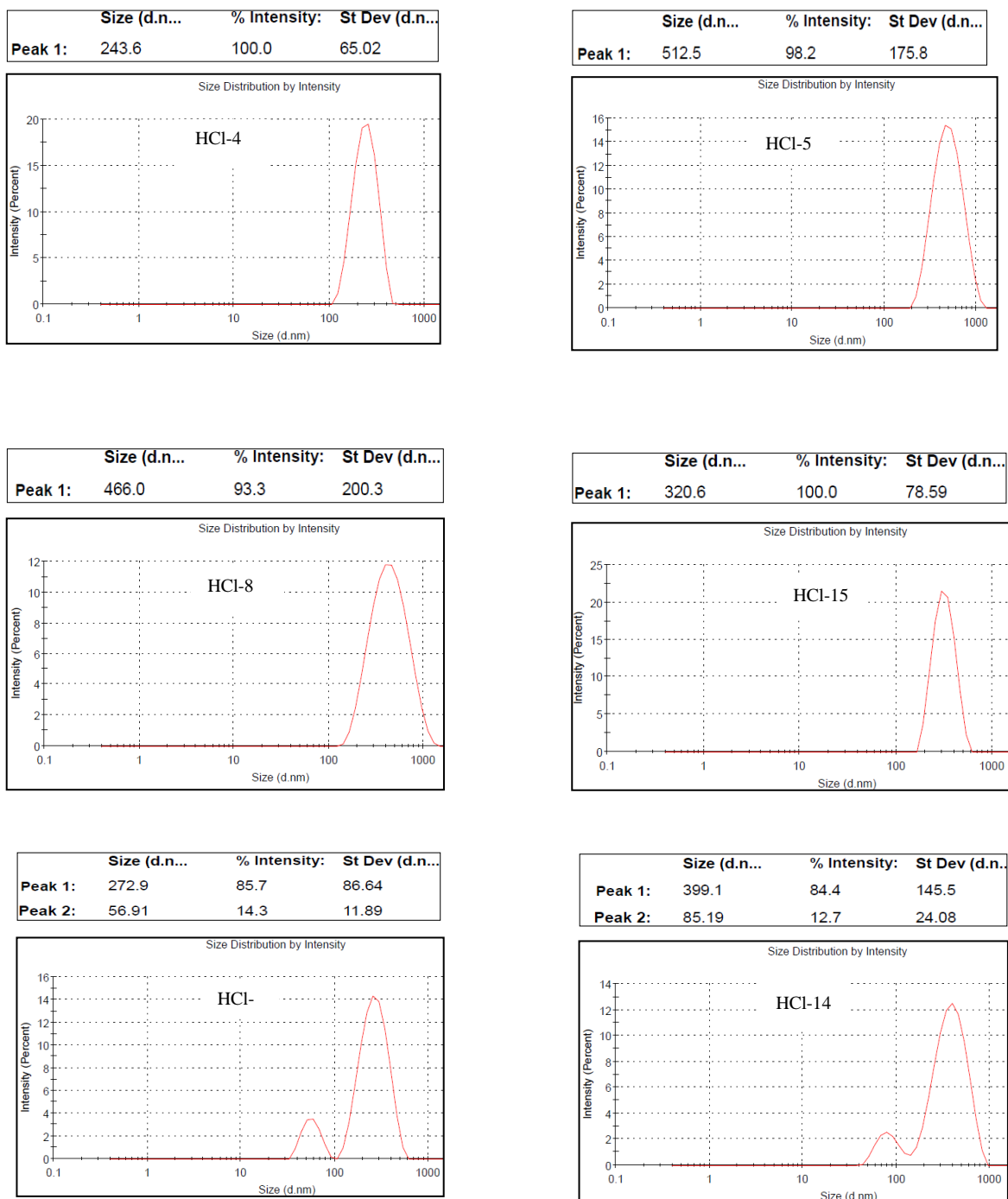


Fig. 5. Particle size distribution obtained by HCl hydrolysis by laser light scattering (DLS)

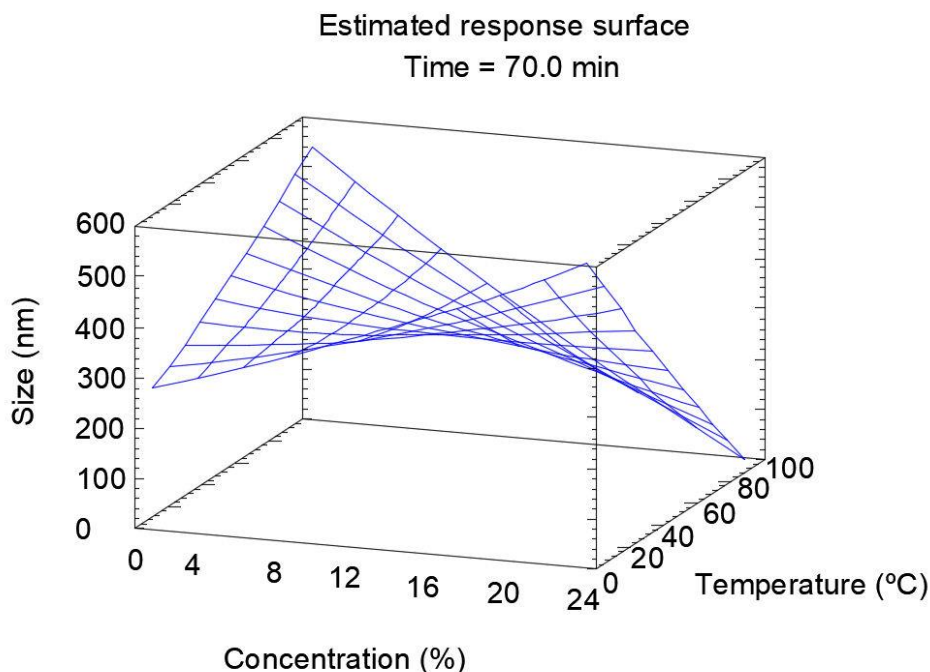


Fig. 6. Plots of response surface design showing various experimental parameters (concentration, temperature, and size of NCCs) obtained with HCl acid

One possible mechanism for the differences found in the dimensions of NCCs produced with sulfuric and hydrochloric acids mentioned above is that the former treatment could have grafted sulphate groups and for this reason the dispersed suspension is maintained. Beck-Candanedo and Gray (2005) have reported that the hydrolysis with sulfuric acid of the native cellulose fibers produces stable suspensions of cellulose nanocrystals. Above a critical concentration, the suspensions spontaneously form an anisotropic chiral nematic liquid phase. The present authors have examined the effect of the reaction time and the acid-pulp relation on the properties of the nanocrystal. Longer hydrolysis times produced shorter and less polydispersed cellulose nanocrystals and this is slightly increased with the critical concentration for formation of the anisotropic phase. The increase in the acid-pulp ratio reduced the dimensions of the nanocrystals thus produced. The critical concentration increased and the two-phase range became narrower. On the other hand, NCCs produced with HCl do not have any grafted group and for this reason they tend to precipitate.

Load density analysis by conductometric titration

Figure 7 shows plots of conductometric studies obtained from the analysis for the NCCs suspensions treated with H_2SO_4 and NaOH (strong base) in experiment 2, which indicated the residual load. In fact, except for experiments 4, 5, 9, 10, and 11, the remaining experiments showed residual loads. It may be noted that the effect of using a strong acid like H_2SO_4 and small quantities of a strong base like NaOH of known concentration on the NCCs suspensions would result in the neutralization of H^+ ions of the acid by the OH^- of the NaOH and formation of water during the titration. In addition, the conductivity decreased because the Na^+ ions had a lower ionic conductivity than the acidic H^+ ions.

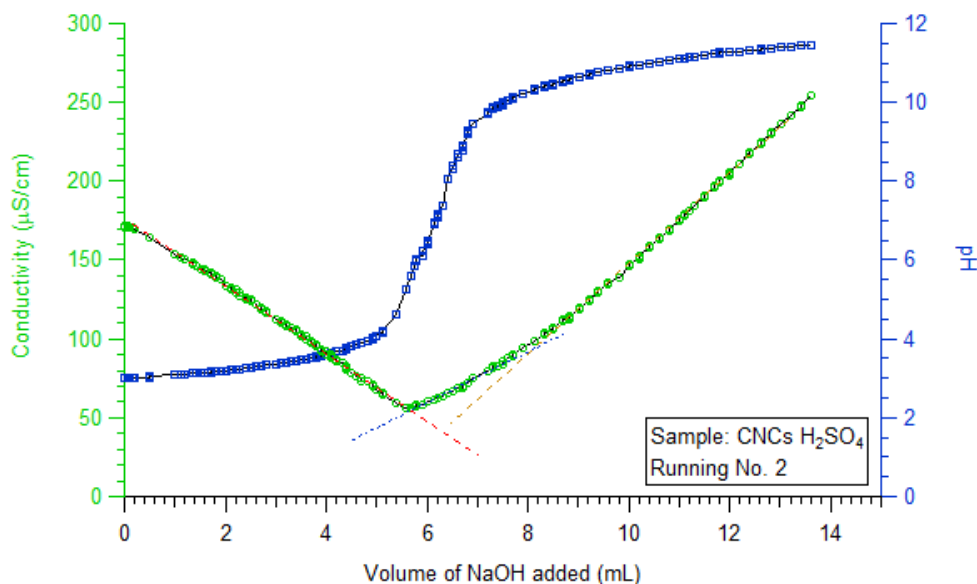


Fig. 7. Plot of conductivity vs. volume of NaOH added during conductometric titration of a suspension of NCCs extracted with H_2SO_4 as a hydrolyzing medium

The conductometric titration curve determines consumption of the NaOH solution to neutralize the total amount of acid groups, including strong and weak acids, in the different conditions in each of the experimental design.

Table 2 shows the quantity of NCCs (in millimoles of sulfate per kg of NCCs) on the surface of nanocrystals. These results indicate the residual charge density of sulphate groups in the different treatments. In fact, some of the values listed in Table 2 are higher than those reported average values of 350 mmol/kg of NCCs (sulfate group content in mmol/kg) obtained from different sources of cellulose, such as wood, cotton, and animal origin (de Souza Lima and Borsali 2004). Further, Table 2 listing the residual load density of the sulfate groups for different treatments mentioned in Table 1(a,b) indicates that higher the concentration of H_2SO_4 , higher the temperature and longer the time of hydrolysis, large quantity of sulfate groups would be grafted on the NCCs.

Table 2. Residual Load Density of Sulfate Groups (millimoles/kg de NCCs)

Sample	Amount of NCCs (mmol sulfate groups/kg)
1	39.78
2	333.75
3	505.05
6	70.98
7	37.62
8	26.90

All experiments except experiment Nos. 4, 5, 10, and 11 showed that the suspensions with H_2SO_4 had acidic pH with charged nanocrystals. In addition, colloidal suspensions extracted with H_2SO_4 had a neutral or slightly alkaline pH (6.38 to 8.71), which is underlined by the absence of charge density in the NCCs. In contrast, the NCCs obtained with HCl did not show any residual charge on their surface in any of the experiments.

Morphology Studies

The morphology of the crystals obtained in “tapping mode” of the controlled hydrolysis of huizache cellulose, shown in Fig. 8a, shows an image obtained with AFM of huizache cellulose nanocrystals using H_2SO_4 as a hydrolyzing medium. Figure 8b shows a similar three-dimensional projection, which physically shows the size and shape of the NCCs.

Figure 3 shows that that the particle size obtained by hydrolysis with H_2SO_4 is between 100 and 300 nm, as measured by DLS method depending on the conditions of the treatment. These values are similar to those obtained by AFM (Fig. 8), suggesting that irrespective of the method used to measure the length of NCCs, the value of length remains the same, which in turn indicates that use of AFM is adequate to measure the length and diameter of the NCCs, as well as to see their shape.

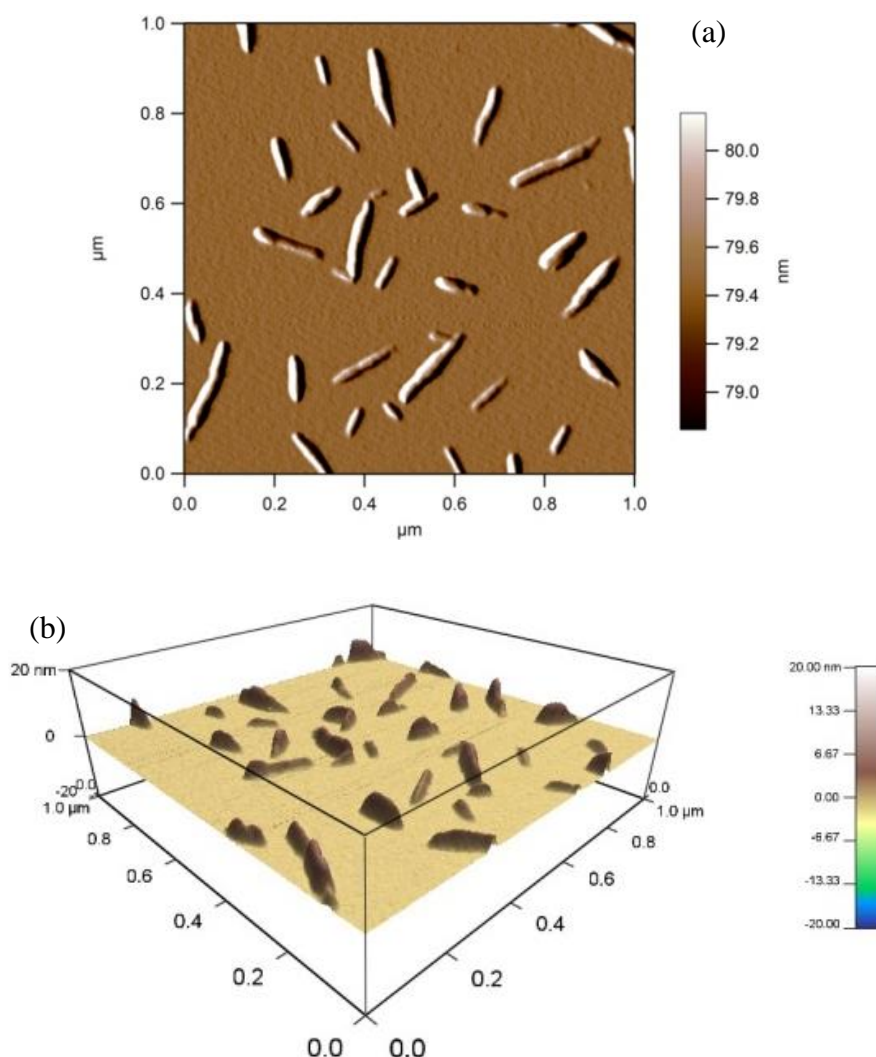


Fig. 8. Morphology of α -cellulose nanocrystals obtained from huizache plant using H_2SO_4 as a hydrolyzing medium; (a) AFM image and (b) Three-dimensional projection

Figure 9 shows the scanned resolution of AFM performed in a scanning area of $2.0 \mu\text{m} \times 2.0 \mu\text{m}$. The details of the crystal of nanocrystals obtained with HCl along with the units in nm shown in the left side bar correspond to the diameter of the nanocrystals.

The scale of this bar ranges from 0 to 12 nm. The experimental conditions used in the design of experiments can be observed in this figure. The topography was obtained in “tapping mode”. In addition, the Y-axis shows the height in nm scale of a real nanocrystal obtained in the experiment. This makes it possible to measure the thickness or height of the NCCs by measuring them along the Y-axis scale. Accordingly, the height (thickness) and average length distribution of the NCCs were approximately 7 nm and 0.2 μm , respectively, and smaller on average.

In Figure 9, the units in nm are shown on the left hand side. It may be noted that for dark color, the value shown is low; while for the light color the value is higher (the color range mentioned is normally called ‘tonality’, which in photography language refers to mid-tone values occupying the scale range between the lightest to the darkest points in the photograph).

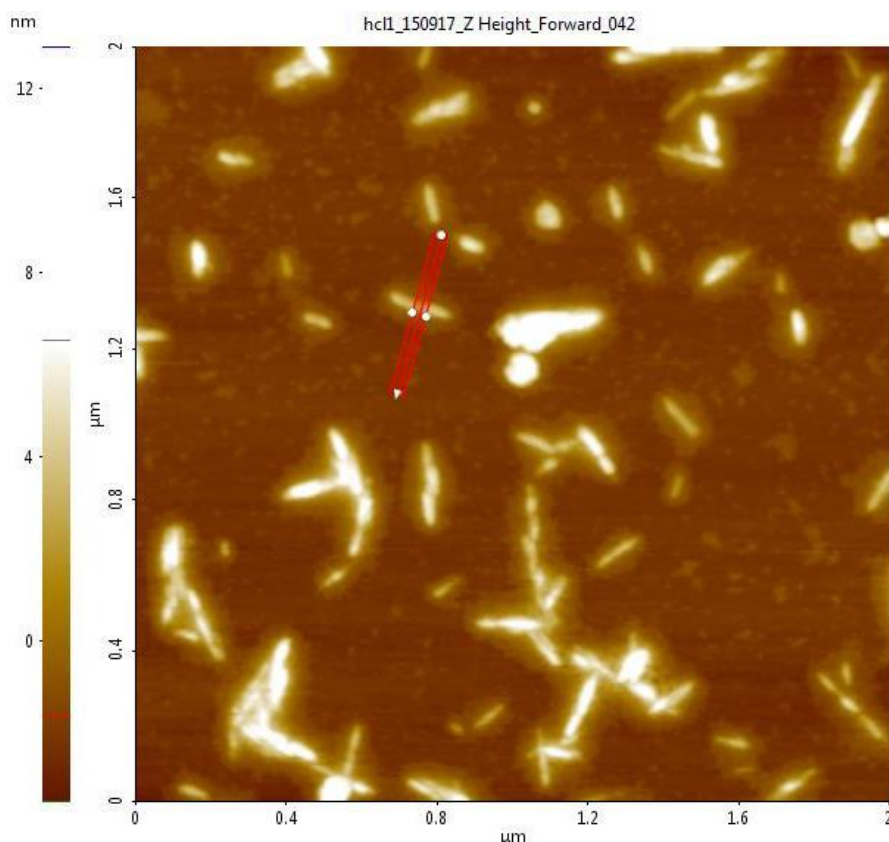


Fig. 9. Topographic AFM image of cellulose nanocrystals obtained from huizache cellulose hydrolyzed with HCl (Experiment 1)

CONCLUSIONS

1. Nanocellulose crystals of different particle sizes and morphologies were produced from huizache soluble grade cellulose (*Acacia farnesiana* L. Willd.) under different conditions of acid hydrolysis based on 2^3 factorial designs of experiments.
2. NCCs particle size distribution was a unimodal or bimodal pattern of the Gaussian type in the case of both acids used for hydrolysis, depending on other

experimental conditions.

3. While the average NCCs particle size obtained by H₂SO₄ hydrolysis was approximately 100 nm, it was 100 nm to 512 nm for HCl hydrolysis. However, it is possible to get larger particle sizes (260 nm) with H₂SO₄ hydrolysis by using an acid concentration of 60%, temperature of 45 °C, and shorter treatment time of 45 min. It was possible to obtain NCCs less than 75 nm by using high H₂SO₄ acid concentration and temperature with a low treatment time.
4. Residual load analysis of sulphate groups in the NCCs suspensions to produce NCCs by H₂SO₄ hydrolysis showed a minimum load of 39 mmol/kg and a maximum of 505 mmol/kg of NCCs.
5. Comparison of the NCCs characteristics obtained by the hydrolysis using both acids indicated the most substantial differences were the size, the density of residual load, and their properties in aqueous suspension. These different properties determined possible applications of NCCs. For example, materials with residual charge benefit the property of dispersion in polymer matrices.
6. Finally, possible uses of the huizache plant to produce cellulose nanocrystals could expand its use and other similar species in various applications of nanotechnology, turning these plants into biomaterials with high added value.

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REFERENCES CITED

- Abe, K., Iwamoto, S., and Yano, H. (2007). "Obtaining cellulose nanofibers with a uniform width of 15 nm from wood," *Biomacromolecules* 8(10), 3276-3278. DOI: 10.1021/bm700624p
- Ahuja, T., and Kumar, D. (2009). "Recent progress in the development of nano-structured conducting polymers/nanocomposites for sensor applications," *Sensor Actuators B: Chemical* 136(1), 275-286. DOI: 10.1016/j.snb.2008.09.014
- Andrade, D. R. M., Mendonça, M. H., Helm, C. V., Magalhães, W. L., de Muniz, G. I. B., and Kestur, S. G. (2015). "Assessment of nano cellulose from peach palm residue as potential food additive: Part II: Preliminary studies," *Journal of Food Science and Technology*. 52(9), 5641-5650. DOI: 10.1007/s13197-014-1684-0
- Araki, J. W. M., Kuga, S., and Okano, T. (1998). "Flow properties of microcrystalline cellulose suspension prepared by acid treatment of native cellulose," *Colloids Surfaces A*. 142, 75-82. DOI: 10.1016/S0927-7757(98)00404-X

- Beck-Candanedo, S. R. M., and Gray, D. G. (2005). "Effect of reaction conditions on the properties and behavior of wood cellulose nanocrystal suspensions," *Biomacromolecules* 6, 1048-1054. DOI: 10.1021/bm049300
- Bolio-López, G. V.-G. A., Veleza, L., and Andreeva, A. (2011). "Whiskers de celulosa a partir de residuos agroindustriales de banano: Obtención y caracterización," *Revista Mexicana de Ingeniería Química* 10(2), 291-299.
- Brinchi, L., Cotana, F., Fournati, E., and Kenny, J. M. (2013). "Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications," *Carbohydrate Polymers*. 94, 154-169. DOI: 10.1016/j.carbpol.2013.01.033
- Casillas, R. R., Rodríguez, K. F. B., Cruz-Estrada, R. H., Dávalos-Olivares, F., Navarro-Arzate, F., and Satyanarayana, K. G. (2018). "Isolation and characterization of cellulose nanocrystals created from recycled laser printed paper," *BioResources* 13(4), 7404-7429. DOI: 10.15376/biores.13.4.7404-7429
- Casillas, R. R., López López, M. C., Aguilar, B. B., Olivares, F. D., and Styanarayana, K. G. (2019). "Obtaining dissolving grade cellulose from the huizache (*Acacia farnesiana* L. Willd) plant," *BioResources* 14(2), 3301-3318.
- Corrales, S. C. (2015). "Synthesis and applications of cellulose nanocrystals," *MoleQla: Revista de Ciencias de la Universidad Pablo de Olavide* 2015(20), 18.
- Cowie, J., Bilek, E. T., Wegner, T. H., and Shatkin, J. A. (2014). "Market projections of cellulose nanomaterial-enabled products--Part 2: Volume estimates," *TAPPI J.* 13(6), 57-69.
- de Souza Lima, M. M., and Borsali, R. (2004). "Rodlike cellulose microcrystals: Structure, properties, and applications," *Macromolecular Rapid Communications*. 25(7), 771-787. DOI: 10.1002/marc.200300268
- Elazzouzi-Hafraoui, S., Nishiyama, Y., Putaux, J. L., Heux, L., Dubreuil, F., and Rochas, C. (2008). "The shape and size distribution of crystalline nanoparticles prepared by acid hydrolysis of native cellulose," *Biomacromolecules* 9(1), 57-65. DOI: 10.1021/bm700769p
- Favier, V., Chanzy, H., and Cavaille, J. Y. (1995). "Polymer nanocomposites reinforced by cellulose whiskers," *Macromolecules* 28(18), 6365-6367. DOI: 10.1021/ma00122a053
- Gardner, D. J., Oporto, G. S., Mills, R., and Samir, M. A. S. A. (2008). "Adhesion and surface issues in cellulose and nanocellulose," *Journal of Adhesion Science and Technology*, 22(5-6), 545-567. DOI: 10.1163/156856108X295509
- Grunert, M., and Winter, W. T. (2002). "Nanocomposites of cellulose acetate butyrate reinforced with cellulose nanocrystals," *Journal of Polymers Environment*. 10(1-2), 27-30. DOI: 10.1023/A:1021065905986
- Habibi, Y., Lucia, L. A., and Rojas, O. J. (2010). "Cellulose nanocrystals: Chemistry, self-assembly, and applications," *Chemical Reviews*. 110(6), 3479-3500. DOI: 10.1021/cr900339w
- Kamide, K. (2005). *Cellulose and Cellulose Derivatives*, 1st Edition, Elsevier Science. DOI: 10.1016/B978-0-444-82254-3.X5000-0.
- Khalil, H. A., Davoudpour, Y., Islam, M. N., Mustapha, A., Sudesh, K., Dungani, R., and Jawaid, M. (2014). "Production and modification of nanofibrillated cellulose using various mechanical processes: A review," *Carbohydrate Polymers* 99, 649-665. DOI: 10.1016/j.carbpol.2013.08.069
- Klemm, D., Heublein, B., Fink, H. P., and Bohn, A. (2005). "Cellulose: Fascinating

- biopolymer and sustainable raw material,” *Angewandte Chemi International Edition* 44(22), 3358-3393. DOI: 10.1002/anie.200460587
- Kumode, M. M. N., Bolzon, G. I. M., Magalhães, W. L., and Kestur, S. G. (2017). “Microfibrillated nanocellulose from balsa tree as potential reinforcement in the preparation of ‘green’ composites with castor seed cake,” *Journal of Cleaner Production*. 149, 1157-1163. DOI: 10.1016/j.jclepro.2017.02.083
- Magalhães, W. L. E., Zaroni, P. R. S., Helm, C. V., Lazzarotto, M., and Satyanarayana, K. G. (2017). “Nanotechnology applied to improve functionality in food,” in: *Nanotechnology in the Agri-Food Industry*, A. Grumezescu (ed), Elsevier Inc, Cambridge, USA, pp. 177-219. DOI: 10.1016/B978-0-12-804304-2.00005-6
- Majoinen, J., Kontturi, E., Ikkala, O., and Gray, D. G. (2012). “SEM imaging of chiral nematic films cast from cellulose nanocrystal suspensions,” *Cellulose* 19(5), 1599-1605. DOI: 10.1007/s10570-012-9733-1
- Mendoza Uribe, G., and Rodríguez-López, J. L. (2007). "Nanoscience and nanotechnology: An ongoing revolution," *Latin American Profiles* 15(29), 161-186.
- O’Sullivan, A. (1997). “Cellulose: The structure slowly unravels,” *Cellulose* 4, 173. DOI: 10.1023/A:1018431705579.
- Parrotta, J. A. (2000). “*Acacia farnesiana* (L.) Willd. Aroma, huisache,” Bioecology of native and exotic trees of Puerto Rico and the West Indies. General Technical Report IITF-115. USDA Forest Service International Institute of Tropical Forestry, Rão Piedras, PR, 6-10.
- Peng, B. L., Dhar, N., Liu, H. L., and Tam, K. C. (2011). “Chemistry and applications of nanocrystalline cellulose and its derivatives: A nanotechnology perspective,” *The Canadian Journal of Chemical Engineering*. 89(5), 1191-1206. DOI: 10.1002/cjce.20554
- Ponce-Reyes, C. E., Chanona-Pérez, J. J., Garibay-Febles, V., Palacios-González, E., Karamath, J., Terrés-Rojas, E., and Calderon-Dominguez, G. (2014). “Preparation of cellulose nanoparticles from agave waste and its morphological and structural characterization,” *Revista Mexicana de Ingeniería Química* 13(3), 897-906.
- Rehman, N., de Miranda, M. I. G., Rosa, S. M. L., Pimentel, D. M., Nachtigall, S. M. B., and Bica, C. L. D. (2014). “Cellulose and nanocellulose from maize straw: An insight on the crystal properties,” *Journal of Polymers and the Environment*. 22(2), 252-259. DOI: 10.1007/s10924-013-0624-9
- Rosli, N. A., Ahmad, I., and Abdullah, I. (2013). “Isolation and characterization of cellulose nanocrystals from *Agave angustifolia* fibre,” *BioResources* 8(2), 1893-1908. DOI: 10.15376/biores.8.2.1893-1908
- Satyanarayana, K. G., Anupama, R., Prasad, V. S., and Magalhaes, W. L. E. (2017). “Preparation, characterization, and applications of nanomaterials (cellulose, lignin, and silica) from renewable (lignocellulosic) resources,” in: *Handbook of Composite from Renewable Materials*, V. K. Thakur, M. K. Thakur, and M. R. Kessler (eds.), Scrivener Publishing LLC, New Jersey, USA, pp. 1-66.
- SCAN-CM 65:02 (2002). “Total acidic group content,” SCAN-test Methods recommended by KCL, PFI and STFI-Packforsk for the pulp, paper and board industries in Finland, Norway and Sweden.
- Šturcová, A., Davies, G. R., and Eichhorn, S. J. (2005). “Elastic modulus and stress-transfer properties of tunicate cellulose whiskers,” *Biomacromolecules* 6(2), 1055-1061. DOI: 10.1021/bm049291k
- Thakur, V. K. (Ed.), (2014). *Nanocellulose Polymer Nanocomposites: Fundamentals and*

Applications, Scrivener Publishing LLC, Massachusetts, USA.

DOI: 10.1002/9781118872246

Wegner, T. H., and Jones, P. E. (2006). "Advancing cellulose-based nanotechnology,"

Cellulose 13(2), 115-118. DOI: 10.1007/s10570-006-9056-1

Zeni, M., Favero, D., Pacheco, K., and Grisa, A. (2016). "Preparation of microcellulose (MCC) and nanocellulose (NCC) from eucalyptus kraft ssp pulp," *Polymer Sciences*.

1(1), 7. DOI: 10.4172/2471-9935.100007

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