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Original Article

Effect of Process Variables on the Development and Characterization of Nanocellulose as Novel Biopolymer

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ABSTRACT

Nanocellulose has excellent mechanical, physical, and biological properties; it is an ideal material for many applications. The present study aimed to develop nanocellulose from cellulose by hydrolyzing with sulphuric acid to yield nanocellulose. In order to prepare nanocellulose, two factors were considered: the concentration of sulphuric acid and the variation in temperature (AHNC1 to AHNC5). Optimized NC was characterized by its size, zeta potential, TEM, XRD, and FT-IR. NCs had a size range between 134 to 644 nm. AHNC4 and AHNC5 showed respective Zeta potentials of -40.6 and -37.4. XRD diffractograms showed crystallinity indices in the 55-66% range. The TEM showed that the nanocellulose was semicrystalline. FT-IR measurement showed a peak at 1177 cm^{-1} due to O-H association bond of nanocellulose, which is similar to cellulose's peak. This work analyzed that the nanocellulose preparation involves the optimization process to get the desired size of particles by altering process parameters in the acid hydrolysis method like concentration of acid and temperature maintained during the reaction, which depend on the source of cellulose used in the development of nanocellulose.

Keywords: Nanocellulose, Acid hydrolysis, CNC, CNF, Nanocrystals.

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INTRODUCTION

Among the natural polymers, cellulose is most abundantly available as a polysaccharide as it is a significant component of all plant materials.[1] Because of its chemical nature and intramolecular or intermolecular hydrogen bonding, it is insoluble in water and many other organic solvents.[2] Most cellulose comprises large polymeric chains with varying molecular weights with the formula $(C_6H_5O_5)n$, where n denotes the degree of polymerization.[3] The degree of polymerization of the cellulose mainly depends on the source of plant material and the methodology adopted during manufacture. Data reveals the degree of polymerization between 1500 to 3500.[4]

Several types of nanocellulose were found, and they found their high significance in many aspects. Nanocellulose (NC) or Cellulose Nanocrystals (CNC) are in whisker–shaped and rod-like structures. These are found in crystalline regions of cellulose. These crystalline cellulose provide a high aspect ratio.[4] Nanocellulosic fibrils, NCF, are thread-like polymers containing hydrogen bonding between the cellulose and OH groups that interact intra-molecularly. They can be prepared easily by a mechanical method.[5] Reducing the size of cellulose to nano level yields nanosize particles and provides high crystallinity. This can be achieved by various methods like acid hydrolysis, tempo oxidation, and ionic liquid.[6]

In the present evolving trend, nanocellulose is the tool for various branches like pharmacy, medical field, engineering, tissue engineering, chemical engineering, etc. Nanocellulose is one of the best carriers in the pharmaceutical field because it can be used as natural super disintegrants,[7] drug delivery carrier,[8] thickening agent in the hydrogel,[9] aerogels,[10] wound healing,[11] transdermal films,[12] and ophthalmic drug delivery.[13]

Several types of research are being found nowadays on developing nanocellulose from various sources of plant materials, bio waste, and agricultural residues. This study focuses on the acid hydrolysis process parameters in developing nanocellulose and, for this purpose, compares results of acid hydrolysis and size of nanocellulose by available literature as tabulated in Table 1. The particle size of nanocellulose is significantly influenced by the acid content, reaction temperature, and reaction duration as per the observation from Table 1. In addition to this method of approach, knowledge of the source of cellulose employed for the investigation is necessary.

MATERIALS AND METHODS

Materials

Cellulose and sulphuric acid (98%) were purchased from SD Fine Chem. All chemicals are 98.0% pure and used without

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Table 1: Influence of methodologic factors and source of cellulose on particle size of nanocellulose.

Sl. No	Research Papers	Source of Cellulose	Concentration of acid	Temperature	Particle Size
1	Nanocellulose prepared by acid hydrolysis of isolated cellulose from sugarcane bagasse.[14]	Sugar Cane bagasse– CNC	50% V/V H_2SO_4	40°C 30 min	111 nm
2	Acid Hydrolysis-Mediated preparation of Nanocrystalline Cellulose from Rice Straw.[15]	Rice straw –CNC	64% V/V H_2SO_4	45°C for 60 min	129.9 to 292.0 nm length
3	Preparation and Characterization of Cellulose Nanocrystal Extraction From Pennisetum hydridum Fertilized by Municipal Sewage Sludge via Sulfuric Acid Hydrolysis.[16]	Pennisetum hydridum– CNC	65% V/V H_2SO_4	37°C 120 min	272.5 nm
4	Isolation and Characterization of Nanocellulose Obtained from Industrial Crop Waste Resources by Using Mild Acid Hydrolysis.[17]	Pineapple leaf	30% V/V H_2SO_4	50°C for 6 hr	250 nm
			60% V/V H_2SO_4	50°C for 30 min	160 nm
		Banana rachis	30% V/V H_2SO_4	50°C for 6 hr	459 nm
			60% V/V H_2SO_4	50°C for 30 min	296 nm
		Sugarcane bagasse	30% V/V H2SO4	50°C for 6 hr	730 nm
			60% V/V H_2SO_4	50°C for 30 min	334 nm
5	Preparation and characterization of cellulose nanocrystal extracted from ramie fibers by sulfuric acid hydrolysis.[18]	Ramie fibers CNC	58% V/V H_2SO_4	45°C for 30 min	145.61 nm length and 6.67 nm Width
6	Isolation and Characterization of Nanocrystalline Cellulose Isolated from Pineapple Crown Leaf Fiber Agricultural Wastes Using Acid Hydrolysis.[19]	Pineapple crown leaf fiber	1 M H_2SO_4	45°C for 60 min for 1 to 3 hr	11.55 to 20.21 μm
7	Production of Nanocellulose Crystal Derived from Enset Fiber Using Acid Hydrolysis Coupled with Ultrasonication, Isolation, Statistical Modeling, Optimization, and Characterizations.[20]	Enset Fiber	51.6% H_2SO_4 V/V	47°C for 66.5 min	66 nm
8	Physicochemical Properties of Nanocellulose Isolated from Cotton Stalk Waste.[21]	Cotton Stalk	65% V/V	50°C	
				40 min	90.5 nm
				50 min	270.4 nm
				60 min	664.9 nm
9	Facile synthesis and characterization of nanocellulose from *Zea mays* husk.[22]	*Zea mays* husk	60% W/V H_2SO_4	45°C for 60 min	14 to 29 nm

purification. GEM Laboratories was kind enough to provide the lab-grade water for research.

Methods

The acid hydrolyzed nanocellulose (AHNC) was prepared by keeping the temperature and concentration of the acid as the independent parameters. To study the effect of acid on the development of nanocellulose,35,41,44,47 and 50% v/v sulphuric acid concentrations were chosen to study. NC was prepared according to the general acid hydrolysis method. Briefly, 1g of cellulose sample was dispersed slowly in the sulphuric acid at different concentrations. The temperatures that were maintained for the various concentrations are shown in Table 2. The reaction was carried out under constant stirring with the aid of a magnetic stirrer for 2.5-3 hr. The reaction was quenched by the addition of 10 folds of ice-cold water. The change of colour of mixture

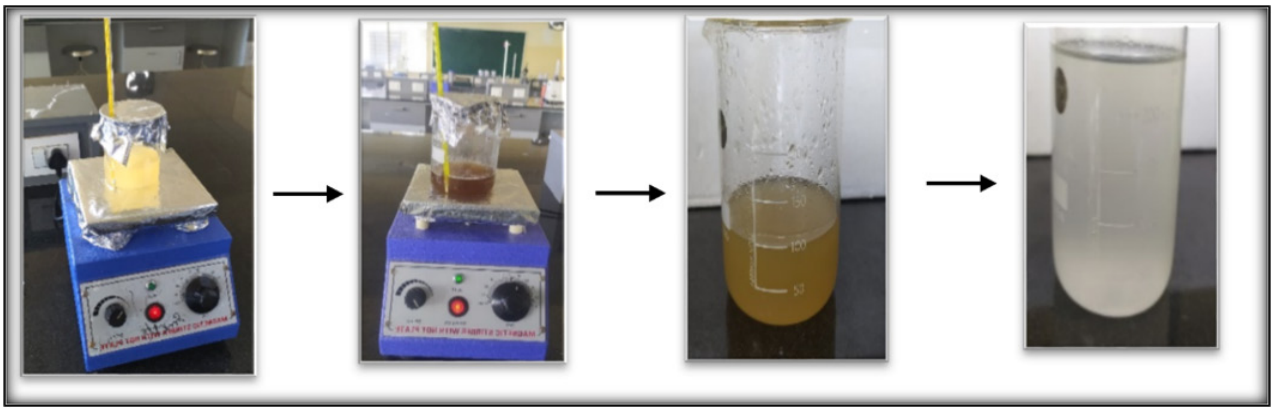

Figure 1: Change of colour during the process of reaction.

Table 2: Parameters considered for development of NC and results.

Formulation Code	Concentration of Sulfuric Acid v/v	Temperature in °C
AHNC1	35%	30
AHNC2	41%	35
AHNC3	44%	36.5
AHNC4	47%	40
AHNC5	50%	43.5

during the reaction shown in Figure 1. The obtained product was washed until the supernatant reached a neutral pH. The filtrate was checked for sulphate ions through a barium chloride test. The suspension was centrifuged and collected. The sample was stored in the refrigerator for future use (Figure 2). Methodology is graphically represented as shown in Figure 3.

Characterization of Nanocellulose

Fourier transform infrared spectroscopy

The presence of functional groups in cellulose and nanocellulose was determined using Fourier Transform Infrared Spectroscopy and spectrum obtained from Bruker at poornayu research lab, Bangalore, Karnataka, India. The range obtained from 500 to 4000 cm^{-1}, with the KBr pelletization method, is adopted during analysis.

Dynamic light scattering

Dynamic light scattering analysis is one of the well-known techniques to determine a nanoparticle's average particle size and intensity size distribution. Through the various treatments, the cellulosic polymeric chains break down into smaller units, changing the size of cellulose to nanocellulose. The samples were diluted with purified water and shaken for 15 min in a shaker bath to remove any clumps formed during the transit. This was followed by filtration to analyze the sample using a glass cuvette and disposal of zeta cell for zeta potential by Malvern zeta sizer.

X-Ray Diffractometer

The crystallinity index of the sample was analyzed for the samples for a desired size range. The X-Ray Diffractograms (P-XRD) were recorded with MiniFlex 300/600. The nanosuspensions were dried using the solvent exchange method by using acetone as a solvent. The Crystallinity index calculated by using following formula,

$$\text{Crystallinity Index } (\%) = \frac{\text{Area of crystalline peaks}}{\text{Total area of peaks}} \text{ x } 100 \ldots\ldots(1)$$

Transmission electron microscopy (TEM)

The morphological studies of optimized nanocellulose suspension were observed by TEM analysis. TEM images were recorded using the FEI TECNAI G2 TEM @200KV, a Field Emission Gun (FEG), and a +/-80-degree tilted computer-controlled LiN cryostage.

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy (FTIR)

The FT IR spectra of nanocellulose, as shown in Figure 4, reveals that the presence of a strong, broad peak at 3334.93 cm^{-1} is due to the -OH stretching present in cellulose. The peak absorption at 1629.73 cm^{-1} was reported as H-O-H and conjugated C=O stretching. The minor peak at 2105 cm^{-1} represents superimposed OH peaks due to intermolecular hydrogen bonding. The peak at 1197.40 cm^{-1} is due to the O-H association bond of cellulose. The peak around 1046.76 cm^{-1} (due to the β-glycosidic linkage) was attributed to the O-C-O stretching during the C-H deformation of cellulose.[22]

Dynamic light scattering method

The DLS method was used to determine the particle size and polydispersity index of nanocellulose produced by the acid hydrolysis method.

Particle size and particle size distribution were examined by DLS, and findings were recorded in Figure 5 and Figure 6. In Figure 7, AHNC 1 and 2 illustrates the tri-modal peaks size of particle ranging from 100 to 10000 nm, respectively. The Z average mean

Figure 2: Nanocellulose suspensions.

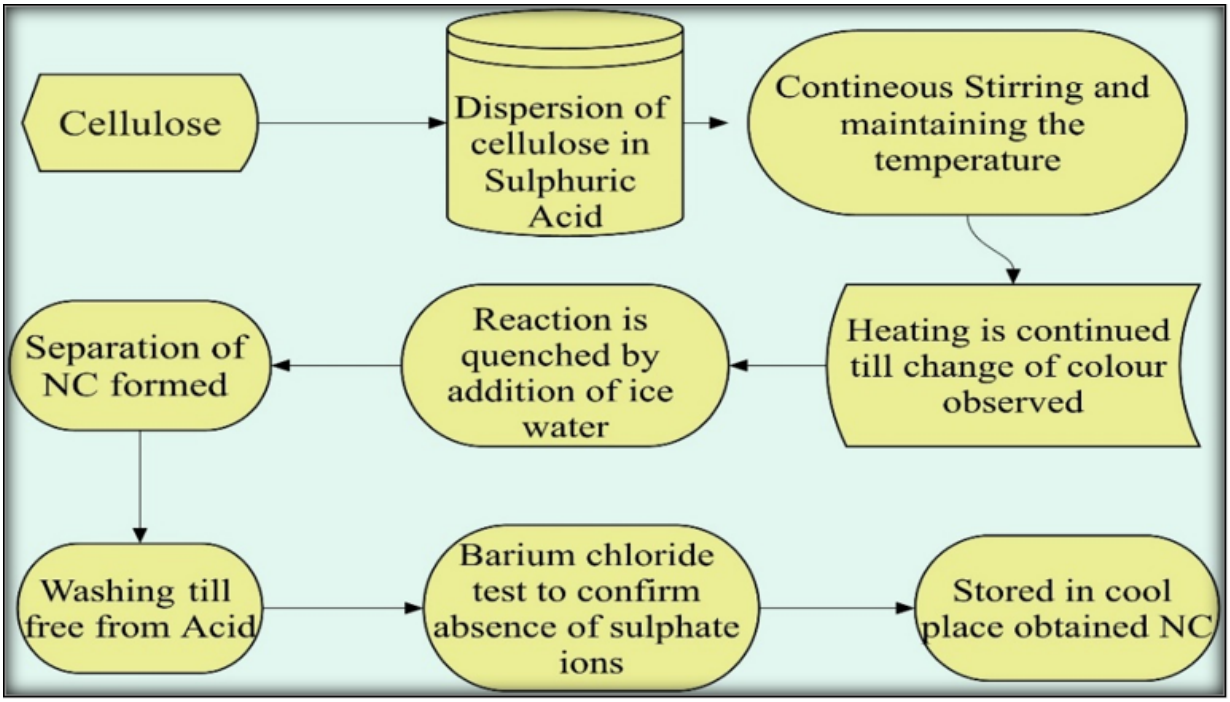

Figure 3: Steps involved in the process of nanocellulose.

recorded by the instrument is 644.5 and 522.5 nm. The results are not in the acceptable range as % the intensity of size distribution varies with tri-modal peaks. Further acid hydrolysis of cellulose continued with increasing the concentration of sulphuric acid and temperature, as mentioned in Table 2. From Figure 7, it was observed that AHNC4 showed a better particle range between 50 to 600 nm with uniform distribution, and its measured Z average mean documented 134 nm for nanocellulose suspension prepared from 47% v/v and 40°C temperature, which is in nanometer scale and desired for the study.

On the other hand, AHNC 3 has shown bimodal peaks with particle sizes ranging from 50 to 400 nm, even though it is within the nanometer scale; because of the nonuniform distribution of particles, it was not considered. Similarly, AHNC 5 has shown particle size between 70 to 900 nm with Z average particle size of 208.3 nm within the nanometer scale. Based on the particle size AHNC4 is considered an optimized formula for further studies.

The intensity of particle size distribution determines the Z average. The polydispersity index (PDI) explains the homogeneity of the sample. As the sample's PDI increases, the sample's dispersibility decreases. Typically, the samples having PDI less than 0.4 with unimodal peak are said to be monodisperse. In the case of more than 0.4, PDI bimodal and trimodal peaks are observed.

Table 3: Z-Average particle size from DLS instrument.

Formulation code	Formulation Code		Size (d. nm)	% Intensity	Zeta Potential
AHNC1	Z-average (d.nm): 644 PDI: 1.0 Result quality: Refer to quality report	Peak 1	1067	64.5	NA
		Peak 2	175.2	20.7	
		Peak 3	4998	14.8	
AHNC2	Z-average (d.nm): 522.5 PDI: 0.826 Result Quality: Refer to quality report	Peak 1	624.9	62.2	NA
		Peak 2	4675	21.3	
		Peak 3	122.7	16.5	
AHNC3	Z-average (d.nm): 255.6 PDI: 0.467 Result Quality: Refer to quality report	Peak 1	235.4	66.4	NA
		Peak 2	85.93	33.6	
		Peak 3	0.0	0.0	
AHNC4	Z-average (d.nm): 134 PDI: 0.341 Result Quality: Good	Peak 1	159.	94.4	- 40.6
		Peak 2	0.0	0.0	
		Peak 3	0.0	0.0	
AHNC5	Z-average (d.nm):208.3 PDI: 0.212 Result Quality: Good	Peak 1	268.0	100	-37.4
		Peak 2	0.0	0.0	
		Peak 3	0.0	0.0	

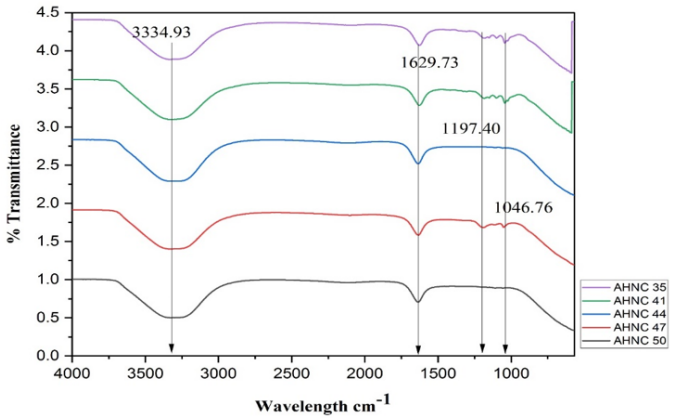

Figure 4: FTIR spectrum of AHNC 1 to 5.

The PDI of the AHNC4 and AHNC 5 prepared from experimental work is found to be 0.341 and 0.212, which confirms the uniformity of the nanocellulose with a single peak.

Zeta potential is used to estimate the long-term stability of the particles. The particles which show the zeta potential of more than ± 30 mV are said to be good as they are less prone to form aggregates.[23]

The Zeta potential of acid hydrolyzed nanocellulose suspension of AHNC4 and AHNC5 is found to be -40.6 and -37.4, which confirms that prepared nanocellulose is more stable in long-term storage if proper storage conditions are maintained. The zeta potential of AHNC 4 and AHNC5 is shown in Figure 7. The average particle size from DLS instrument shown in Table 3.

From Table 1 it was observed the behavior of sulphuric acid and temperature were analyzed for the preparation of nanocellulose by acid hydrolysis method. The temperature was varied in the range of 37°C to 50°C with varying concentrations of sulphuric acid from 30 to 65% v/v. As a result, the particle size of NC has been obtained in the range of 14 nm to 730 nm.

The reason for varying particle size can be explained by understanding the mechanism of acid hydrolysis on cellulose. Cellulose consists of amorphous and crystalline zones alternatively. Amorphous areas in cellulose are considered as structural flaws or chaotic areas. Enzymatic and acid hydrolysis are preferential methods for removing those flaws.[24-28] Comparatively speaking, the amorphous sections hydrolyze more quickly than the crystalline segments.[29] Thus, the concept of controlled hydrolysis was applied to the disruption of the amorphous areas to produce crystalline nanocellulose. Hence, the particle size mainly varies or depends on the source of cellulose and how much it contains amorphous regions. Hence the rate of reaction depends on the concentration of acid and temperature which can be observed from Table 1.

X-Ray Diffractometer

AHNC 4 and AHNC 5 were taken for further studies as they have shown better results. To understand the crystallinity of the sample after chemical treatment was analyzed by XRD. Figure 8(a) and (b) shows intense high peaks at 22.5° and 22.6°, explaining the crystalline nature of the nanocellulose. In both other peaks at 16°, 15.3°, and 34°, 34.7° is observed about an amorphous form so that it may be considered as developed nanocellulose is in semicrystalline. Similar results were obtained by comparing commercial NC, having a peak at 22.66 at 2θ.[22]

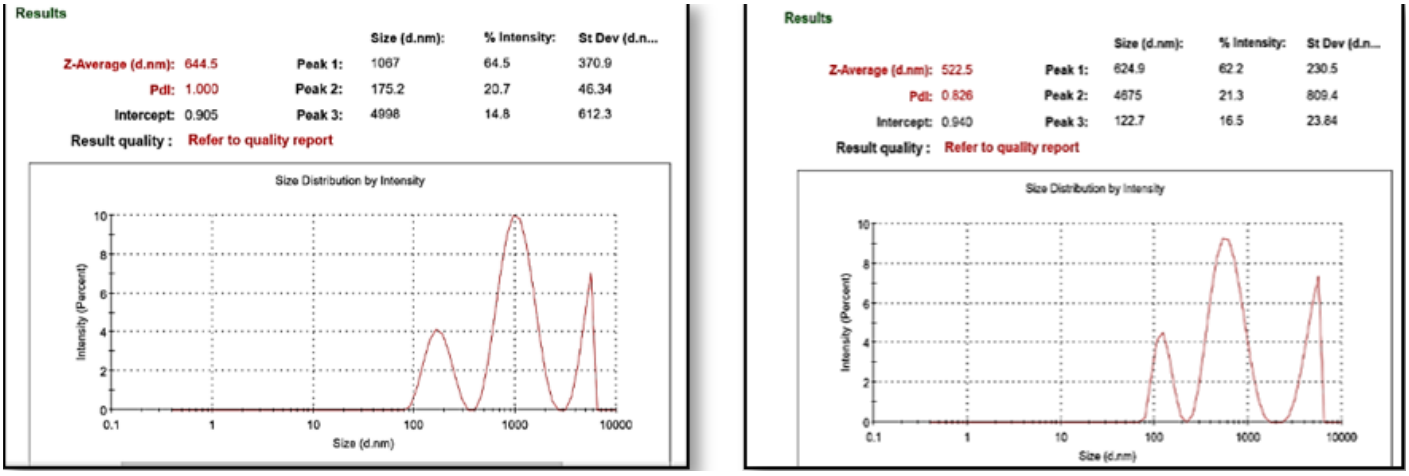

Figure 5: Particle size distribution by DLS method for nanocellulose of AHNC 1 and 2.

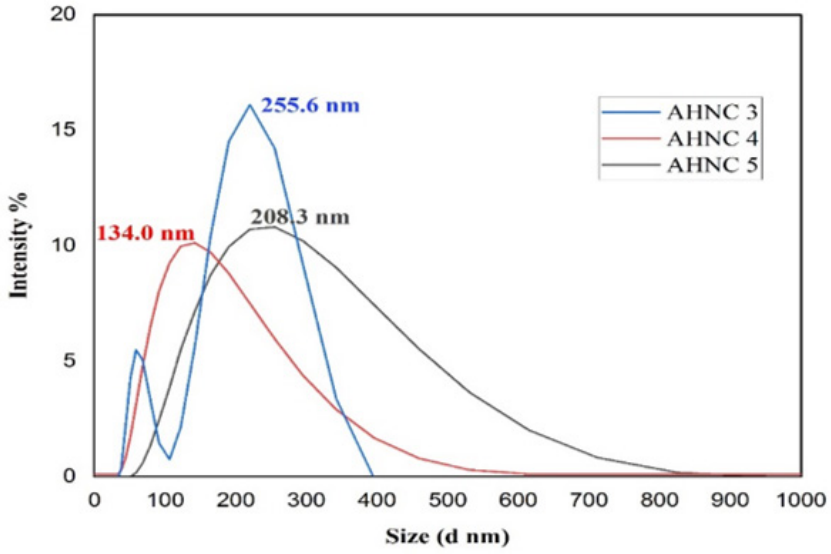

Figure 6: Particle size distribution by DLS method for nanocellulose of AHNC 3 to 5 and varied with temperature and concentration.

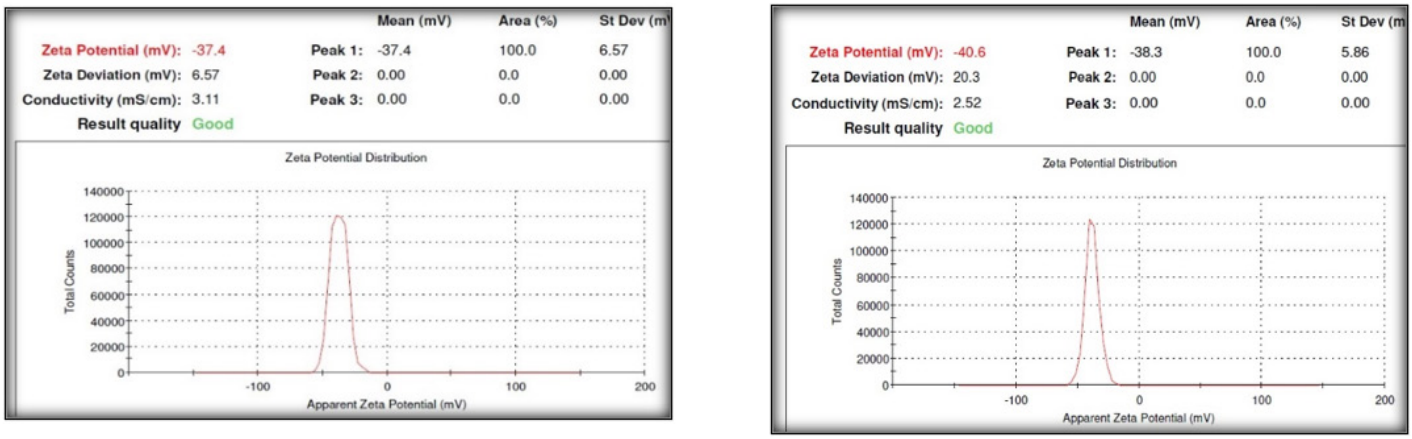

Figure 7: Zeta potential of AHNC 4 and AHNC 5.

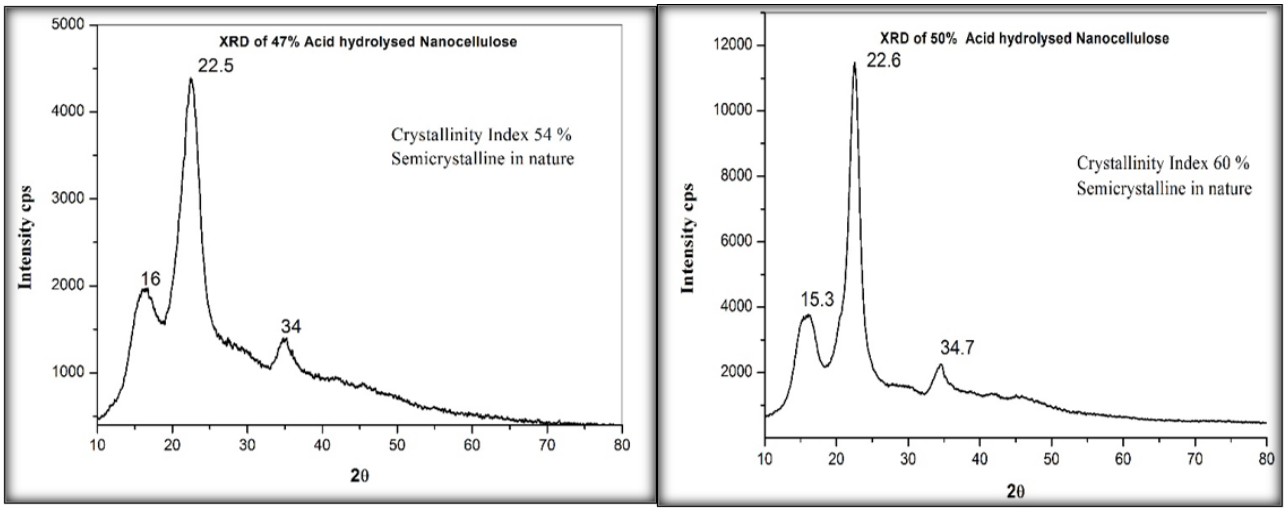

Figure 8: XRD of (a) AHNC 4 and (b) AHNC 5.

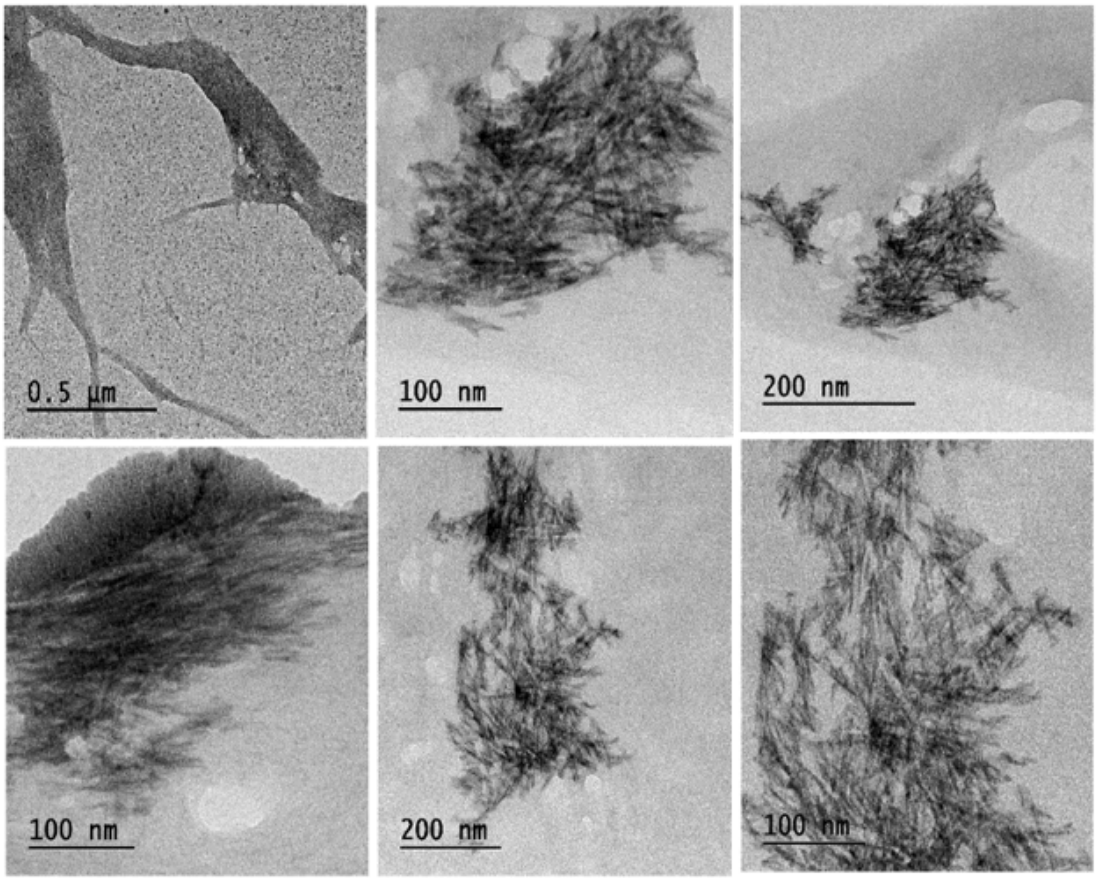

Figure 9: TEM images of AHNC4.

The crystallinity index was calculated by using equation 1 (as discussed in methodology) by using Origin 2019b 64-bit software. It was found that 54 and 60%, respectively, for AHNC4 and AHNC5.

Transmission electron microscopy

The structure and internal morphology of prepared nanocellulose were examined using TEM analysis. The prepared cellulose has a nanofibrous morphology with fibers, as shown in Figure 9. It was simple to see that the nanofibers were clearly separated from one another. The lengths of prepared nanocellulose range from 50 to 300 nm. It can be observed in the fibrous aggregation images. It is inferred that the harshness of the acid hydrolysis process results in the production of rod-like nanocellulose.

CONCLUSION

In this study, commercial cellulose was acid hydrolyzed to produce nanocellulose. The impact of changing acid concentration and temperature on acid hydrolysis was thoroughly investigated. From this work, it was established that an optimal temperature and acid concentration were needed to start the reaction and produce the desired particle size. For any source of cellulose, it is necessary to optimize the factors to obtain the desired size. FTIR measurements confirm the existence of functional groups similar to those found in cellulose. According to PXRD data and referred data, manufactured nanocellulose has the same crystallinity as commercial nanocellulose, and the nature of semicrystalline was seen in TEM images. The presence of fibrous nature with diameters ranging from 50 to 300 nm is confirmed by TEM images. Thus obtained, nanocellulose was further studied for its applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier transform infrared spectroscopy; **PXRD:** Powder X- Ray Diffraction; **TEM:** Transmission electron microscopy; **AHNC:** Acid hydrolysed nanocellulose; **NC:** Nanocellulose; **CI:** Crystallinity Index; **CNC:** Cellulose nanocrystals; **CNF:** Cellulose nanofibrils.

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