Extraction and Characterization of Cellulose and Cellulose Nanowhiskers from Almond Shell Biomass, Metal Removal and Toxicity Analysis

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Abstract: Almond shell is a major agro-industry waste. Cellulose is the major crystalline component of naturally porous almond shell biomass. In this study, cellulose (ASC) was isolated from almond shell (AS) by the dewaxing-alkali treatment-bleaching method, and nanocrystalline cellulose (ASN) was obtained by sulphuric acid hydrolysis of the obtained ASC. Separation efficiency was confirmed by X-ray diffraction and IR absorption studies. ASC exhibited predominantly microporous monolithic structures under a scanning electron microscope. Its porosity resulted in significant absorption of Cu(II) and Pb(II) ions when applied as an absorbent in their solutions. Transmission electron microscopy and atomic force microscopy revealed the formation of ASN nanowhiskers with an average length and diameter of 170 nm and 20 nm, respectively. Zeta potential of -32.4 mV suggested good colloidal dispersibility of the nanowhiskers. No hemolytic toxicity to erythrocyte cells was recorded, which suggested the potential applicability of the obtained nanomaterial in foods and pharmaceuticals. Remarkably high crystallinity and thermal resistance observed from calorimetry and thermogravimetry studies indicated enhanced density of the crystalline moiety during synthesis. ASC and ASN can be developed as effective metal absorption substrates and reinforcement agents in heat-resistant composite materials.

Keywords: almond shell; nanocellulose; thermal; toxicity; metal removal.

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1. Introduction

Cellulose is the most abundant structural biopolymer, generally extracted from the plant cell wall. It is composed of D-glucose units joined by linear β -1,4 glycosidic bonds. Its renewability, biocompatibility, mechanical properties, strength, and biodegradability have attracted significant technological attention in the field of material science and bio-waste management [1]. The crystallinity in semicrystalline cellulose is due to specifically ordered inter and intra-glycosidic chain interactions. The ratio of crystalline fractions to disordered amorphous regions in the polymeric matrix is a function of its origin [2]. Six distinct allomorphic forms of cellulose, namely cellulose I, II, III_I, III_I, IV_I, and IV_{II} have been identified [3]. Of these, cellulose I and II are highly abundant in nature and identified by their

infrared absorption and X-ray diffraction patterns. Inter-conversion of allomorphism in cellulose can occur during chemical and thermal processing. Variation in extraction and synthesis conditions can result in marked differences in physical and physicochemical characteristics of cellulose and its derivatives.

Nanocrystalline cellulose (NCC) is considered the most popular physical derivative of cellulose [4]. Amorphous and crystalline domains are arranged alternately within the semicrystalline matrix of cellulose. Hydrolysis by diluted acids results in selective dissociation of amorphous fractions and release of the crystalline structures as NCC particles. Targeted applications of NCCs owe to their small size, high strength, crystallinity, surface area, aspect ratio, and thermostability [5]. Being biodegradable and inert, cellulose and NCC have been used in food and pharmacological appliances [6]. NCC-based primary packaging films have been successfully developed [7]. Although biological nanomaterials are generally considered safe, corrosive chemicals like acids during their synthesis may impart toxic properties. Toxicity to human cell systems must be analyzed to ensure safety. Nanoparticles, being very small in size, may easily interfere with erythrocyte cells once they entered the bloodstream, causing conditions like anemia and jaundice. Our previous studies analyzed and reported on the nontoxicity of rice husk NCC and applied derivatized carboxymethylcellulose as an effective thickness stabilizer in yogurt [8,9]. Stabilizing effect on microencapsulation systems can also be imparted by ASN, indicating probable use in food and pharmaceutical targeted delivery systems [10].

NCCs have been synthesized from cellulose extracted from a vast range of plant parts [11-17]. Almond (Prunusdulcis sp.) contains an edible fleshy seed covered in a hard shell. The shell is cracked open to obtain the seed. Almond shell constituting about 50% of the total fruit weight is a low-cost, porous, and pitted lightweight waste material. Its popular management approaches include landfilling, animal feed, bedding material, and use as fuel for the boiler. Shell powder has been used as reinforcing filler in various composite materials [18], as growing material for soilless vegetable production [19], and for producing activated carbon [20]. Biosorbent materials are popular due to their bulk availability, non-toxicity, and biodegradability. Almond shell has been used as heavy metal and dye absorbent [21-23]. The shell possesses piths formed by lignified thick-walled sclereid cells of sclerenchyma tissue [24]. Almond shell is a rich source of lignocellulosic components. Functional polysaccharides in the material need to be characterized for the prospective value addition of this food industry waste. A cellulose composition of more than 29% makes almond shell a suitable raw material for cellulose and nanocellulose extraction [25]. Variations in extraction technique parameters could be found in the few available studies [26-28]. Method and process conditions, namely time, temperature, and solvent concentration, play decisive roles in yield, shape, size, and physicochemical characteristics, including those from almond shells [29-31]. For example, the shape of NCCs can vary from whisker, rod-shaped, or spherical. Accordingly, particle sizes can also differ [27,29]. Aspect ratio and degree of crystallinity curb the targeted application of nanocrystalline cellulose [32]. Nanofibers and nano-spheres of almond shell NCC have been reported by Maaloul et al. (2017) and Urruzola et al. (2014), respectively [27,29]. Use of 64% or higher concentrations of sulphuric acid has been reported to be ideal to obtain cellulose nanowhisker, a shape which has never been reported for almond shell NCC [1]. Composite beads formed by crosslinking ASN fibers with sodium trimetaphosphate acted as an effective biosorbent in a study [33]. In a more recent report, the authors have developed a roughly sphereshaped biopolymer composite of almond shell cellulose nanocrystals by the dissolution of the

primary particles in 1-butyl-3-methylimidazolium chloride to find them as effective adsorbent of Cu(II) with absorption capacity as high as 131.16 mg/g in batch process and up to 81% ion removal after four cycles of repeated adsorption and desorption [34]. Similarly, Shu *et al.* (2020) used iron biochar-derived AS for removing hexavalent chromium ions [35]. Chromium ion and Orange G dye removal using polyaniline-AS biomass composite were also reported [36]. These were partially indicative of and hence created the major scope for doing adsorption studies on cheaper, un-derivatized native materials, namely almond shell powder, cellulose, and nanocellulose on multiple toxic metal ions in industrial wastewater streams.

In this research, almond shell cellulose (ASC) was extracted by alkaline processing and bleaching of almond shell (AS) powder followed by reduction to nanowhiskers (ASN) using a 68% solution of sulphuric acid. AS, ASC, and ASN were studied for changes in their physicochemical properties, toxicity on erythrocyte cells, and applicability as copper and lead metal absorbent biomaterials

2. Materials and Methods

2.1. Materials.

Almond shells (AS) procured from an almond seller from New Delhi, India was washed repeatedly with distilled water followed by air drying at 50 °C for 24 h. The shells were pulverized and passed through sieves to obtain flour with 150 to 210 μ m particle sizes. Milled almond shell particles' size can directly affect the extractability of cellulose [31]. The particulate powder samples were stored in a high-density polyethylene container till further use. Analytical grade chemicals were purchased from HiMedia (India), dialysis membranes from Sigma-Aldrich (USA), and glassware from Borosil (India).

- 2.2. Methods.
- 2.2.1. Isolation of cellulose.

Cellulose was isolated from AS powder using modifications of available methods [11, 37-39]. Briefly, the powder was first dewaxed by the soxhlet method using a mixture of benzene and methanol (1:1) at 80 °C for 8 h. After evaporating the residual solvent, 100 g of dewaxed powder was mixed with 400 ml sodium hydroxide solution (3%, w/v) and autoclaved (121 °C, 15 psig) for 8 h. This treatment weakened the bonds between different non-cellulosic components and was repeated twice to ensure better separation. The alkali solution was then strained, and the residual material was washed ten times with distilled water to get rid of all adhering alkali. It was then subjected to bleaching thrice with a solution containing 27 g NaOH, 75 g glacial acetic acid, and 17 g sodium hypochlorite in 1000 ml distilled water, each time for 8 hours in an orbital shaker. Bleaching resulted in delignification of the holocellulose material and solubilization of the non-cellulose components. Off-white-colored neutral ASC was obtained after washing several times with distilled water. The material was dried at 70 °C for 24 h to 5% moisture content and stored in an air-tight container.

2.2.2. Preparation of nanowhiskers.

For nanowhisker production, 5 g ASC was treated with 147 ml of a sulphuric acid solution (68% w/w, 1:20 ASC to acid ratio) at 40 °C for 90 min with stirring at 1500 rpm. The reaction was terminated by pouring the reaction mixture into 1 liter of chilled distilled water.

The solution was centrifuged at 8000 rpm for 10 min. The obtained aliquot was dialyzed against millipore water till neutrality. The suspension was sonicated (300 W) in an ice bath for 30 min, frozen at -40 °C, and then lyophilized for 72 h to ASN particles.

2.2.3. Chemical composition and yield.

Chemical compositions of AS and ASC were analyzed by the methods described by de Oliveira *et al.* (2017) [40]. Lignin was determined by the TAPPI T13m-54 procedure as described by Song *et al.* (2020) [41]. Briefly, a suspension carrying 1 g of a powder sample in 20 ml sulphuric acid (72%) was magnetically stirred for 24 h. The leftover residue containing insoluble lignin and ash was collected by filtering the suspension through a reweighted sintered glass funnel (Borosil, G4) and consequently dried (70 °C, 24 h) and weighed. Holocellulose (cellulose + hemicellulose) and cellulose contents were determined as per Song *et al.* (2020) [41]. A suspension carrying powdered sample (3 g), sodium hypochlorite (2.5 g), glacial acetic acid (1 ml), and distilled water (120 ml) was maintained at 70° C in a water bath with stirring for 1 h. The sequence was repeated twice with the addition of a fresh solution. After storing at 4 °C for 3 h, the supernatant containing suspended holocellulose material was filtered through sintered glass funnel, washed rigorously with distilled water followed by methanol, and dried at 40 °C in a vacuum oven till constant weight. Ash content was determined by a standard AOAC (2006) method [42].

2.2.4. Yield of nanocrystalline cellulose.

The yield of ASN in sample suspension was determined gravimetrically [12]. A measured aliquot volume was taken and dried at 90 $^{\circ}$ C for 24 h in a hot air oven. The yield was determined by the ratio of the mass left after drying to the representative mass of AS taken for its preparation.

2.2.5. Electron microscopy.

The morphologies of AS and ASC samples were observed under a scanning electron microscope (SEM, model EVO-18, Zeiss, Germany). Powder samples were fixed on doublesided adhesive carbon tapes, vacuum dried, sputter-coated with gold (Au), and scanned at an accelerating voltage of 20 kV under 5000x magnification. A transmission electron microscope (TEM, XFlash 6T130, Bruker, USA) operating at 120 kV voltage was used to assess the surface dimensions of the ASN particles at higher magnifications. A drop of 0.2% aqueous suspension of ASN was deposited on a copper grid (360 mesh) and allowed to dry under a vacuum. The copper grid was then inserted into the instrument, and magnified images were captured.

2.2.6. Atomic force microscopy.

An atomic force microscope (AFM, NT-MDT, SOLVER NANO, Russia) was used to capture 2D and 3D surface images of ASN particles. The sample was prepared by depositing a drop of 1% aqueous suspension of ASN on a glass slide, followed by drying. Tapping mode scanning was performed using a standard silicon probe at room temperature ($RT = 25\pm2$ °C).

2.2.7. Zeta potential.

The value of zeta potential indicates the surface charge of nanoparticles, suggesting their electrophoretic mobility. An aqueous suspension (0.005%, w/w) of the freeze-dried ASN

(2)

particles was analyzed in a Zeta Sizer Nano ZS (Malvern Panalytical, UK) instrument at RT, applying a wavelength of 659 nm.

2.2.8. Infrared spectroscopy.

A Fourier transform infrared (FTIR) spectrometer (Cary 630 FTIR, Agilent Technologies, USA) with KBr optics was used to estimate the chemical bonding patterns of AS, ASC and ASN (1:100, sample:KBr). Scanning was performed within the wavenumber range of 400-4000 cm⁻¹ at a resolution of 4.0 cm⁻¹, using inbuilt Resolution Pro software (version 2.5.5, Agilent Technologies, USA) to obtain the absorption data.

2.2.9. X-ray diffraction.

Wide-angle X-ray diffraction (XRD) was performed to determine the crystallinity of the samples. A MiniFlex 600 (Rigaku) instrument with $\lambda = 1.54$, acceleration potential = 40 kV, current =15 mA fitted with a copper target was used to measure the diffraction over a scanning range of $2\theta = 2^{\circ}$ to 60° at a scan speed of 3° /min. Percentage crystallinity (Xc) was determined using the amorphous subtraction method [11].

$$X_{C} = A_{c} / (A_{C} + A_{m}) \times 100$$
(1)
Where X_{c} = percentage crystallinity

where $X_c =$ percentage crystallinity

 A_c = area under crystalline peaks A_m = area under the amorphous portion

The crystallinity index (CrI) was determined according to the Segal equation (Segal, Creely, Martin, & Conrad, 1959) as

$$CrI = (I_{200} - I_{am})/I_{200} \times 100$$

Where,

 I_{200} = intensity of the crystalline peak corresponding to crystalline plane 200 (2 θ = 22.7°) I_{am} = intensity of the amorphous peak between crystalline planes 200 and 110 (2 θ = 18.8°) Average crystal thickness (t) of cellulose I was determined using Scherrer's equation

 $t = K\lambda/\beta Cos\theta$

Where,

K = correction factor value of 0.91

 $\lambda = 0.154 \text{ nm}$

 $\theta = diffraction angle$

 β = full width at half maximum of the peak corresponding to crystalline plane 200

2.2.10. Thermogravimetric analysis.

The samples' thermal degradation-dependent weight loss patterns were assessed using a thermogravimetric analyzer (TGA, TG209 F3 Tarsus, NETZSCH) under N₂ atmosphere. Approximately 10 mg of powder sample was heated from 20 °C to 600 °C at a rate of 20 °C/min under a gas flow rate of 60 ml/min.

2.2.11. Differential scanning calorimetry.

Differential scanning calorimetry (DSC) of the samples was carried out using a DSC-1 STAR^e System (Mettler Toledo, USA). The machine was periodically calibrated by flushing with standard indium for heat flow and temperature. Approximately 10 mg sample was taken https://biointerfaceresearch.com/

in a hermetically sealed DSC pan, heated from 20 °C to 500 °C at a rate of 10 °C/min under N₂ atmosphere against an empty reference pan. The onset (To), peak (Tp), and conclusion (Tc) temperatures and enthalpy of crystallite melting (Δ H, J/g) were recorded from the thermal transition curve generated by the inbuilt software.

2.2.12. Hemolytic activity.

The hemolytic activity of nanomaterials suggests their toxicity. Kalita *et al.* (2015) described a method slightly modified to measure the hemolytic activity of ASN [44]. Briefly, fresh goat blood was collected in a sodium heparin-coated tube, centrifuged at 5000 rpm for 5 minutes, and the supernatant discarded. The precipitate containing erythrocyte cells was washed twice with sterile phosphate buffer saline (PBS, pH = 7.4). Two milliliters of the solution were transferred to a tube containing 5 mg of ASN, and volume was made up to 50 ml with phosphate buffer. Another tube with 0.2% Triton X-100 was also inoculated with the same volume of erythrocytes, considering the complete lysis of the cells. After 80 min of incubation at 37 °C, the tubes were centrifuged (5000 rpm for 5 minutes), and the supernatants' absorbance was recorded at 415 nm. The absorbance of PBS was the negative control. Hemolysis percentage (H, %) was calculated using the equation

$$H = [(As - An) / (A_{100} - An)] \times 100$$
(3)

Where,

As = Absorbance of ASN hemolysed sample A_{100} = Absorbance of Trion X-100 hemolysed sample An = Absorbance of negative sample

2.2.13. Metal sorption property.

AS, ASC, and ASN powders were assessed for their copper and lead absorption capacity by a batch method modified from Ronda *et al.* (2013) [45]. Two metal solutions were prepared by dissolving 1 g of Cu(NO₃)₂.3H₂O and Pb(NO₃)₂ in 1000 ml distilled water. 100 ml portions of these solutions were taken in 150 ml conical flasks, and 1 mg powder samples were added, pH adjusted to 5.0, and incubated at 25 ± 1 °C for 120 min in a shaking incubator. After filtration, final concentrations of Cu(II) and Pb(II) in the solutions were measured by atomic absorption spectrometry (AAS-ICE 3500, Thermo Scientific, UK), and the percentage of ion removal was calculated.

3. Results and Discussion

3.1. Chemical composition and yield of fibers.

Weight-based compositions of α -cellulose, hemicellulose, lignin, and ash in AS were 32.46%, 29.54%, 31.70%, and 3.10%, respectively. The isolation process and process variables decide the final yield of cellulose from its source [30]. A comparison of these values to those reported by other authors using different extraction methods is given in Table 1. ASC had a cellulose content of 90.24% and hemicellulose and lignin proportions of 4.78% and 0.56%, respectively, suggesting efficient removal of non-cellulosic components during alkali and acid treatments used in this synthesis method.

Cellulose	Hemicellulose	Lignin	Ash	References
32.46	29.54	31.70	3.10	Present study
29.90	25.10	30.10	3.40	[27]
29.10	35.20	32.70	3.40	[25]
50.70	26.40	20.40	1.63	[24]
40.50	19.70	27.20	-	[59]

 Table 1. Composition (average, weight basis %) of cellulose and non cellulosic components recorded for almond shell biomass in different studies.

3.2. Yield of nanocellulose.

The yield of ASN was 41.53%, which suggested maximum removal of amorphous fractions, indicating an efficient time, temperature, and acid concentration combination for cellulose hydrolysis. The yield was higher than previously reports on nanocrystalline cellulose extracted from coconut husk (12.0%), soy hull (20.0%), pinewood (2.3%) and corn cob (6.0%) [12,46,47] and lower than those extracted from cotton linters (74.5%), spruce wood (61%), Chinese silver grass (55.6%), eucalyptus wood (63%) and sugarcane bagasse (50-58%) [48,49].

3.3. Scanning electron microscopy.

Morphological features of 5000x magnified AS and ASC samples are shown in Figure 1 (a, b, and c). AS exhibited a smooth, uniformly dotted surface. The dotted layer was removed during dewaxing and delignification. ASC mostly exhibited pitted and porous monolithic structures with pore sizes ranging between 0.8 μ m to 2.0 μ m and very few rod-like structures (Figure 1 b, c). Khili *et al.* (2018) reported the occurrence of only rod-like fibrillary structures with breadth and length of 10-20 μ m and 80 μ m respectively, which were further composed of nano-rods held together [26]. Delignification in their method involved high temperature, which could cause degradation of the basic porous cellulose bodies. The porous structures dominated the scan area in the present study, even after multiple repetitions of extraction and SEM observation.



Figure 1. 5000x SEM micrographs of (a) AS; (b) ASC porous structures; (c) ASC rod structures; (d) 8000x and (e) 50000x TEM micrographs of ASN; (f) 2D; (g) 3D AFM images of ASN.

It was opined that cellulose fibers in AS repeatedly interlock with each other, forming its naturally porous surface. This peculiar structure of ASC is being reported for the first time. Porous cellulose monoliths could be a suitable absorbent substrate for separation, purification, and biocatalysis. This microporous structure could be considered the factor behind almond shell biomass's high absorption efficiency [21].

3.4. Transmission electron microscopy.

Roughly spindle-shaped whisker structures with lengths ranging from 130 nm to 470 nm and diameter from 10 nm to 30 nm were observed under TEM (Figure 1 d, e). This was contrary to previous reports [27, 34], which reported 200 nm-sized spherical structures of ASN particles. Differences in hydrolytic acid concentration, temperature, and time have been related to differences in nanoparticle morphology [50]. The nanowhiskers exhibited a trivial clustering tendency due to H-bonding [11]. At 50000x magnification, ASN exhibited transparency, suggesting porosity (Figure 1 e). Hence, the typical lightweight of almond shell biomass could be attributed to extensive porosity ranging from macro, micro, to nano-scales of its natural construction.

3.5. Atomic force microscopy.

Both 2D and 3D AFM images of ASN exhibited spindle-like structures with ovalshaped smooth central portions (Figure 1 f, g). As was also observed under TEM, aggregation of particles at endpoints caused limited aberration in structural distinctiveness in the AFM images. Particle lengths recorded between 230 nm to 450 nm were within the size range observed under TEM.

3.6. Zeta potential.

The stability of nanoparticles in aqueous colloidal suspension is determined by their surface charges. ASN presented an average zeta potential of -32.4 mV. The application of sulphuric acid for hydrolysis causes sulfate esterification of hydroxyl groups of the cellulose molecules [51]. The presence of negative charges results in electric repulsion, restricting them from coagulating. This substantially negative zeta potential value (lower than -25 mV) suggested good colloidal stability of the nanoparticles, as was also indicated by the limited self-adherence of the particles prepared for TEM and AFM studies [52]. This property could make way for ASN in applications requiring suspension of the bio-nanomaterial in the aqueous phase.

3.7. Functional groups.

FTIR spectra of AS, ASC, and ASN are shown in Figure 2. Characteristic bands of cellulose were observed for all samples. The prominent peaks at 1517 cm⁻¹, 1589 cm⁻¹, and 1234 cm⁻¹ bands in AS were ascribed to the unsaturated linkages and C=C stretching vibrations of aromatic ring structures of lignin [11,13]. Another band at 1734 cm⁻¹ of AS is associated with the C=O stretching of acetyl and uronic ester groups of hemicellulose and xylans or carboxylic group ester linkages of ferulic and p-coumaric acids in lignin [40,53]. The absence of all these bands and increased prominence of the band at 2921 cm⁻¹ (C-H stretching vibration of cellulose) for ASC and ASN depicted successful removal of non-cellulosic components during delignification [14,48]. In holocellulose matrix, these components inhibit inter-cellulose

H-bonding. The broad peak between 3000-3700 cm⁻¹, associated with -OH stretching vibration of cellulose, became sharper in ASC and ASN, suggesting a higher concentration of purer cellulose per unit of matrix space. Absorption bands at 1432 cm⁻¹, 1060 cm⁻¹, and 897cm⁻¹ which were characteristic of $-CH_2-(C_6)$ - bending, C-O stretching of pyranose ring, and bending vibration of β -glycosidic linkages, respectively, were depictive of unaltered cellulose I structure [11].



Figure 2. FTIR spectra of AS, ASC, and ASN.

3.8. X-ray diffraction.

XRD graphs of AS, ASC, and ASN are presented in Figure 3. AS exhibited major diffraction peaks at 2 θ values of 16.4°, 21.6°, and 35.0°. The peak at 21.6° shifted to 22.7° in ASC and ASN and developed distinct prominence. This shift could be attributed to the removal of hemicellulose and lignin portions from the crystalline matrix of holocellulose that are known to cause crystalline diffraction interference [16,50].



Figure 3. XRD curves of AS, ASC, and ASN. Crystallinity (%) and crystallinity index (%) are plotted in insets I and II, respectively.

Significant increase in Xc from AS (35.7%) to ASC (51.8%) and enhanced prominence of all the three peaks occurred due to the removal of largely amorphous lignin and

hemicellulose (Figure 3 Inset I). Cellulose I crystal structure with 110, 200, and 004 diffraction planes were thereby confirmed [54]. No allomorphic transformation was found with the prevalence of cellulose I throughout the synthesis process. ASN showed the highest Xc value of 70.4% due to removing hydrolyzed amorphous portions of cellulose. Values of CrI for AS, ASC, and ASN were 52.45%, 68.21%, and 80.15%, respectively (Figure 3 Inset II). This was in accordance with the result of ASN yield and FTIR study, which indicated a more compact packing of cellulose crystallites in ASN. The value was higher than the crystallinity percentage of ASN reported by Maaloul *et al.* [27,34]. The crystal thickness of ASN corresponding to the crystalline plane 200 was calculated to be 6.29 nm, which was also supported by the TEM results.

3.9. Thermal properties.

The TGA thermograms of AS, ASC, and ASN are shown in Figure 4 (a). Each sample showed an initial weight loss below 100 °C, which was assigned to the release of partially bound inter and intramolecular chemisorbed water [55]. Thermal degradation of vapor-free AS, ASC, and ASN initiated at T_{onset} of 224 °C, 245 °C, and 220 °C, respectively. This second stage of weight loss corresponds to multistep thermal degradation (decoiling + depolymerization + intermolecular dehydration + decomposition) of glycosidic chains. Lower T_{onset} of AS was attributed to the early decomposition of hemicellulose and lignin with lower structural stability than cellulose. The increased proportion of cellulose in ASC was reflected by a sharper drop in percentage mass than AS [56]. ASN showed the lowest T_{onset} (220 °C) but exhibited the longest retention of structural integrity with T_{max} value of 463 °C. This was higher than T_{max} of ASC (359.4 °C) and AS (382.1 °C). Sulfate esterification of cellulose nanoparticle surfaces causes alteration in size and density of the crystalline moiety [57]. In the TGA curve of ASN, the second stage of thermal degradation had two distinct sub-stages. The second sub-stage ranging from 344 °C to 463 °C was contributed by degradation of the lowest molecular weight carbonic residual components in ASN [11].



a b Figure 4. (**a**) TGA; (**b**) DSC curves of AS, ASC and ASN samples.

Two endothermic peaks were observed in each DSC thermogram (Figure 4 b). The first peak below 130 °C was contributed by the evaporation of bound water [11,55]. The second

peak within the range of 300-450 °C was associated with the pyrolysis of all organic components in the samples. The peak was broader in AS and ASC with markedly lower To values of 304 °C and 341 °C, respectively, suggesting initial melting of the amorphous materials present in the samples [58]. The corresponding Δ H values were 7.5 J/g and 12.8 J/g, respectively. The peak for ASN was narrower, intense, and sharper with To, Tp, and Tc values of 359 °C, 393 °C, and 444 °C, respectively, and Δ H value of 19.9 J/g. The highest Tf value of ASN was due to improved crystalline structure during delignification and hydrolysis, which was consistent with the results of XRD and TGA. The results indicated significant development of thermal resistance in the bionanomaterial after acid treatment, suggesting its potential applicability as a reinforcement agent in heat-resistant solid composite materials [13].

3.10. Hemolytic activity.

Hemolytic assays describe the toxicity and biocompatibility of nanomaterials. *Kalita et al.* (2015) reported the non-toxicity of rice husk cellulose nanoparticles [44]. ASN caused 0% hemolysis (H = 0) in the erythrocyte solution showing the same light absorption value as the negative control. Hence, the ASN particles were without any hemolytic toxicity in the state they were analyzed and could be considered safe for use in food and pharmaceutical formulations for intravenous usage.

3.11. Metal sorption property.

The percentage removal of Cu(II) and Pb(II) ions from their aqueous solutions after suspending AS, ASC, and ASN flour particles are plotted in Figure 5. Each sample absorbed Pb(II) more than Cu(II), indicating a higher affinity of the former to the cellulose matrix. ASC exhibited the highest absorption of both the bivalent metals (86.3% and 93.1%), marginally followed by AS (84.2% and 89.1%).



Figure 5. Cu(II) and Pb(II) metal removal capacities (%) of AS, ASC and ASN.

This suggested that the cellulose component served as the major metal ion absorption substrate in both samples. An increase in surface area due to exposure of the porous cellulose surface in ASC could have enhanced the removal efficiency. The reduction of ASC to ASN substantially reduced the sorption property (39.0% and 41.3%). It suggested that the minor negative potential developed on the ASN particles' surfaces after reduction by sulphuric acid treatment and porosity could not improve its metal sorption capacity as sorption is also

governed by substrate's surface size integrity was higher in ASC. However, adsorption efficiency may be further improved after suitable composite development with organic or inorganic components, as reported by other authors [33,35,36].

4. Conclusions

Nanowhiskers were obtained after hydrolysis of the cellulose with 65% sulphuric acid. The extraction method could effectively isolate cellulose from the almond shell and gave sufficient yield and purity of nanocrystalline cellulose (Figure 6). The obtained porous bead-like cellulose structures showed good dicationic metal absorption properties. The porosity of ASC can be projected for absorption and filtration materials to treat industry effluent. The isolation method imparted significant thermal resistance in the nanomaterial. The particles were non-toxic to erythrocytes and can be used as an inert bionanomaterial for application in food and pharmacological industries.



Figure 6. Schematic presentation of the cellulose extraction and nanocrystalline cellulose isolation process.

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Conflicts of Interest

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