Physico-chemical characteristics of nanocellulose at the variation of catalytic hydrolysis process

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ABSTRACT

In this work, the influence of various sulfuric acid (H₂SO₄) concentration is studied towards the crystallinity, particle size distribution, thermal stability, and morphology of the synthesized nanocellulose (NCC) during the esterification process. Different concentrations of H₂SO₄ (40%, 58%, 64% and 78%) was utilized to achieve the optimal properties of NCC. The as-produced NCC was characterized by Fourier Transmission Infra-Red (FTIR) analysis that confirmed the attachment of sulphate ions (SO₃⁻) to C-6 of the glucose ring. Moreover, the hydrogen ions (H⁺) weakened the C-6 of cellulose chains by attacking the glycosidic linkages resulting in the formation of NCC. The X-Ray Diffraction (XRD) analysis revealed an increase in the crystallinity index with increasing H₂SO₄ concentration till 78%. NCC represented a needle shaped like structure having a particle size of 10–18 nm in diameter as observed under Atomic Force Microscopy (AFM) and Fourier Emission Scanning Electron Microscopy (FESEM). Furthermore, Dynamic Light Scattering (DSL) analysis recorded the particle size of the NCC as less than 20 nm in diameter. Thus, owing to various H₂SO₄ concentration the particle size, crystallinity, and features of NCC are substantially affected.

1. Introduction

Cellulose is one of the most abundant renewable natural resource in the world and has nearly one trillion tonnes of annual biomass production. It is present in a wide range of living organisms such as plants cell walls, microorganisms like bacteria, fungi, algae etc. [1, 2, 3]. Irrespective of the nanocellulose (NCC) resource, cellulose is a homopolysaccharide consisting of a β linked D-glucose units connected together by β 1–4 glycosidic bonds. The repeating element of cellulose is a dimer of glucose units grouping together consisting of primary and secondary alcohol groups at C-6, C-2, and C-3 position, respectively. The primary alcohol reacts ten times faster than the secondary one [5]. Though, the surface reactivity might be influenced by a reactant or solvents used during NCC synthesis. Also, the NCC structure consists of three OH groups depicting different surface reactivity in the order of OH-C₆ > OH-C₂ > OH-C₃, correspondingly [6].

Cellulose has a wide-ranging applications in pharmaceutics, veterinary food, packaging, wood and paper, cosmetics and textile industries [4]. Nevertheless, NCC has given tremendous applications in biomedical [6, 7], packaging, tissue engineering, ornamental coating materials, security papers [8], and life science devices [7]. NCC depicting remarkable characteristics like elemental nano dimensions, high surface area, unique morphology, high crystallinity index, mechanical reinforcement, biocompatibility and eco-friendliness [4, 5, 9]. Various kind of NCC such as nanofibril cellulose, nanocrystalline cellulose, nano whiskers and bacterial NCC can be produced by different methods i.e., mechanical treatment, chemical reaction, and bacterial interaction.

Normally, the NCC isolation process is achieved using acid hydrolysis treatment. An amorphous region inside the NCC structure is disrupted during the treatment whilst the crystalline region remained intact. Various parameters affect the hydrolytic cleavage such as the acid species, acid concentration, time, and temperature of the hydrolysis reaction. Under defined conditions, NCC is produced using sulphuric acid (H₂SO₄) as a catalyst that allows the attachment of support groups, induces a negative electrostatic layer covering the cellulose chains [10, 11, 12, 13]. In the literature reports different raw materials are being used for NCC synthesis namely cotton, linen [9], avicel, wood pulp, and kenaf [14]. Nanoparticles (NP) production from acid treated cellulose have gained valuable attention when it was studied that, in suspension, they could form stabilized chiral nematic phases similar to highly textured...
organizations of microfibrils found in native systems. Steric stabilization of the nanocrystals with either surfactants or surface chemical modifications recently allowed research on cellulose whiskers suspension in apolar solvents and in a medium with high salinity [15].

The geometric properties of NCC structures such as shape, length and diameter depend upon the cellulose resource and the extraction/isolation process. In addition, the hydrolysis condition including time, temperature, kind of acids and pH play a pivot role in NCC production with different nanodimensions, shape, and length [8, 16]. NCC morphology is crucial to explore their potential applications like fillers in biopolymers. From previous reports, the size distribution and shape of three cellulose types (cotton, avicel and tunicate) was studied using acid mediated hydrolysis reaction [15]. Findings revealed that cotton and avicel cellulose showed maximum width size distribution as compared to tunicate, indicating that using 64% H2SO4 resulted in NCC formation with heterogeneous alterations. Another reports evaluated optimal hydrolysis conditions using microcrystalline cellulose (MCC) derived from Norway spruce (Picea abies) [16]. Researchers observed that at 2 h with a H2SO4 concentration 63% (w/w), it is possible to achieve nanocrystals with a different nanodimensions, shape, and length [8, 16]. NCC morphology is determined with increasing H2SO4 concentration from 40% to 78%. Secondly, the NCC samples were analysed using X-ray diffraction (XRD) having theta ranges from 5°–40° with a 0.02 step. To characterize the crystallinity of the different samples, the crystallinity index CI was determined using the following Eq. (1).

\[ CI(\%) = \left( \frac{I_{200} - I_{am}}{I_{200}} \right) \times 100 \]

- I200: maximum intensity of the principal peak (200) having a reference value of 22.7° ± 20
- Iam: intensity scattered by the sample amorphous part where the lattice diffraction peak is located at 18° ± 20

2.3. Fourier Transmission Infra-Red (FTIR)

X-ray diffraction was used to determine the crystallinity of the synthesized NCC obtained with various acid concentrations. The samples were analysed using X-ray diffractometer (D8-Advance Bruker AXS GmbH) at 25 °C with monochromatic Cu Kα radiation source. Wave-length of radiation source (Cu Kα) was 0.154 nm. Step scan mode is used having theta ranges from 5°–40° with a 0.02 step. To characterize the crystallinity of the different samples, the crystallinity index CI was determined using the following Eq. (1).

\[ CI(\%) = \left( \frac{I_{200} - I_{am}}{I_{200}} \right) \times 100 \]

- I200: maximum intensity of the principal peak (200) having a reference value of 22.7° ± 20
- Iam: intensity scattered by the sample amorphous part where the lattice diffraction peak is located at 18° ± 20

2.3.3. Dynamic Light Scattering (DLS)

The particle diameter of samples was measured by means of DLS (Malvern 110). A sample (0.001%w/v) was dispersed in water and sonicated to prevent the agglomeration of nanocellulose fibers.

3. Result and discussion

3.1. NCC characterization

3.1.1. Fourier Transmission Infra-Red (FTIR)

FTIR characterization (Figure 1) is achieved to comprehend the mechanism and surface chemistry of synthesized NCC at various acid concentration. The analysis of samples (MCC, 40NCC, 58NCC, 64NCC and 78NCC) were observed at wavelength of 3500 cm⁻¹ (OH stretching vibration of the OH groups in cellulose chains) [17], 2890 cm⁻¹ (alkyl group–CH) and 1640 cm⁻¹ (NCC/H–OH stretching bend of absorbed water in cellulose) [18].

Table 2 shows a series of FTIR spectra for the NCC-characteristics. The peaks at around 1430-1420 cm⁻¹ (–CH2 scissoring and deformation vibrations of CH) referred towards the specific features of cellulose polymeric chains. This phenomenon was brought by the hydrolysis reaction during the NCC synthesis. There are three different possible mechanisms occurred during NCC production. First, SO4²⁻ attacked C-6 of cellulose pyranose ring as less steric interruption is occurred at C-6 than other carbon ring locations. This can be confirmed via 1230 cm⁻¹ peak obtained with increasing H2SO4 concentration from 40% to 78%. Secondly,
the H⁺ ions attacked the glycosidic linkages, broken down the cellulose chains that resulted in NCC formation. Lastly, there is a possibility that the hydrolysis reaction occurred at C-O-C pyranose ring skeletal and disrupted the cellulose ring. The phenomenon can be proved by the intensity reduction of 1150 cm⁻¹ band (C-O-C-pyranose ring) as observed in Figure 2.

### 3.1.2. X-ray diffraction (XRD)

The crystallinity of produced NCC is further analysed using XRD with the consideration that, micro and nano sized cellulose have both the crystalline and amorphous phases. Formation of the NCC crystalline structure is due to the inter and intra hydrogen bonding as well as the van der Waals interaction between the polymeric chains [19]. However, during the hydrolysis of MCC detachment of inter linkages consequently results in the re-arrangements of polymeric linkages [20].

Figure 3 shows the diffraction patterns obtained from microcrystalline cellulose (MCC) having different H₂SO₄ concentrations (40%, 58%, 64% and 78%). The crystallinity index (Cθ) of the analyzed samples are summarized in Table 3. The highest value of Cθ was recorded at 86.6% for 78NCC produced at 78% H₂SO₄ concentration. Also, at 20 the strongest and the sharpest peak obtained was 22°. The achieved results were strongly supported by the FTIR analysis and the possible hydrolysis mechanisms during the NCC synthesis.

During the hydrolysis reaction, the H⁺ ions were capable to penetrate the more accessible amorphous regions of cellulose, allowed the lytic cleavage of glycosidic bonds thus resulted in individual crystallinity [21]. Moreover, the parallel realignment of mono-crystal and rearrangement of cellulose chains could improve the crystallinity [22]. This phenomenon could be supported by the narrowing of the diffraction peaks. The increment in crystallinity is also expected to increase the mechanical properties (toughness, firmness).

### 3.1.3. Fourier Emission Scanning Electron Microscopy (FESEM) and Atomic Force Microscopy (AFM)

The surface morphological properties of synthesized NCC were observed under FESEM analysis. Figure 4 shows the FESEM images of the MCC and NCC at different concentrations of H₂SO₄. From the series of images, some of the obvious changes noticed regarding particle size, features, and surface area from MCC to NCC as the concentration of H₂SO₄ increased (40%–78%). Results elucidated that the morphological features of MCC remain unchanged with the H₂SO₄ concentration till reached 40%. However, as the concentration of H₂SO₄ raised to 58%, there were some significant changes noticed such as formation of microfibril’s structures. From the observations it can be anticipated that, the H⁺ ions attacked the glycosidic linkages of MCC cellulose chains, caused the scissoring effects of polymeric chains and resulted in single fibers structure formation.

AFM micrographs topographical analysis exposed that in 40NCC structures, there were clumpy fibrous structures appeared. With increasing acid concentration to 64% and 78%, the morphology of the fibres (64NCC, 78NCC) changed from single fibres to needle shaped like structures. Also, the surface area of the fibres were found noticeably increased with decreasing fibres sizes (~74 nm), especially for 64NCC and 78NCC.

As mentioned before, during MCC hydrolysis the function of H₂SO₄ is to cleave and eliminate the amorphous regions of microfibrils that leads to NCC formation having a diameter range from micron to nano-sized [22]. Figure 4(c) shows the cellulose image of 40NCC in which the fibres are not fully separated, and the amorphous region is not eliminated as validated from Cθ. With 78% H₂SO₄ concentration, needle shaped like morphology was observed for 78NCC as displayed in Figure 4(d). The findings are similar with the micrographic images of FESEM and AFM analysis.

### 3.1.4. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was carried out to investigate the effects of SO₃⁻ ions concentration on the thermal stability and material decomposition under N₂ atmosphere [23]. The decomposition curves were plotted over temperature as shown in Figure 5. From the thermographs, it can be depicted that the decomposition temperature of MCC was found lowered from 320 °C to 180 °C with increasing H₂SO₄ concentrations (40%–78%). 64NCC and 78NCC samples presented the decomposition temperature almost similar to the previous reports under optimal conditions [16], as at 64% H₂SO₄ concentration all the C-6 in glucose units reacted with SO₃⁻ ions.

Generally, all the evaluated samples revealed a similar mass decomposition (less than 7%) at 25 °C-100 °C. As water content loosely bound to the NCC hydrophilic surface responsible for bonding between the remnants OH groups inside NCC chains. From Figure 5, the on-set temperature of decomposition was reduced significantly over the varying H₂SO₄ concentrations. Theoretically, NCC hydrolysed using H₂SO₄ possessed sulphate groups (SO₃⁻) on the outer surface of NCC cellulose chains. The presence of the SO₃⁻ noticeably decreased the thermal stability via the dehydration reaction [23]. Therefore, 78NCC and 64NCC having higher SO₃⁻ ratio subsequently lowered the temperature of thermal degradation. This phenomenon became more significant with the fact, an increment in short NCC chains resulted in lower thermal degradation energies and vice versa [3, 24]. Another reports studied the effect of SO₃⁻ groups on thermal

### Table 2. Functional groups and compounds present in NCC.

| Entry No. | Wavelength (1 cm) | Functional Group | Compound                      |
|-----------|-------------------|------------------|-------------------------------|
| 1         | 3300              | OH               | Acid                          |
| 2         | 1640              | NCC-OH           | Adsorbed water                |
| 3         | 1430–1420         | CH₂              | CH₂ scissoring motion in cellulose |
| 4         | 1270–1232         | C-O-C            | Aryl-alkyl ester              |
| 5         | 1150              | C-O-C            | Pyranose ring skeletal        |
degradation and observed similar results \cite{21}. However, there is some effort required to improve the thermal stability of cellulosic chains due to $\text{SO}_4^{2-}/\text{CO}_3^{4-}$ groups reported by Wang et al., \cite{23}. The de-sulfonation and neutralization by alkaline solution to end sulphate group content and the results proved an increase in thermal stability.

3.1.5. Dynamic Light Scattering

Particle analysis was executed to estimate the measurement or size of produced NCC as shown in Figure 6 (a-d). Mainly, all the synthesized NCC demonstrated a mono modal normal distribution and asymmetric pattern having <100 nm size. 40NCC produced by 40% $\text{H}_2\text{SO}_4$ indicated the major distribution between 30-50 nm with 30% at 40 nm peak. The wide particles size distribution as depicted in Figure 6a was observed to be lowered than other concentrations. Figure 6c shows the unimodal distribution of 64NCC at 64% $\text{H}_2\text{SO}_4$ concentration which was the maximum concentration recorded than earlier studies \cite{12, 16, 25}. According to another reports, with 64% $\text{H}_2\text{SO}_4$ concentration the produced cellulosic whisker exhibited a length between 200 nm–400 nm and a width <10 nm \cite{16}. In this study, distribution at 78% concentration was narrower than other samples such as 40NCC, 58NCC and 64NCC, respectively.

The highest concentration of $\text{H}_2\text{SO}_4$ (78%) recorded the smallest particle size distribution within 5–35 nm range and the highest peak obtained between 10-15 nm at 13%. The smallest range and narrower distribution showed that the NCC particle size was uniform and dispersed well in water. Theoretically, by increasing $\text{H}_2\text{SO}_4$ concentration the $\text{SO}_4^{2-}$ would increase significantly. In $\text{SO}_4^{2-}$ presence, NCC become highly negative charged exhibiting maximum dispersion as

![Figure 2](image2.png)

**Figure 2.** Proposed chemical reaction on the hydrolysis process of microcrystalline cellulose (MCC) with sulphuric acid, a) sulphate ions attacking C-6 in cellulose chain, b) hydrogen ions break pyranose ring, and c) hydrogen ions attacked glycosidic linkages and break the cellulose chains.

![Figure 3](image3.png)

**Figure 3.** Diffractogram of nanocellulose (40NCC, 58NCC, 64NCC and 78NCC) at different acid concentration and microcrystalline cellulose precursor (MCC).

| Table 3. Degree of crystallinity of the produced NCC. |
|---------|------|------|-----------------|
| Entry No. | Samples | $I_{m}$ | $I_{py}$ | Degree of Crystallinity |
| 1 | MCC | 22.706 | 16.6 | 74.9% |
| 2 | 40NCC | 22.781 | 16.749 | 76.4% |
| 3 | 58NCC | 22.758 | 16.452 | 83.3% |
| 4 | 64NCC | 22.855 | 16.452 | 85.8% |
| 5 | 78NCC | 22.781 | 16.675 | 86.6% |
observed specially in water [6]. Furthermore, as stated previously 78NCC demonstrated minute fraction of particles size within small range. Breaking down of the cellulose chains and NCC agglomeration depend upon the acid concentration. Findings of Das et. al. demonstrated that 64% H$_2$SO$_4$ was able to produce MCC of 10–50 nm particle size [24].

Figure 4. SEM Morphology of nanocellulose with various acids concentration, a) 40NCC, b) 78NCC; and AFM c) 40NCC d) 78NCC.

Figure 5. Comparative Thermogravimetric Analysis (TGA) of nanocellulose samples (40NCC, 58NCC, 64NCC and 78NCC) with their corresponding microcrystalline cellulose (MCC) precursor.
4. Conclusion

NCC with a targeted range of particle size and uniform distribution was successfully produced and controlled using acid hydrolysis approach. Experimental results revealed that 78% concentration of H$_2$SO$_4$ was able to produce exceptionally fine particles within 8–35 nm range and subsequently enhanced the cellulose fibres surface area. 64NCC and 78NCC exposed that NCC is in needle shaped form. While 40NCC and 58NCC, the fibre was not fully cleaved as acid concentration was not sufficient to break the cellulose chains. XRD analysis elucidated that 78NCC depicted a higher crystallinity index compared to other samples as expected. NCC lowered thermal stabilities at higher acid concentration was due to the splitting of SO$_4$$^2$- groups during thermal degradation process.

Declarations

Author contribution statement

Erfan Suryani Abdul Rashid: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ayesha Gul: Analyzed and interpreted the data; Wrote the paper.

Wageeh Abdul Hadi Yehya: Analyzed and interpreted the data.

Nurhidayatullaili Muhd Julkapli: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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