Extraction and analysis of cellulose nanocrystals from cotton balls by acid hydrolysis

K. G. R. Samarawickrama[1*], U. G. S. Wijayapala[2] and C. A. N. Fernando[3]

[1,2]Department of Textile and Apparel Engineering, Faculty of Engineering, University of Moratuwa, Moratuwa, Sri Lanka
[3]Department of Nano Science Technology, Faculty of Technology, Wayamba University, Kuliyapitiya, Sri Lanka

*Corresponding author: rumeshsnt@gmail.com

https://orcid.org/0000-0003-2364-0716

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Abstract Cellulose is one of the most widely used natural polymers developed in eco-friendly methods, which has been used in various industrial processes and products since ancient times. The sources of cellulose materials are plant and wood fibers. Cellulosic materials are converted into cellulose nanocrystals (CNCs) using mechanical or chemical methods. In this study, the CNCs were obtained from cotton balls by acid hydrolysis method using sulfuric acid. The sulfuric acid hydrolysis method was performed with 64% (w/w) sulfuric acid and combined using a liquor ratio of 1:20 with cotton balls while being subjected to vigorous stirring at 50 ^{o}C for 60 minutes. The cellulose nanocrystals were characterized by Transmission Electron Microscopy (TEM), Fourier Transform Infrared (FTIR) spectroscopy analysis and X-ray Diffraction (XRD) techniques. The extracted cellulose nanocrystals had needle-shaped particles with a 6.35 nm average diameter and a length of 108.8 nm on average. The functional groups of the extracted cellulose nanocrystals were shown to have been evaluated through analysis of the FTIR spectra. Therefore, it was confirmed that the cellulose nanocrystals were successfully extracted from cotton balls using sulfuric acid hydrolysis. The distinctive crystalline cellulose phase of artificial cellulose nanocrystals was recognized using the XRD spectrum.

Keywords: Acid hydrolysis, Cellulose nanocrystals, Cellulosic materials, Cotton fibers, Sulfuric acid.

Introduction

New environmental regulations and increasing global environmental concerns have made it necessary to look for new environmentally friendly materials. Cellulose is the most abundant natural polymer available on the earth and naturally occurring cellulose in wood, natural fibers, tunicate, algae fungi, bacteria, and invertebrates. Primary sources of cellulose for manufacturing procedures are wood and cotton (Rajinipriya et al., 2018; Pandi et al., 2021). Cellulose is a polysaccharide composed of linear glucan chains joined together by -1, 4-glycosidic linkages with cellobiose remnants serving as the repeating unit at different levels of polymerization. These glucan chains are bundled into microfibrils and joined by intramolecular hydrogen bonds and intermolecular Van-der Waals interactions (Habibi et al., 2010; Mandal & Chakrabarty, 2011). Cellulose materials are extensively used in many industrial applications, due to their innate properties including biodegradability, renewability, cost-effectiveness, lightweight and environmental benefits (Tingaut & Zimmermann, 2012; George & Sabapathi, 2015). Cellulose has been also frequently used in various applications such as food, chemicals, textile, biomaterials, electronics and pharmaceuticals etc (Atakhanov et al., 2019; Yang et al., 2019).

Cellulose-based nanomaterials are sustainable and renewable materials. There are two major structures of cellulose nanomaterials that consist of micro fibrillated cellulose (MFC) and cellulose nanocrystal (CNC) and which differ in extraction methods and morphology (Fortunati et al., 2013). The nanoscale configuration of cellulose known as cellulose nanocrystals (CNCs) can be created in a variety of morphological shapes such as spherical, rod, ribbon and needle-like shapes (Lavoine et al., 2012; Samarawickrama et al., 2021). The CNCs have strong crystallinity, high levels of molecular orientation and contain many hydroxyl groups on a large surface area. The cellulose chains in the CNCs are packed into a compact and ordered molecular

arrangement maintained by intramolecular and intermolecular hydrogen bonds. The micro fibrillated structure of the CNCs is also highly reactive, multiscale, and organized hierarchically (Klemm et al., 2011; Tonoli et al., 2019). Cellulose nanocrystals (CNCs) have attracted a lot of attention due to their physical and chemical properties, renewable nature, sustainability and use in composite materials (Azizi Samir et al., 2005; Dimas et al., 2020). The extraction process of CNCs can be carried out using numerous methods such as acid hydrolysis, temp-mediated oxidation, mechanical disintegration and enzyme-assisted hydrolysis. The most efficient among these techniques is the acid hydrolysis method. The fibers of cellulose are exposed to concentrated acid to hydrolyse the amorphous regions of the cellulose chains leaving the crystalline regions unaffected (Habibi et al., 2010; Sacui et al., 2014). Furthermore, CNCs can be extracted using a variety of strong acids including hydrobromic, sulfuric, phosphoric, hydrochloric, and nitric acids. However, the hydrolysis method frequently uses sulfuric acid, which produces a simple and less reaction time than other strong acids. Additionally, CNCs extracted using this method also have a functionalized surface, excellent crystallinity and strong colloidal stability in water (Chakrabarty & Teramoto, 2018; Dimas et al., 2020). Cellulose nanocrystals are mostly extracted from rich natural cellulose materials such as wood, straw, bamboo pulp, coconut husk, rice husk, and sugarcane bagasse etc. (Tang et al., 2017). The excellent properties and biodegradability of cellulose nanocrystals are attractive for numerous manufacturing applications such as paints, coatings, adhesives, food, lacquers, cosmetics, transparent paper and medicines (Klemm et al., 2005; Lavoine et al., 2012). In this study, we used to extract the cellulose nanocrystals from the rich cellulose material of cotton balls and which are made from 100% premium quality cotton fibers. Cotton balls (Non-Discarded) have a number of applications such as removing makeup and cleaning wounds (Xu et al., 2021). The sulfuric acid hydrolysis method is used in this study to separate cellulose nanocrystals from the cotton balls. The characterization of resultant cellulose nanocrystals was performed by Transmission Electron Microscopy (TEM), Fourier Transform Infrared Ray (FTIR) spectroscopy and X-ray Diffraction (XRD) techniques.

Methodology

Materials

Cotton balls (Non-Discarded Cotton fiber balls) were utilized in this study and purchased from the local pharmacy in Sri Lanka. The 98% Sulfuric acid (AR Grade – Sigma-Aldrich, USA) and dialysis membrane tubing (Carolina Dialysis Tubing, USA – Molecular weight cut off 12,000-14,000 Dalton) used in this work were purchased from local chemical suppliers in Sri Lanka. The distilled water was prepared by using Distilled Water Dispenser in the laboratory.

Extraction process of cellulose nanocrystals

The cotton balls were hydrolysed in 64% (w/w) sulfuric acid to extract the cellulose nanocrystals. The acid hydrolysis was carried out by adding 64% (w/w) sulfuric acid dropwise to the pulp of cotton balls (liquor ratio of 1:20) with strong magnetic stirring in an ice water bath at 20 °C. After the addition of the acid, the mixture was heated to 50 °C for 60 minutes while being constantly stirred. After the hydrolysis process, the suspension was diluted with cold distilled water (at 4 °C) in a ratio of 1:10 (v/v) to complete the hydrolysis process. CNCs were separated from the mixture by centrifuging the suspension at 6000 rpm for 15 minutes and the centrifugation is repeated two or three times. Followed by the centrifugation, the supernatant solution was removed and the precipitate solution was rinsed with distilled water in a dialysis membrane until the pH was neutral (pH 6.5 - 7.5). After the dialysis procedure, the suspension was sonicated with an ultrasonic probe sonication homogenizer for 30 minutes in an ice water bath. Finally, the suspension was freeze-dried to obtain the CNCs and stored at 4 °C.

Characterization of extracted cellulose nanocrystals

Transmission electron microscopy (TEM)

The morphology and diameter of cellulose nanocrystals were analyzed by means of the Transmission Electron Microscope (JEM-2100 Transmission Electron Microscope, Japan) with an accelerating voltage of 200 kV. The copper grid with carbon coating received a drop of the aqueous solution of CNCs and before the TEM examination, the sample was dried at ambient temperature.

Fourier transforms infrared (FTIR) spectroscopy

The functional groups of the CNCs sample were identified using Fourier transform infrared spectroscopy. The analysis of functional groups was carried out by the Thermo Scientific Nicolet IS5-ATR (Attenuated Total Reflectance) spectrometer at wavelengths ranging from 600 - 4000 cm^{-1} with a scanning resolution of 4 cm^{-1} in transmission mode.

X-Ray Diffraction (XRD) spectroscopy

A spectrum of X-ray diffraction (XRD) was captured by using Bruker D8 Focus X-Ray Diffraction Spectrometer at the scanning rate of 2°/min from 2θ range of 5° - 60° with Cu Kα radiation (λ = 1.5418 A°) utilizing 40 kV and 40 mA for voltage and current. The extracted CNC sample's crystallinity index (CI) was calculated using the Segal crystallinity index equation given below (Segal et al., 1959; Liu et al., 2017).

$$\mathrm{CrI} = \frac{I_{200} - I_{am}}{I_{200}} \times 100$$

CrI = Crystallinity index

$I_{(200)}$ = Maximum intensity value for the crystalline cellulose (2θ = 22.7°)

I_{am} = Background scatter intensity (2θ = 18°)

Results and Discussion

Evaluation of Transmission Electron Microscopy (TEM) analysis

The morphology and dimensions of CNC samples were examined by TEM observations (Figure 1). The image clearly shows that the extracted CNCs have a morphology similar to needles (or needle-like) and ascertain their effective extraction from cotton balls using the 64% sulfuric acid hydrolysis method. The TEM picture was analysed using the digital image analysing software of Image J, which was possible to determine the typical diameter and length of the extracted CNCs sample and which verified that the CNCs were extracted at the nanometre range. Based on the analysis, extracted CNCs have a diameter of 6.35 nm and a length of 108.8 nm on average.

Evaluation of Fourier transform infrared (FTIR) spectroscopy analysis

Several literature papers on the FTIR data of cellulose nanocrystals provide a list of peak assignments. Figure 2 shows an FTIR spectrum of extracted CNCs from cotton balls using the 64% sulfuric acid hydrolysis method in the wavelength range of 600 - 4000 cm^{-1}. The vibrations recorded at 3443 cm^{-1} were assigned to the intermolecular O-H group present in CNCs (Popescu et al., 2011; Solihin et al., 2018). The stretching vibration modes of the -CH, $-CH_2$, and $-CH_3$ groups are responsible for the wavenumber peaks in the wavenumber range of 2889 - 1651 cm^{-1} (Poletto et al., 2011). The vibrations of stretching and bending the $-CH_2$, $-CH_3$, -OH, and C-O bonds in CNCs are responsible for the absorbency bands at 1425, 1366, 1157, 1027, and 898 cm^{-1}. The CNCs' degree of crystallinity is connected to the band at 1420 - 1430 cm^{-1} (Ponce-Reyes et al., 2014). These different vibration bands can be identified correctly and extracted by purifying cellulose nanocrystals from cotton balls using the 64% sulfuric acid hydrolysis method.

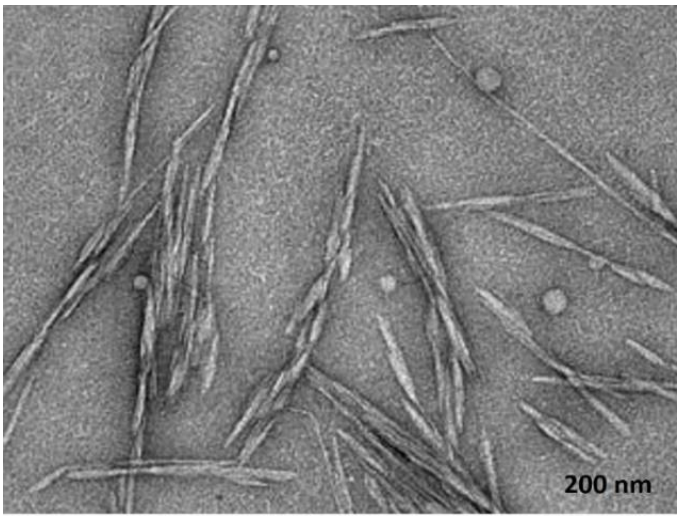

Figure 1: TEM image of extracted CNCs from cotton balls using 64% sulfuric acid hydrolysis

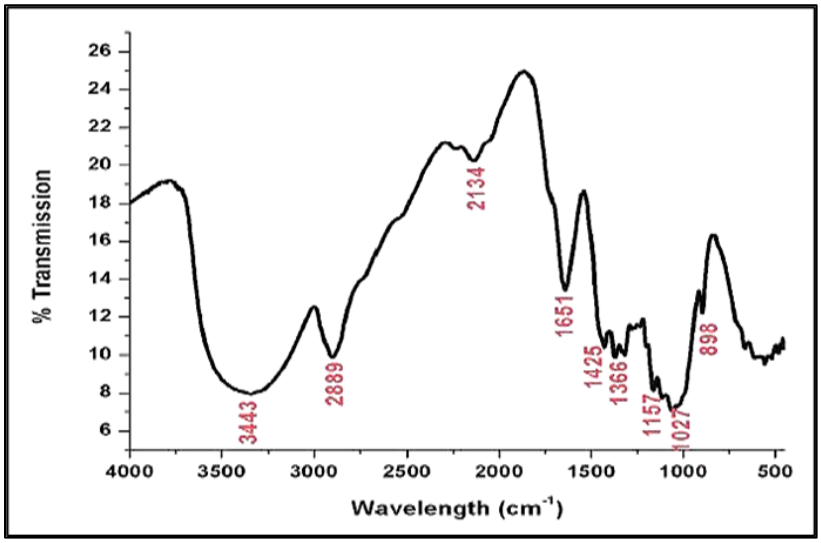

Figure 2: FTIR spectra of extracted CNCs from cotton balls using 64% sulfuric acid

Evaluation of X-Ray Diffraction (XRD) analysis

The crystalline structure and the crystallinity index of extracted CNCs made from cotton balls using the 64% sulfuric acid hydrolysis method shows in Figure 3. According to the XRD analysis, the main characteristic peaks occur at 2θ values of around 14.8°, 16.5°, 22.7° and 34.6°, which correspond to the (110), (110), (200) and (004) planes. These XRD results represent the normal cellulose I structure (Plermjai et al., 2018). According to the Segal crystallinity index equation, the crystallinity index (CrI) of the extracted cellulose nanocrystal sample was respectively calculated to be 77.6%, which was close to that reported in previous studies for cellulose nanocrystal extracted from the cellulose sources using the sulfuric acid hydrolysis method (Plermjai et al., 2018; Bao et al., 2021).

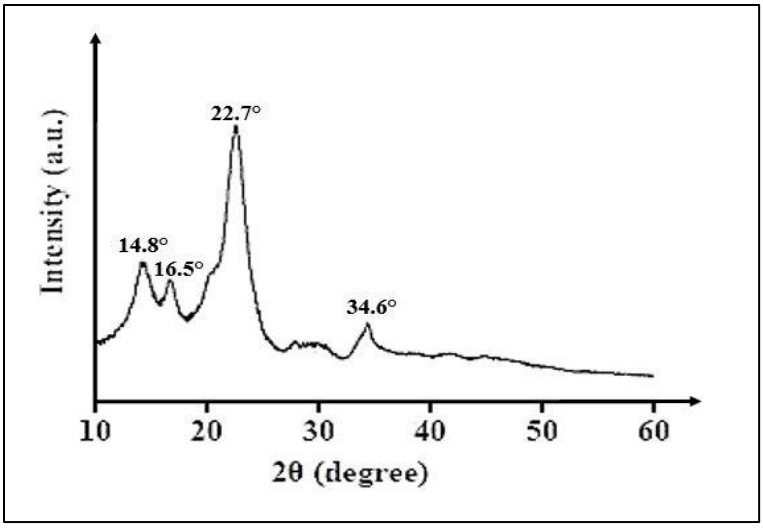

Figure 3: X-ray diffractogram of extracted CNCs from cotton balls using 64% sulfuric acid

Conclusions

In this study, cellulose nanocrystals were successfully extracted from cotton balls using a 64% (w/w) sulfuric acid hydrolysis method. The extracted cellulose nanocrystals were characterized for their morphology, functional groups, crystallinity structure, and crystallinity index by using TEM, FTIR, and XRD analysis. The TEM image showed needle-shaped CNCs with a 6.35 nm average diameter and length of 108.8 nm on average. The study of the FTIR spectra showed the elimination of the functional groups of extracted cellulose nanocrystals. The results from the X-ray diffraction analysis showed the crystallinity structure and index of the extracted cellulose nanocrystals. The characteristic crystalline cellulose phase of synthesized cellulose nanocrystals was identified using the XRD spectrum. Additionally, the extracted cellulose nanocrystal sample showed a crystallinity index (CrI) of 77.6%. The cellulose nanocrystals have a lot of potential for engineering and manufacturing applications.

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Author contributions

RS: writing – original draft, methodology, data curation and visualization. SW: writing – review editing and supervision. NF: writing - review editing and supervision.

Conflicts of interest

The authors declare no conflicts of interest.

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