

Isolation and Characteristics of Nanocelluloses from Microcrystalline Cellulose by Acid Hydrolysis

Mi-Jung Cho^{*}, Byung-Dae Park

Department of Wood Science and Technology, Kyungpook National University, Daegu,
702-701, Korea
jomil86@knu.ac.kr

Abstract

This study attempted to isolate nanocelluloses from commercial microcrystalline cellulose (MCC) by sulfuric acid hydrolysis, and to characterize their morphology, size, crystallinity, and chemical properties. The acid hydrolysis provided rod-like nanocelluloses from the MCC. Wet particle size analysis resulted in an average size of about 340 nm. But, the measurements of individual nanocelluloses under transmission electron microscopy (TEM) gave the dimension of about 6.96 ± 0.87 nm wide and 178 ± 55 nm long. The liquid crystal of the nanocellulose was confirmed by birefringence under polarized light. The crystallinity of the nanocellulose was about 85.2% relative to MCC 82.3%. FT-IR measurements of nanocellulose showed the typical cellulose bands.

1. Introduction

In recent years, the use of natural fibers as reinforcements in polymers and composites has attracted much attention due to the environmental concerns. Among the natural fibers, cellulose is the most abundant, renewable and biodegradable natural polymer in the world. Cellulose fibers are present in plant cell walls in combination with hemicelluloses, lignin, waxes, etc. commonly known as nano sized microfibrils. These nano sized fibrils are again combined of a crystalline and

amorphous part. The crystalline region consists of bundles of microfibrils where the cellulose chains are stabilized laterally by hydrogen bond between hydroxyl groups. So, they have high structural strength and stiffness. It was reported that the e-glass fiber's elastic modulus is 73 GPa (Nabi Saheb and Joe, 1999) but the native cellulose I crystal regions had elastic modulus of 167.5 GPa (Tashiro and Kobayashi, 1991) and elastic modulus for tunicin whiskers gave a value of 143 GPa (Šturcová *et al.* 2005). Because of these good mechanical properties, nanocellulose has generated a great deal of interest as a source of nano size reinforcements.

Various methods, like chemical and mechanical treatments have been used to isolate cellulose microfibrils from various sources. The chemical method, such as acid hydrolysis, removes the amorphous regions of cellulose fiber and produces nano-size fibrils. Wood pulp (Araki *et al.* 1998), sea animals (Favier *et al.* 1995), sisal (Rodriguez *et al.* 2006) and sugar beet pulp (Azizi Samir *et al.* 2004), Microcrystalline cellulose (MCC) (Bondeson *et al.*, 2006) were used as raw materials to isolate nanocelluloses by acid hydrolysis. Mechanical method includes a high-pressure homogenizer treatment (Herrick *et al.* 1983), a Freeze-cryocrushing method (Bhatnagar and Sain 2005) and a high intensity ultrasonication (Cheng *et al.* 2009). Fibrils made by these mechanical methods could be bundles of micro-fibrils, or cellulose nanofibers.

Among the nanocellulose raw material, the MCC is a commercially available material which is mainly used as a binder in tablets and capsules. Nanocellulose is commonly produced from hydrolysis of the MCC with strong acid. The nanocellulose from previous studies has a diverse ranges of their size, depending on how to make nanocellulose and what raw materials are.

In this paper, we have investigated properties of nanocellulose isolated from the MCC by sulfuric acid hydrolysis. The morphology of nanocellulose was investigated by wet particle size analysis and transmission electron microscopy (TEM). Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD) were performed to determined chemical analysis of isolated nanocellulose.

2. Materials and Methods

2.1 Materials

A commercially available microcrystalline cellulose (MCC) (Avicel[®] PH-101, Fluka, U.S.A) was used as raw material for the isolation of nanocelluloses. It was pure microcrystalline cellulose with particle sizes from 14 to 35 μm . Sulphuric acid (95%, DC chemical Co. Ltd.) was used for the acid hydrolysis of MCC.

2.2 Preparation of Nanocelluloses

A 10.2 g MCC was mixed with a 100 mL deionized water. The water/MCC suspension was then put in an ice bath under continuous mechanical stirring. 100 mL sulfuric acid was added by drops. After adding sulfuric acid, the suspension was hydrolysed at 44°C in 130.3 min. The suspensions were then washed with deionized water for 20 min at 5000 rpm, using repeated centrifugation (Hanil Centrifuge Co., Ltd, Korea). The supernatant was removed from the sediment and replaced by new deionized water and mixed. The centrifugation step was continued until the pH value of the supernatant became 1. The last wash was conducted using dialysis with deionized water to remove the last residue of the sulfuric acid until the wash water maintained at constant pH and became pH 4. The ultrasonication (Sonosmasher, Jeio Tech, Korea) was carried out for 20 min at power 30% in an ice bath to avoid overheating, which can cause desulfonation of the sulfate groups on the cellulose (Dong *et al.* 1998).

Yield was calculated as a percentage of the initial weight of the MCC after hydrolysis. After dialysis, the total of suspension was freeze dried and compared to the initial weight of the MCC.

2.3 Characterization

2.3.1 Morphology

Wet particle size analysis was conducted using particle analyzer (Beckman

Coulter N5/LS-13320, U.S.A) to approximate measure of the size of the nanocellulose. The solution of nanocellulose exposed for 200s using a 25mV Helium-Neon Laser Light source.

Field Emission Scanning Electron Microscopy (FE-SEM) (HITACHI S-4300 & EDX-350, Japan) was used to observe sample's morphology. The raw MCC powder was attached on carbon tape and observed at an accelerating voltage of 15 kV. Freeze dried sediment, Microfibrillated cellulose fiber (MCF) was diluted to a concentration of 1 _{w/w}%. Three drops of diluted solution were put on a center of cover glass. The sample was dried in the vacuum oven (Fisher Scientific 281A, U.S.A) at 50°C overnight. After gold coating the images were taken with and accelerating voltage of 5kV for the MCF sample.

Transmission electron microscopy (TEM) (H-7600, HITACHI, Japan) observations were performed using at an accelerating voltage of 100 kV. The sample was diluted to a concentration of 0.5_{w/w}%. To examine the nanocelluloses, Three droplets of suspension was put on a Cu-grid coated with a thin carbon film and allowed to dry at 70°C for 10 min. To enhance the contrast in TEM, the nanocellulose was stained by allowing the grids to float in a 3wt% solutions of uranyl acetate for 3 min and were dried at 70°C for 10 min. The particle dimensions were measured by counting individual nanocellulose from the TEM micrograph. The distributions of particle length and width were achieved by counting several particles in the same micro photograph.

2.3.2. Flow birefringence

Flow birefringence of the suspensions containing nanocellulose was investigated by using a lamp and two crossed polarization filters. Flow birefringence is a method, which can be used to investigate if nanocelluloses are liquid crystal in a suspension.

2.3.3. Crystallinity measurements

In order to investigate the crystallinity of nanocelluloses, X-ray diffraction (XRD) patterns of the MCC, nanocellulose and MCF were obtained using a X-ray diffractometer (Rigaku D/Max-2500, Japan). Powdered samples were prepared by crushing the freeze-dried MCF and nanocellulose. The milled sample powders were analyzed at ambient temperature using a CuK_α-1 X-ray source with a wavelength (λ) of 1.541 Å. The angle of incidence was varied from 5° to 30° by step of 0.02° and scanning rate of 6°/min. The Segal method was used to calculate the crystallinity of the samples (Thygesen *et al.* 2005). The sample crystallinity, X_{CR}, has frequently been determined by the Equation (1) using the height of the 200 peak (I₂₀₀, 2θ=22.7°) and the lowest height between the 200 and 110 peaks (I_{AM}, 2θ=18°). I₂₀₀ represents both crystalline and amorphous material while I_{AM} represents amorphous material only.

$$X_{CR} = \frac{I_{200} - I_{AM}}{I_{200}} \times 100 \quad \text{----- (1)}$$

The expression requires that the amorphous material diffracts with the same intensity at 18° and 22.7°, and that the crystalline cellulose does not contribute to the intensity at 18° (Segal *et al.* 1959).

2.3.4. Fourier transform infrared(FT-IR) spectroscopy

The FTIR spectra were recorded on an attenuated total reflectance fourier transform infrared (ATR-FTIR) instrument (Bruker Co. Ltd., Alpha-p, German) with DTGS detector in the range of 400-4000cm⁻¹. Spectral outputs were recorded in the transmittance mode as a function of wavenumber and 24 scans with resolution of 4 cm⁻¹ at room temperature.

3. Results and Discussion

3.1 Morphology

Fig. 1 is the particle size analysis result of nanocellulose obtained after the sulfuric acid hydrolysis. After measuring more than 1.27 million particles, an average size of the nanocelluloses was 340.6nm.

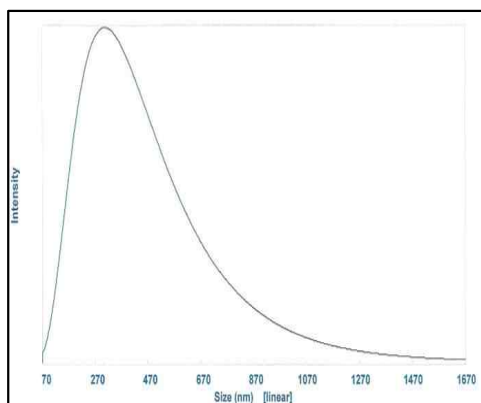


Fig. 1 The result of nanocelluloses particle size analysis

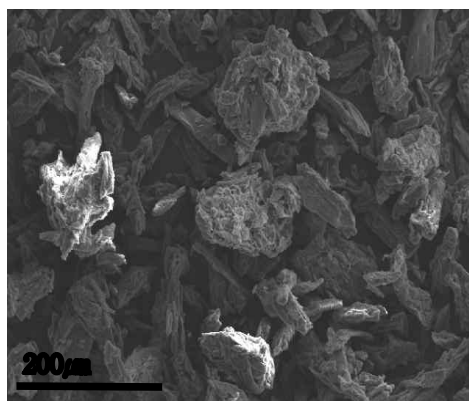


Fig. 2 FE-SEM image of Microcrystalline cellulose(MCC)

Fig. 2 shows this study's raw material, MCC with particle size 14~35 μm . The MCC is a commercially available material which is mainly used as a binder in tablets and capsules. It is prepared from native cellulose by hydrolysis, back-neutralization with alkali and spray-drying. Strong hydrogen bonding between the individual cellulose crystals produced promotes reaggregation during drying procedures (Levis and Deasy, 2001). Thus, the MCC produced consists of aggregated bundle of crystallites with different particle size. To utilize cellulose crystals as reinforcement, the hydrogen bonds between the crystals must be broken and the cellulose crystals must be well dispersed in the matrix (① Bondeson *et al*, 2006).

Fig. 3 represents a FE-SEM image of freeze dried microfibrillated cellulose fiber (MCF). As shown, fibers have smooth surface because of acid hydrolysis process.

However, those fibers still have large diameter of 3~9 μm and well below nanosized fiber. It means that hydrolysis conditions were not enough to make nanocellulose.

In fact, the yield of nanocellulose was determined as 9.7%, which was lower than other study (②Bondeson *et al*, 2006).

Fig. 4 shows TEM image of rod liked nanocellulose prepared by acid hydrolysis of MCC. The analysis of TEM revealed that isolated nano celluloses have width(d) of 6.96 ± 0.87 nm and length (L) of 178 ± 55 nm, giving a aspect ratio (L/d) of 25. These dimension are in agreement with the results found by Bondeson *et al* (2006) ①.

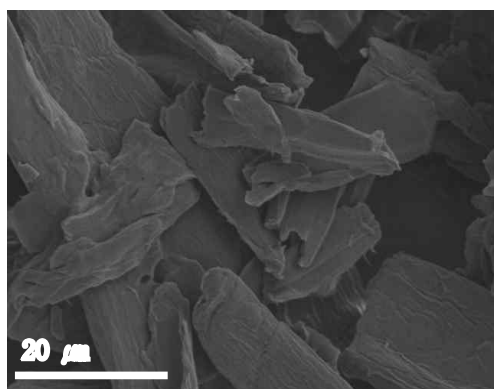


Fig. 3 FE-SEM image of microfibrillated cellulose fiber (MCF) after acid hydrolysis

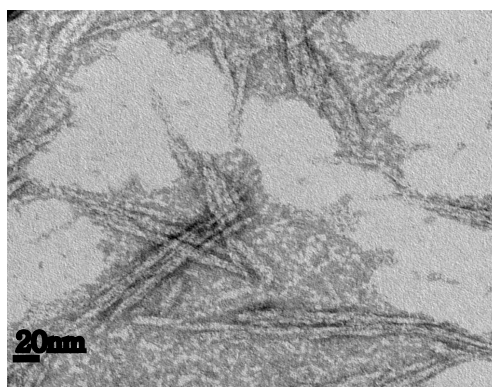


Fig. 4 TEM image of nanocellulose isolated MCC by hydrolysis

3.2 Flow-birefringence

Fig. 5 shows flow birefringence for the suspension made by acid hydrolysis of the MCC under cross polarized light. This indicates a nematic liquid crystalline alignment and reveals the existence of nanocelluloses (Ort *et al*. 1998).

3.3 Crystallinity measurements

Fig. 6 shows the X-ray spectrum of the MCC, MCF, and nanocellulose. As shown in Fig. 6, the intensity of nanocellulose is the highest, indicating that the

crystallinity of nanocellulose was increased after the hydrolysis. This result suggest that the amorphous region was disappeared and remained the crystalline region during the acid hydrolysis. The diffractogram of cellulose shows three peaks around $2\theta=15.5^\circ$ (peak 1), 16.5° (peak 2) and 22.6° (peak 3) which are typical of cellulose I. The crystallinity of nanocellulose was calculated to be 85.2% from the Segal Method, which was reasonable according to the published values of 86.7% for commercial MFC (Microfibrillated cellulose) (Lu *et al*, 2008).



Fig. 5 Flow birefringence of the nanocellulose dispersed in water under polarized light

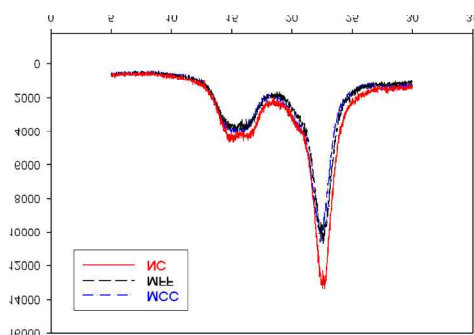


Fig. 6 X-ray diffraction patterns of MCC, MCF, and nanocellulose

3.4 Chemical properties

FT-IR spectroscopy is a non destructive method for studying the physicochemical properties of ligno cellulosic materials (Alemdar and Sain, 2008). The FT-IR spectra of nanocellulose isolated by acid hydrolysis is shown in Fig. 7. The absorbance at 897cm^{-1} is related to the C-H rocking vibration of cellulose (Alemdar and Sain, 2008). The FT-IR spectra show characteristic cellulose peaks around $1000\text{--}1200\text{ cm}^{-1}$ (Zhbakov *et al.* 2000, Langkilde *et al.* 1995). The peak at 1160cm^{-1} is antisymmetric bridge stretching of C-O-C groups. The 1314cm^{-1} could be ascribe to CH_2 - wagging vibration in cellulose and hemicellulose. The peak of 2891cm^{-1} reflects stretching vibration of C-H. The band near 3400cm^{-1} is representative of

O-H vibration (Cao and Tan, 2004)

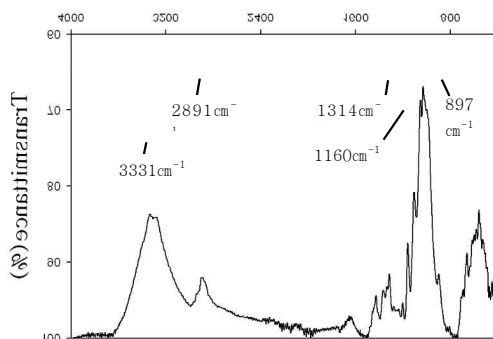


Fig 7. FT-IR spectrum of the nanocellulose

4. Conclusions

In this work, nanocellulose was isolated from the MCC by sulfuric acid and chemical properties. Experimental results showed that rod like nanocellulose has width of 6.96 ± 0.87 nm and length of 178 ± 55 nm by TEM. An average size is 340.6nm by wet particle size analysis. Also, nanocellulose in water showed flow birefringence. The crystallinity of the nanocellulose was increased by 85.2% relative to that of the MCC (82.3%). FT-IR measurements of nanocellulose showed the typical cellulose bands and revealed that in case of hydrolysis, the molecular structure of cellulose was not changed.

References

- 1)Nabi Saheb D., Joe J.P., natural fiber polymer composites:A review. *Advances in Polymer technology* 18: 351-363(1999).
- 2)Tashiro K., Kobayashi M., Theoretical evaluation of three dimensional elastic constants of native and regenerated cellulose : role of hydrogen bonds *Polymer* 32: 1516-1526

(1991).

- 3) Šturcová A., Davies G.R., Eichhorn S.J., Elastic modulus and stress transfer properties of tunicate cellulose whiskers, *Biomacromolecules* 6: 1055–1061 (2005).
- 4) Araki J., Wada M., Kuga S., Okano T., Flow properties of microcrystalline cellulose suspension prepared by acid treatment of native cellulose *Colloid and surfaces A* 142: 75–82 (1998).
- 5) Favier V., Canova G.R., Cavaille J.Y., Chanzy H., Dufresne A., Gauthier C., nanocomposite materials from latex and cellulose whiskers, *Polymer for Advanced technology* 6: 351–355 (1995).
- 6) Rodriguez N.L.G., Thielemans W., Dufresne A., Sisal cellulose whiskers reinforced polyvinyl acetate nanocomposites, *Cellulose* 13: 261–270 (2006).
- 7) Leitner J., Hinterstoisser B., Wastyn M., Keckes J., Gindl W., Sugar beet cellulose nanofibril-reinforced composites, *Cellulose* 14: 419–425 (2007).
- 8) ① Bondeson D., Kvien I., Oksman K., Strategies for preparation of cellulose whiskers from microcrystalline cellulose as reinforcement in nanocomposites, *American chemical society symposium series* 938 ;10–25 (2006).
② Bondeson D., Mathew A., Oksman K., Optimization of the isolation of nanocrystals from MCC by acid hydrolysis, *Cellulose* 13: 171–180 (2006).
- 9) Herrick F.W., Casebier R.L., Hamilton J.K., Sandberg K.R., Microfibrillated cellulose: Morphology and accessibility, *Journal of applied polymer science: Applied polymer symposium* 37: 797–813 (1983).
- 10) Bhatnagar A. and Sain M., processing of cellulose nanofiber-reinforced composites, *Journal of reinforced plastics and composites* 24: 1259–1268 (2005).
- 11) Cheng Q., Wang S., Rials T.G., Poly(vinyl alcohol)nanocomposites reinforced with cellulose fibrils isolated by high intensity, *Composites:Part A* 40: 218–224 (2009).
- 12) Dong X. M., Revol J.F., Gray D.G., Effect of microcrystallite preparation conditions on the formation of colloid crystals of cellulose, *Cellulose* 5: 19–32 (1998).
- 13) Thygesen A., Oddershede J., Lilholt, On the determination of crystallinity and cellulose content in plant fibers, *Cellulose* 12: 563–567 (2005).

- 14) Segal L., Creely J.J., Martin A.E. Conrad C.M., An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Research Journal* 29: 786-794 (1959).
- 15) Levis S.R. and Deasy P.B., Production and evaluation of size reduced grades of microcrystalline cellulose, *International Journal of Pharmaceutics* 213: 13-24 (2001).
- 16) Ort W.J., Godbout L., Marchessault R.H. Revol J.F., Enhanced ordering of liquid crystalline suspensions of cellulose microfibrils: A small angle neutron Macromolecules 31: 5717-5725 (1998).
- 17) Lu J., Wang T., T. Drzal L., Preparation and properties of microfibrillated cellulose polyvinyl alcohol composite materials, *Composites: Part A* 39: 738-746 (2008).
- 18) Alemdar A. and Sain M., Isolation and characterization of nanofibers from agricultural residues - Wheat straw and soy hulls, *Bioresource Technology* 99: 1664-1671 (2008).
- 19) Zhabankov R.G., Firson S.P., Korolik E.V., Petov P.T., Laplocski M.P., Tsarenkov V.M., Marchewka M.K., Ratajczak H., Vibrational spectra and the structure of medical biopolymers, *Journal of Molecular Structure* 555: 85-96(2000)
- 20) Langkilde F.W., Svantesson A., Identification of celluloses with Fourier-Transform (FT) mid-infrared, FT-Raman and near-infrared spectrometry, *Journal of Pharmaceutical and Biomedical Analysis* 13: 409-414 (1995).
- 21) Cao Y. and Tan H., Structural characterization of cellulose with enzymatic treatment, *Journal of Molecular Structure* 705: 189-193 (2004).