Particle Size Distribution and Yield Analysis of Different Charged Cellulose Nanofibrils Obtained by TEMPO-mediated Oxidation

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Abstract: Cellulose Nanofibrils (CNF) was successfully obtained by TEMPO (2,2,6,6-tetramethylpiperidine -1-oxyl radical)-mediated oxidation with the addition of different oxidant namely NaClO (Sodium Hypochlorite) i.e. 3, 4, 7, 10 and 15 mL followed by ultra-sonication treatment. Size distribution of nanocellulose was observed using particle size analyzer, while surface charged was measured using zeta potentiometer. At different level of oxidant, CNF obtained different charged with different size distribution. As the amount of oxidant increased, the size distribution of CNF increased which correlated to the higher CNF yield, however it decreased at maximum oxidant addition. Although, in general the yield for nanocellulose was very low. With zeta potential value about -48 mV, it showed very stable suspension in water for more than 8 months observation. An optimum oxidant level promoted thinner and longer CNF which further beneficial for better entanglement in the hydrogel formation application.

1. Introduction

Cellulose as the most abundant biopolymer on earth with productivity reaches \(10^9\) – \(1.5 \times 10^{12}\) tones/year shows myriad potential applications in energy sector, advanced material, medical and personal care field, environment issues and food industry sector [1-3]. Cellulose is responsible for reinforcing wood cell wall and protecting organelle of the wood cell [4]. Due to its complex structure in wood cell wall interlinked with lignocellulose components, it is challenging to obtain pristine high purity cellulose. Recently, studies focus on extraction of cellulose in nano dimension structure which further categorize the cellulose into several nomenclature such as cellulose nanofibrils (CNF), cellulose nanocrystals (CNC) and microfibrillated cellulose (MFC) [5]. Those variations depend on the methods used to isolate the cellulose.
Acid hydrolysis results in dominant crystalline phase of cellulose, needle-like morphology, therefore it is named as nanocrystal cellulose. It includes the use of strong acid such as H$_2$SO$_4$, HCl and H$_3$PO$_4$ for the hydrolysis process [6,7]. On the other hand, cellulose nanofibril (CNF) still consists of amorphous and crystalline part, spaghetti-like morphology. The fibrillation process is resulted from mechanically treatment such as high-pressure homogenizer or microfluidizer. Chemically treatment by esterification, TEMPO-mediated oxidation produced high surface charged cellulose which lead internal electrostatic repulsion results in lowering the total energy for fibrillation process. While, microfibrillated cellulose (MFC), commonly isolated nanocellulose without harsh chemical involved. It obtains no charge on the cellulose surface therefore the total energy to produce MFC is relatively high. The morphology of MFC is quite the same with CNF, however due to the average size of MFC is higher than that of CNF. MFC is more opaque than CNF which is quite transluscent.

In our research group, we are developing a concept to produce nanocellulose with eco-technology approach. Figure 1 illustrates several keywords to obtain commercially and eco-friendly method to scale up nanocellulose production. The concepts follow and summarize the parameter that have been established in the literatures such as high yield [8-10], one step processing which includes surface modification and extraction in one step method [11], the incorporation of catalyst to reduce reaction time and effectively process [12], using low cost sources as the raw materials such as sugarcane bagasse [13], onion skins [14], grape skins [15] and industrial crops waste [16]. Instead of using harsh reagent, the choice to use some of organic acid i.e. formic acid, formic acid with FeCl$_3$, maleic acid, citric acid and HCl, oxalate and HCl is undergoing main selection.

**Figure 1.** Diagram mapping of keywords for eco-technology concept for nanocellulose production at our Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI)

One of the existing method that we use to isolate CNF is using TEMPO-mediated oxidation. Previously, we have reported that CNF could be prepared by varying the amount of oxidant level [17]. The carboxyl contents was reported increased from 1.1 to 1.9 mmol/g by increasing the oxidant level from 4 to 15 mL NaClO, respectively, affected the rheology behavior such as shear viscosity, shear stress, storage modulus (G') and loss modulus (G''). It was hypothesized that at low level oxidator, CNF
was obtained at relatively thick size, longer fibrils compared to that CNF obtained from higher level oxidator. Entanglement phenomena for CNF at low degree of carboxylation suggested that hydrogen bonding interaction among cellulose nanofibrils occurred. Meanwhile, at high degree of carboxylation electrostatic repulsion dominated the interaction within nanocellulose resulted in shear rate dependence. For that case, we did not reported the size distribution of CNF, the oxidation yield and zeta potential of our samples. We also kept our nanocellulose suspension in the refrigerator to observe the stabilization of fiber dispersion regarding the storage life.

The aim of this study is to characterize the size distribution by particle size analyzer of CNF which obtained from TEMPO-mediated oxidation under different oxidant addition. In this study, the addition of NaClO was varied from very low (3 mL) to very high (15 mL). That parameter was determined to calculate the yield of nanocellulose production.

2. Materials and Methods

2.1. Materials
Dried hardwood bleached kraft pulp (HW-BKP) was obtained from Moorim Paper Co., Ltd. Korea, and stored in a humidity chamber at 25 °C before use. TEMPO, sodium bromide (NaBr), and sodium hypochlorite (NaClO) with chlorine content 12.5 % solutions were purchased and used as received from Sigma-Aldrich, Korea. Reverse-osmosis purified water with a conductivity of 6 µS/cm was obtained from a water purification system (Ultra-pure, ROTech, Korea).

2.2. Cellulose nanofibril preparation
Nanocellulose was obtained by TEMPO/NaBr/NaClO oxidation system at pH 10.5 and room temperature [18]. In brief, cellulose fibers (2 g) were dispersed in reverse osmosis-purified water (150 mL) containing diluted TEMPO (0.025 g) and NaBr (0.25 g). The oxidation reaction started to occur after NaClO solution was added drop-wise with different amount addition namely 3, 4, 7, 10 and 15 mL. Small NaClO addition resulted in very short reaction time. For example, at 3 mL addition, the reaction need only 15 minutes to complete while at 7 mL addition about 75 minutes were required. For 15 mL addition, the reaction was completed after 150 minutes. The reaction was maintained at constant pH 10.5 by adding NaOH 0.5 M solution. The complete oxidation reaction was indicated by no more changes in pH during process. Therefore, the reaction was quenched by adding ethanol and amount of distilled water. Further, oxidized pulp slurry was ultrasonicated using sonicator at 20 kHz for 40 minutes at 30% power. After that the supernatant was separated from its residue by centrifugation (Labogene 1580 centrifuge, Gyrozen Co., Ltd., Daejeon, Korea). Finally, the CNF suspension was stored at refrigerator 4-8 °C up to more than 8 months. The amount of negatively surface charged of cellulose was calculated by conductometry titration using a portable conductometer (SevenGo, Mettler-Toledo, China) [26].

2.3. Cellulose nanofibril yield
Yield of nanocellulose was calculated using gravimetry method. The basis weight, 2 g, for initial dry weight of HWBKP was used as the basis for yield calculation. An estimation of small volume and dried nanocellulose contents was multiply with the total volume nanocellulose suspension obtained from 2 gram of cellulose by oxidation reaction. In order to minimize the degradation of cellulose due to the carboxyl and aldehyde content on cellulose surfaces which resulted in lowering the weight of cellulose, the nanocellulose drying was conducted in freeze dryer rather than in oven conventional drying [19]. The yields of nanocellulose were calculated by ratio of sample weight before and after extraction as perform in equation 1.
\[ \text{Yield} (\%) = \frac{\text{sample weight of CNF}}{\text{initial weight (2 g)}} \times 100 \] (1)

2.4. Particle size distribution
Very low concentration of nanocellulose in water (0.05 mg/mL) was prepared and observed using particle size distribution apparatus (Beckman Coulter, USA). Temperature: 20 °C, Angle: 90°, water solvent, and refractive index: 1.333.

2.5. Zeta potential analysis
Surface charge of obtained nanocellulose was analyzed using Malvern Zetasizer Nano ZEN5600, temperature 25 °C, equilibrium time: 120 seconds. The concentration of solution was determined in 0.05 mg/mL.

2.6. Morphology
Morphology characterization was observed using Scanning Electron Microscope (SEM) Hitachi SU-3500, Japan with very low voltage 3 kV. Prior to analyze, nanocellulose was small dropped in a thin glass preparator and let it air dried. After drying, samples were placed in gold-palladium sputter coater (Denton vacuum Inc, DESK IV) before observation.

3. Result and Discussion
3.1. Cellulose nanofibril yield
TEMPO-mediated oxidation facilitated the formation of negative surface charge on the surface of cellulose. However, the amount of carboxyl is limited due to the high selectivity of oxidation that only occurred in primary hydroxyl groups (C6). The strong intra- and intermolecular hydrogen bonding of cellulose caused that the oxidation only took place on the surface of cellulose fibril therefore maintaining the crystal structure of native cellulose. In this case, TEMPO-mediated oxidation could provide no change in crystallinity index which favorable to be used as reinforcing agent in composite materials, improving barrier properties and rheological modifiers [18]. The yield of nanocellulose obtained in this study are listed in Table 1. In general, our results show very low nanocellulose yield especially at low level of oxidant. The optimum yield is obtained at 7 mL addition of NaClO which comparable the result from others reference [20]. As the oxidant level increase the yield of nanocellulose increased, but lowered at maximum oxidant addition. At low oxidant addition, there is no sufficient conversion of hydroxyl to carboxyl which facilitated internal osmotic pressure leading to internal fibrillation of CNF. Therefore, such mechanical treatment applied, had no effect on the CNF production. At maximum oxidant addition level, the process required longer time resulted in the increased of solubility of cellulose which further reduce the yield of nanocellulose.

The yield in this study were affected by some parameters such as the dried raw material, chemical composition and non-effective ultrasonication. Irreversible agglomeration of cellulosic components during drying is known as hornification, which leads to generate hydrogen bonds and prevent the penetration of chemical into cellulose. However, recent study from Adu et al. (2018) [21] concluded that paper mill sludge can be used in a dried state for CNF production to reduce transportation and storage challenges posed on industrial scale. In this case, they used ultrafine mechanical grinding, a super mass collider MKCA6-3. This indicated that perfect combination between chemical and mechanical treatment will lead to higher nanocellulose yield.
Chemical composition of raw material also influences the yield of fibrillation [22]. Although we did not measure the chemical compound of raw material that we used in this study; however, the presence of hemicellulose in this study was qualitatively and quantitatively showed by FTIR spectra peak at 1750 cm\(^{-1}\) and 0.45 mmol/g, respectively [26]. Those results showed very low hemicellulose contents. Some studies show that the amount of hemicellulose facilitated the nanofibrillation of wood pulp which improved the yield of nanocellulose production [23]. The proposed mechanisms for high nanocellulose yield are as follow: Alila et al. (2013) proposed that hemicelluloses are linked to the cellulose microfibrils and are assumed to span the gap between them. Their presence is therefore likely to reduce the interaction through hydrogen bonding among the cellulose microfibrils thus facilitating the penetration of chemical reagent, separation and the individualization of the cellulose during mechanical shearing action.

Bian et al. (2017) [24] studied the effect of lignin on the production of lignin containing cellulose nanocrystals (LCNC) and nanofibrils (LCNF). They also reported very low yield of CNC production from 2.1 to 5.94%. Due to lignin is more hydrophobic and it protects cellulose from esterification which resulted in LCNC and LCNF that was less carboxylated compared to those lignin-free CNC and CNF and therefore had lower charges and lower yield.

| Amount of NaClO addition | Yield (%) | Size distribution (nm) | Zeta potential (mV) |
|--------------------------|-----------|------------------------|---------------------|
| 3 mL                     | 1.26      | 90 – 2290              | n/a                 |
| 4 mL                     | 5.72      | 100 – 2400             | n/a                 |
| 7 mL                     | 30.46     | 170 – 4270             | - 48                |
| 10 mL                    | 28.30     | 320 – 7720             | n/a                 |
| 15 mL                    | 19.44     | 260 – 6260             | n/a                 |

3.2. Particle size distribution
The correlation between size distribution of CNF and the intensity at different level of oxidant is presented in Figure 3 and summarize in Table 1. As the amount of oxidant increase, the size distribution of CNF increases which correlate to the higher CNF yield, however it decreases at maximum oxidant level addition due to the breakdown of degree of polymerization cellulose (DP) [18]. At higher oxidant level, the conversion of hydroxyl groups into carboxyl groups promote the chain scission of cellulose, lowering the DP, improve the solubility of cellulose. CNF obtained from 15 mL showed CNC-like appearence with chiral nematic behavior. As reported [17] earlier, it also showed drastically changed in shear rate dependency. Therefore this findings support the rheology characteristic of previous report. Interestingly, from particle size analyzer, the lowest average size was obtained from low charged CNF with the lowest CNF yield. Those indicated that an optimum oxidant level facilitated thinner and longer CNF as it is presented in Table 1 and previously reported in Masruchin et al. (2017) [17]. There are some benefits of using CNF instead of CNC on the application of cellulose. With higher aspect ratio (length to diameter) or thinner and longer of CNF, it promotes the entanglement possibility within fibril therefore improve the mechanical strength of composites, improving shear strength and shear viscosity of suspension. The longer fibril also facilitate the possibility of doping many site for nanoparticles, drug loading rather shorter structure of CNC.

From Figure 2, we obtain the average size with the optimum intensity (Figure3). It shows a clearly trend that as the oxidant addition increase the average size of CNF increase reached to optimum addition of oxidant (10 mL). Those trend can be explained by the optimum conversion of hydroxyl to carboxyl groups as follow: in one anhydroglucose unit (AGU) or the monomer of cellulose, there is only one reactive site for carboxylation (OH-C\(_6\)) which means there is only 1 mol -COOH in 1 mol AGU. If 1
gram AGU equals to 6.13 mmol, therefore the maximum conversion to carboxyl theoretically reached 2.04 mmol/g. However according to the report from Okita et al. the optimum carboxyl content in TEMPO/NaBr/NaClO system only reached 1.7 mmol/g [25]. In this study, we could obtain 1.58 - 1.9 mmol/g for 10 - 15 mL addition of NaClO [17, 26]. It can be concluded that optimum oxidant level promote better size distribution.

![Figure 2](image)

**Figure 2.** Size distribution of cellulose nanofibril from different oxidant addition

### 3.3. Zeta potential

In general, due to the presence of acetyl groups from hemicellulose, cellulose fibers are anionic charge in water. After pulping process the functional groups may decreased or introduced with new functional group on the pulp fiber [27]. We have noticed to our HWBKPA raw material that have 0.45 mmol/g
carboxyl content [26]. The carboxyl contents substantially influencing the surface charge of cellulose fibers and the fiber dispersibility in water. The zeta potential measurement for 7 mL NaClO addition showed the value was -48 mV, indicated a very stable suspension of CNF in water solvent. The stable zeta potential for suspension in water was reached at ±30 mV value [18]. Unfortunetaly, we did not measured the other sample for zeta potential. However, after storing the suspension in refrigerator for about 8 months. We observed that the suspension and consistency of CNF was maintained. That result indicated that the surface charged of nanocellulose were sufficient enough for better dispersion in water solvent.

Figure 3. Average size at optimum intensity measurement using particle size analyzer for CNF at different oxidant addition

Figure 4. SEM image for CNF at 7 mL addition of NaClO at (a) 1000x and (b) 10.000x magnification

3.4. Morphology
Figure 5 shows the SEM image of cellulose nanofibrils at 7 mL addition of NaClO. Larger microfibril is still present figure 5a (pointed with white arrow), while nanofibrils at varied size in diameter and length of fibrils are obtained in figure 5b. However, since SEM samples were prepared by air dried prior
to analyze, some agglomeration occurred. This image suggested that homogenous nanofibrillation is still challenging. From Table 1 and Figure 3, it also proof that the variation in size distribution require a proper mechanical treatment to obtain homogenous size of nanocellulose. Abe et al. (2007) propose several passing cycles using super mass grinder to unmodified wood fibers to obtain uniform 15 nm size nanocellulose [28]. Wakabayashi et al. (2020) controlling the degree of fibrillation of TEMPO-oxidized cellulose by high-pressure homogenization with water jet-type apparatus by collision with a ceramic ball with one to five pass cycle [29]. The nanofibrillation yield increased from 39 to 100%. This recent report suggested that mechanical treatment after surface oxidation are important to obtain high yield and uniform size and dispersibility of cellulose nanofibrils.

4. Conclusion
The optimum charged density and yield of CNF was obtained at 10 mL NaClO level. It obtained a stable suspension in water solvent with -48 mV zeta potential for more than 8 months in refrigerator. It also facilitated thinner and longer CNF which further beneficial for composite reinforcing agents due to the high of aspect to ratio of CNF. Ultrasonication treatment produce low yield nanocellulose therefore it suggested to combine oxidized cellulose with super mass grinder mechanical treatment.

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