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Manipulation of Surface Carboxyl Content on TEMPO-Oxidized Cellulose Fibrils[1]

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ABSTRACT

Simple methods of conductometric titration and infrared spectroscopy were used to quantify the surface carboxyl content of cellulose fibrils isolated by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation. The effects of different cellulose sources, post or assisted-sonication oxidation treatment, and the amount of sodium hypochlorite addition on the carboxyl content of cellulose were reported. This study showed that post sonication treatment had no influence on the improvement of surface carboxyl charge of cellulose macrofibrils (CMFs). However, the carboxyl content increased for the isolated cellulose nanofibrils (CNFs). Thus the carboxyl content of CNFs is different from those of their corresponding bulk oxidized cellulose and CMFs. Filter paper as a CNF source imparted a higher surface charge than did hardwood bleached kraft pulp (HWBKP) and microcrystalline cellulose (MCC). It was considered that the crystallinity and microstructure of the initial cellulose affected oxidation efficiency. In addition, the carboxyl content of cellulose was successfully controlled by applying sonication treatment during the oxidation reaction and adjusting the amount of sodium hypochlorite.

Keywords : Carboxyl content, TEMPO, conductometric titration, infrared spectroscopy, cellulose nanofibrils

1. INTRODUCTION

Currently, the increasing trend in nanocellulose research and applications is indisputable (Cho and Park, 2010; Dufresne, 2013). The main reason is to seek sustainable materials as substitutes for petroleum-based materials. Thus, the materials of choice are cellulose nanofibers, which are the most abundant polysaccharides on Earth, with a total biomass production of 1×10^{12} tons per year (Klemm *et al*., 2011). By mimicking the structure of wood materials, strong and flexible biocomposites from nanolevel extracted cellulose could be produced in the presence of various matrices (Berglund and Peijs, 2010; Cho *et al*., 2011, 2012; Park *et al*., 2014). For these purposes, higher crystallinity, high modulus, and low thermal expansion of cellulose are exploited.

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Furthermore, researchers have investigated the application of nanocellulose for smart materials, biomedical applications, and other functional materials that require one-step surface modification of cellulose and easy extraction of cellulose nanofibers simultaneously.

One of the obstacles to commercializing nanocellulose is high energy requirement, which implies a high cost of production. In order to overcome this challenge, enzymatic treatment (Paako *et al*., 2007) and an enzymatic process combined with low-cost recycled paper sources have been studied (Filson *et al*., 2009). These studies found that enzyme treatment facilitated disintegration by partially digesting both the crystalline and amorphous regions and improved the nanocellulose yield. In addition, an integrated concept of bioethanol residue utilization has been proposed and studied (Duran *et al*., 2011; Oksman *et al*., 2011; Herrera *et al*., 2012) with respect to cost efficiency. However, these treatments do not specifically modify the surface of cellulose directly. In addition, de Nooy *et al*. (1995) have reported the oxidation of the primary alcohol groups on water-soluble glucans using 2,2,6,6-tetramethylpiperidine-1-oxoammonium ion (TEMPO) as a catalyst in the presence of hypochlorite/bromide. Furthermore, TEMPO has been used to oxidize cellulose materials and it has been reported that TEMPO oxidation selectively converts the C6 primary hydroxyl groups of cellulose into C6 carboxylate groups (Chang and Robyt, 1996; Isogai and Kato, 1998; Saito *et al*., 2004, 2005). This method promotes surface charge modifications of cellulose that cause the formation of completely individualized CNFs dispersed in water. At the same time, this decreases the energy required for its disintegration (Isogai *et al*., 2011; Syverud *et al*., 2011). Interestingly, CNFs can be obtained almost in the absence of mechanical energy (Tejado *et al*., 2012) if the oxidation is sufficiently within the threshold of the carboxylate group of approximately 3 mmol/g; however, some of the cellulose is dissolved.

Instead of decreasing the energy requirement, carboxylate groups on the surface of cellulose provide reactive sites for further functionalization such as the conjugation of CNCs with 2-aminoanthracene, which resulted in a novel fluorescent nanomaterial that might be further applied in biosensing (Leung *et al*., 2011). Other studies of carboxylated-cellulose surfaces have led to the synthesis of nanoparticles on the surfaces of CNFs (Uddin *et al*., 2014), the increased water uptake of cellulose (Barzyk, 1996), and formation of nanocellulose based hydrogel for drug release study (Masruchin *et al*., 2015a). However, although carboxylate groups do not influence cellulose crystallinity, which is beneficial for the strength of reinforcing agents, they decrease the thermal stability of CNFs, thus limiting further application (Fukuzumi *et al*., 2010). TEMPO is also an expensive and toxic chemical compound; however, it can be recycled and reused following a desalination process reported by Isogai *et al*. (2011).

Because of the many advantages of carboxyl

groups on CNF surfaces, this study was aimed to measure carboxyl group content on oxidized cellulose and especially CNFs for its further functionalization using simple methods, conductometric titration and infrared spectroscopy. In addition to quantify carboxyl group content, conductometric titration has been applied to measure the sulfate groups upon acid hydrolysis of cellulose nanocrystals (Abitbol *et al*., 2013) and aldehyde group content (Saito *et al*., 2004). The latter were converted to oximes via Schiff base formation with hydroxylamine (NH_2OH), and then elemental CHN composition was determined by atomic absorption spectroscopy. However, CHN analysis produced a value that was approximately eight times higher than that normally obtained using conductometric titration (Mihranyan, 2013; Saito *et al*., 2004; 2007). Moreover, according to the literature, the carboxyl content of the bulk oxidized cellulose has similar value than that of oxidized nanocellulose (Saito *et al*., 2004; 2005; 2007; Besbes *et al*., 2011; Rattaz *et al*., 2011; Benhamou *et al*., 2014); however, the results might be different if dried cellulose is used as the raw material and especially when oxidation was conducted at low degrees of oxidation. There have been no reports on the separate measurements of the carboxyl contents of bulk oxidized cellulose and isolated nanocellulose.

In this study, cellulose was oxidized from different sources, underwent time-dependent ultrasound- post or assisted oxidation at different sonication times and with different amounts of sodium hypochlorite (NaClO). Those treatments were conducted in order to obtain cellulose with different levels of surface charges. Further, CNFs was isolated from the cellulose macrofibrils suspension (CMFs) and carboxyl content of oxidized cellulose were quantified, respectively. The carboxyl charge content of cellulose was quantitatively measured by conductometric titration, while infrared spectroscopy analysis was used to qualitatively indicate the presence of carboxylate groups on the surface of cellulose. In addition, recent study reported that fourier transform infrared spectroscopy (FTIR) was used as quantitative analysis to determine thirteen wood species composition (Emandi *et al*., 2011). Therefore, attenuated total reflectance-infrared (ATR-IR) spectroscopy results were calculated and, interestingly, the result agreed with that of conductometric titration.

2. MATERIALS and METHODS

2.1. Materials

Three kinds of cellulose were used, namely dried hardwood bleached kraft pulp (HWBKP), microcrystalline cellulose (MCC), and filter paper (Whatman™, catalog number 1001-110). HWBKP was obtained from Moorim Paper Co. Ltd. MCC was purchased from Fluka under the trade name Avicel PH-101. TEMPO and others chemical such as NaClO solution and sodium bromide (NaBr) were purchased and used as received from Sigma-Aldrich. All water used in this work was purified water from a reverse os-

mosis system (Upure, Rotech, Daegu, Korea) with conductivity of 6 μS/cm.

2.2. TEMPO-mediated oxidation

Oxidation of cellulose was conducted in a TEMPO/NaBr/NaClO oxidation system at pH 10.5 and room temperature (23℃) as reported by Saito *et al*. (2004) with slight modification. That is, TEMPO (0.025 g) and NaBr (0.25 g) were dissolved in water (150 mℓ), and subsequently, cellulose fibers (2 g) were dispersed. A predetermined volume (2, 5, 8, 10, and 15 mℓ) of NaClO solution (12.5% purity) was added dropwise, in order to maintain a pH of 10, to the suspension to initiate the reaction. After the addition of NaClO, owing to the formation of aldehyde and carboxylic groups, the pH of the suspension decreased drastically. Therefore, the pH was maintained at 10.5 by adding a 0.5 M NaOH solution (Montanari *et al*., 2005). The pH was monitored using a pH meter (SevenEasy, Mettler-Toledo GmbH, Switzerland). When addition of NaOH was no longer necessary, the reaction was complete. The reaction took approximately 75 min. Then, hydrochloric acid (0.5 M) was added to neutralize the suspension. Afterwards, the oxidized cellulose fibers were filtered and washed several times with distilled water. Finally, a 2% suspension of insoluble oxidized fibers was obtained. When studying the effect of different cellulose sources: HWBKP, MCC, and filter paper were used in this work.

2.3. Post-sonication oxidation treatment

A suspension of oxidized cellulose fibers was subjected to sonication treatment for 40 min. Sonication was conducted at intervals of 10 min to overcome heat generated during the process. In addition, large amounts of fibrillated cellulose were produced and agglomerated around the tip of the probe. Therefore, it was necessary to disperse the suspension by stirring in order to increase sonication efficiency. The suspension obtained via the sonication process was designated as CMFs. Furthermore, CNFs were separated from the CMF suspension by centrifugation (Labogene 1280 at 12,300 *g* for 40 min); the CNFs were in the supernatant and un-fibrillated celluloses were in the sediment.

2.4. Sonication-assisted oxidation treatment

In order to increase the formation of carboxylate groups on the surface of the crystalline cellulose source (MCC), sonication treatment was applied for 10, 20, or 30 min during the TEMPO oxidation of cellulose. A Sonomasher (ULH 7005, ULSSO Hi-Tech, Korea) with a probe diameter of 1 cm, power of 30%, and selected frequency of 20,320 Hz was used in this work. To avoid heating throughout the treatment, sonication was stopped every 5 min while the pH of the solution was maintained at 10.5.

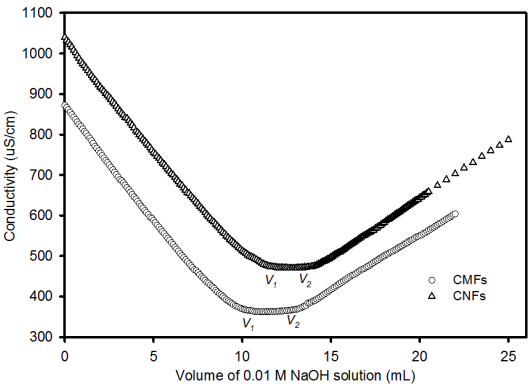

Fig. 1. Typical curves of conductometric titrations of CMFs and CNFs.

2.5. Carboxyl content measurement

Carboxylate groups content on the surface of oxidized cellulose were quantitatively measured as follows; an aqueous cellulose suspension (0.05% solid content) was obtained by adding 80 mℓ of reverse osmosis water and 5 mℓ of a 0.01 M NaCl solution to a CMF or CNF suspension. And then, a 0.1 M HCl solution was added drop wise in order to decrease the pH into around 2.5. Titration process was started by adding a 0.01 M NaOH solution (0.1 mℓ/min) until the pH reached 11. During the titration, the changes of conductivity were recorded using a portable conductometer (SevenGo, Mettler-Toledo, China). Fig. 1 shows graphical correlation between conductivity and the addition of NaOH for CMF and CNF suspensions. The conductivity decreased gradually owing to the neutralization of acid by the addition of NaOH (V_1, mℓ). During the neutralization of carboxylic groups, the conductivity remained unchanged. At the end of the neutralization step (V_2, mℓ), the accumulation of NaOH would increase the conductivity. V_2 and V_1 was determined using least square equation (R^2 = 0.999). All measurements were performed in two replications. The carboxyl content was calculated using equation 1:

$$Carboxyl\ content\ \left(\frac{mmol}{g}\right) = \frac{(V_2 - V_1) \times M\ NaOH}{weight\ of\ cellulose} \quad \cdots\cdots \quad (1)$$

2.6. ATR-IR measurement

The functional groups on the oxidized cellulose surface were detected by attenuated total reflectance-infrared (ATR-IR) spectroscopy (ALPHA-P, Bruker Optics, Germany). Sample was prepared from freeze dried oxidized cellulose (freeze drier, FDA8508, Ilshin BioBase Co., Ltd., Korea). The transmittance was measured from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution with 24 sample scans time.

3. RESULTS and DISCUSSION

In this study, several terms are used to identify the cellulose form, such as raw material sources; namely HWBKP, MCC and filter paper; HWBKP-TEMPO was named for HWBKP after TEMPO-oxidation process in the absence of sonication treatment. A series post sonication (40 min) was applied to HWBKP-TEMPO to obtain CMFs, a mixture of fibrillated and un-fibrillated cellulose. Further, CMFs was separated using centrifuge to obtain the fibrillated cellulose (CNFs) in the super-

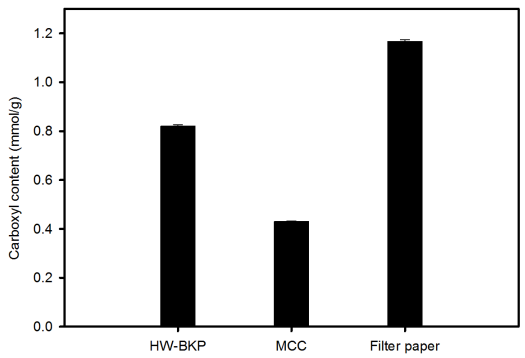

Fig. 2. Carboxyl contents of CNFs from different sources with the addition of 8 mℓ of NaClO and 40 minutes post-sonication oxidation treatment.

natant while un-fibrillated cellulose was in the sediment fraction.

The oxidation reaction was successfully performed on the surface of cellulose. The carboxyl content on HWBKP-TEMPO and CNFs could be manipulated through several methods. The following discussion summarizes the simple effect that could control the amount of carboxyl groups on cellulose, which was quantitatively determined by conductometric titration.

3.1. Effect of different raw material sources

In this study, in order to obtain different carboxyl content of cellulose, CNFs was extracted from different raw materials. Fig. 2 shows the different carboxyl contents of CNFs from different cellulose sources, which were treated with post-oxidation sonication for 40 min and isolated by centrifugation. Thus, different sources showed different carboxyl contents. CNFs from filter paper generated higher carboxyl contents than did those from HWBKP and MCC. Since the reaction conditions for all cellulose sources had been adjusted through the same parameter, the differences in measured carboxyl contents might be caused by the differences in the morphologies and crystallinities of the three types of cellulose. MCC, for example, consisting of highly crystalline cellulose regions, is produced by acid hydrolysis of the amorphous part of cellulose until a level-off degree of polymerization (LODP) is achieved. MCC particle agglomerates are composed of microcrystals, of which the particle size distribution and moisture content vary depending on the drying process. Therefore, it is reasonable that MCC generated the lowest carboxyl content on its surfaces. The crystalline part of cellulose is resistant to enzymatic, chemical, or biological treatment. Owing to its low carboxyl content, the CNF yields obtained from MCC are also lower than those from the other cellulose sources.

On the other hand, the structures of HWBKP and filter paper are composed of amorphous fraction from hemicelluloses and a small part remaining from lignin structures, which are easier to oxidize (Qian *et al*., 2012) and lead to subsequent oxidations on remaining cellulose surfaces. In addition, although filter paper obtained higher carboxyl content, in terms of commercialization, HWBKP would be the best candidate for economic reason to be used for CNFs mass production. Therefore, for further study, we used HWBKP as raw material for production of CNFs.

Above all, in this study, CNFs were produced from dried cellulose sources, which probably caused the low carboxyl contents (< 1.2 mmol/g) on the cellulose surfaces compared to never-dried cellulose from the literature (Saito *et al*., 2007). However, again, the raw sources prepared from dried cellulose are nearly effective and economical enough for the commercialization of CNFs. In a recent study, Besbes *et al*. (2011) successfully produced a high yield of CNFs from dried cellulose instead of never-dried cellulose, even though this resulted in slightly lower carboxyl content (1 mmol/g). In addition, they reported that there was no intrinsic difference between never-dried and once-dried cellulose, as long as the carboxylate content of TEMPO reached approximately 1.5 mmol/g. Therefore, it is necessary to adjust the carboxyl content of dried cellulose sources through further treatments such as ultrasonic assisted oxidation and the different initiator levels, which will be discussed in the next section.

3.2. Effect of ultrasonic assistance during TEMPO-mediated oxidation

Since the carboxyl content of MCC was low, owing to its high crystalline fraction and aggregated structure, which rendered it inaccessible for oxidation, assisted sonication at different durations were applied during the oxidation reaction. Some studies have shown that assisted sonication can be used to obtain CNFs through TEMPO oxidation with an ultrasonic bath system (Qin *et al*., 2011; Rattaz *et al*., 2011; Mishra *et al*., 2011). Furthermore, Mishra *et al*. (2012) compared between the probe and bath ultrasonic systems for oxidation; however, these systems were applied after TEMPO-mediated oxidation. They reported that ultrasonic probes can deliver much higher ultrasonication intensities (greater cavitation effect) than the ultrasonic baths. In a bath ultrasonic system, the sample container is immersed in an ultrasonic bath, while an ultrasonic probe is immersed directly in the sample container. The ultrasonic bath system required a long reaction time (reaction was completed after 14 h), which meant that maintaining the reaction conditions would be difficult (Qin *et al*., 2011). This could explain the greater efficiency of nanocellulose production with the ultrasonic probe. Therefore, in this study, probe-type sonication was used as a mechanical assisted oxidation reaction process.

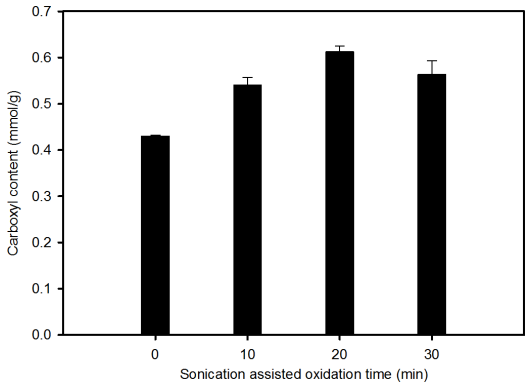

Fig. 3. Carboxyl contents of CNFs obtained from MCC with the addition of 8 mℓ of NaClO via different sonication assisted oxidation time.

Fig. 3 depicts the effect of assisted sonication time on carboxyl contents of CNFs from MCC.

Sonication was applied 10 min after the last addition of NaClO solution when the consumption of NaOH gradually increased, which indicated that a high degree of oxidation was occurring. As the sonication time increased, the carboxyl content increased and reached the optimum at 20 min. Increasing the sonication time to 30 min decreased the carboxyl content. This is reasonable since the sonication treatment produces hot spots from acoustic cavitation, which is the formation, growth, and collapse of bubbles in a liquid (Li *et al*., 2012). Based on the results of studies by Mishra *et al*. (2011; 2012), the optimum temperature for TEMPO/NaBr/NaClO system oxidations is 25℃. The same result was reported by Dang *et al*. (2007); the carboxyl content of cellulose decreased above 30℃ and an optimum temperature was obtained at 23℃. Performing the oxidation reaction with a lengthy assisted sonication treatment decreased the reactivity of the reactants.

In addition, the effect of assisted sonication on carboxyl content could be explained as follows: the formation of acoustic cavitation and free radicals ($H_2O \rightarrow H^*, OH^*$) provides high amounts of energy to induce cellulose folding. Erosion on its surface and external fibrillation occurred (Li *et al*., 2011; Mishra *et al*., 2011), which will improve the accessibility of reactants to the C6 hydroxyls and reactivity of the cellulose. Our previous study showed the formation of a balloon-like structure when TEMPO-mediated oxidation pulp was subjected to ultrasound treatment (Masruchin *et al*., 2015b). Further ultrasound treatment will simultaneously detach and completely fibrillate the fibrils as a result of the electrostatic repulsion and/or osmotic effects between the anionic charges.

Because a probe system with high frequency was used, the nanocellulose yield will probably decrease due to simultaneous dissolution of the oxidized nanofibrils Mishra *et al*. (2011; 2012). However, in this study, we used higher-crystallinity raw cellulose, which has high resistance to mechanical and chemical destruction. Here, a high-crystallinity cellulose nanowhisker with highly functionalized carboxylate groups on the surface could be produced via TEMPO-mediated oxidation of MCC and an assisted sonication process.

3.3. Effect of post-sonication time

Post-oxidation sonication treatment was applied to defibrillate the oxidized pulp and obtained CNFs, especially when a low to moderate volume of NaClO was added. These treatments might be conditioned in order to prevent the abrupt decrease in DPv (degree of polymerization) of cellulose when a high volume of NaClO is used (Puangsin *et al*., 2013). Fig. 4 shows the carboxyl contents of raw HWBKP, HWBKP-TEMPO, CMFs, CNFs and filter paper-TEMPO. The measured carboxyl content of HWBKP was 0.15 mmol/g, which indicated the presence of ester linkages of the carboxylic group in the ferulic and *p*-coumaric acids of lignin and/or hemicelluloses (Plackett *et al*., 2014). It is known that raw bleached hardwood kraft pulp still contains at least 10-15% hemicelluloses

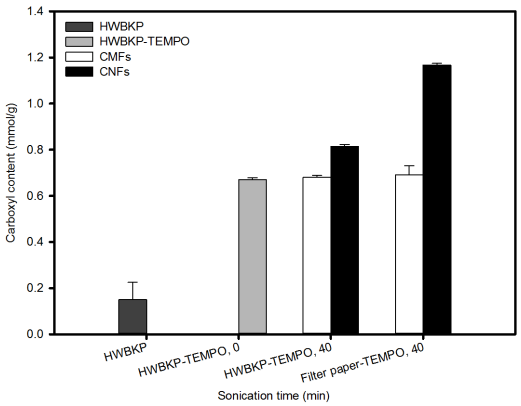

Fig. 4. Carboxyl contents of CMFs and CNFs from HWBKP and filter-paper cellulose with the addition of 8 mℓ of NaClO after 40 minutes post-sonication oxidation treatment.

(Mishra *et al*., 2011). After the oxidation reaction, the carboxyl content increased to 0.68 mmol/g which indicated that carboxylate groups were formed in the surface of cellulose pulp. Thus HWBKP-TEMPO further was subjected to ultrasonication treatment.

In our previous report, HWBKP oxidized with 8 mℓ of NaClO maintained its pulp shape, which indicated that fibrillation during oxidation did not occur on that level of addition. Much debris from the small part of the pulp (fines) appeared in the same shape as unoxidized (Masruchin *et al*., 2015b). However, after post-oxidation sonication treatment for 40 min, the pulp fibers converted into fibrillated and un-fibrillated cellulose (CMFs). The reported carboxyl contents of CMFs were unchanged compared to those without sonication treatment (Fig. 4). This indicated that after the oxidation reaction was complete, physical treatment of oxidized cellulose surfaces did not change the functional groups. No chemical changes on the cellulose chain due to ultrasound treatment were also reported by Mishra *et al*. (2012). They also proved that sonication pretreatment of cellulose prior to TEMPO-mediated oxidation does not significantly improve the carboxyl content after oxidation. Therefore, the possibility of utilizing assisted sonication during the oxidation reaction which has been discussed previously will be an effective way to improve the carboxyl content of oxidized cellulose.

Interestingly, as shown in Fig. 4, CNFs isolated by centrifugation showed quantitatively higher carboxyl contents. This result indicated that nanolevel fibrillated cellulose expose the carboxylate groups on the surface of cellulose, which is beneficial over CMFs since this provides more functional groups over a large surface area for further functionalization. The oxidation reaction occurred specifically at the C6 primary hydroxyl group at the surface of cellulose and not the internal cellulose I crystallites; therefore, sonication treatments peeled cellulose fibrils from pulp fibers to produce single dispersed fibrils with high densities of carboxylate groups on the surfaces of CNFs (Montanari *et al*., 2005; Saito *et al*., 2005; Okita *et al*., 2010). Our previous work reported that the diameter of CNFs obtained by TEMPO observed using transmission electron microscopy (TEM) was 7.09 ± 0.99 nm (Park *et al*., 2014), which was quite similar to the elementary fibril size of cellulose aggregate of 3-5 nm (Saito *et al*., 2006; Isogai *et al*., 2011). Centrifugation left behind a sediment fraction

comprising cellulose that was inaccessible for oxidation reaction.

The same result trend was obtained from a different cellulose source, filter paper (Fig. 4.). However, distinct carboxyl content for its CNFs was higher compare to CNFs from HWBKP. It could be explained that the native cellulose samples isolated from various origins, which may have different characteristics in crystal structure ($I\alpha$ and $I\beta$ ratio), crystal size, and microfibril morphology that led to different efficiency for converting the hydroxyl groups to carboxylate groups (Okita *et al*., 2010).

3.4. Effect of different amounts of sodium hypochlorite (NaClO)

Fig. 5 shows the effect of different amounts of primary oxidant, NaClO, on the carboxyl contents of CMFs and CNFs from HWBKP. As the amount of NaClO increased, the carboxyl contents on cellulose increased, except when 2 mℓ of NaClO was added. Even after increasing the sonication time to 60 min, we could not obtain CNFs. The yield of CNFs was too low. At low levels of NaClO addition, the conversion of primary hydroxyl groups to carboxylates via aldehydes did not occur. The reaction reached a constant pH of 10.5 too early and the amount of NaOH consumption as an indicator of the oxidation reaction process was very low. According to Isogai *et al*. (2011), the minimum amount of NaClO is 0.9 mmol/g; however, in our case, the amount was relatively high since dried cellulose was used as the raw material.

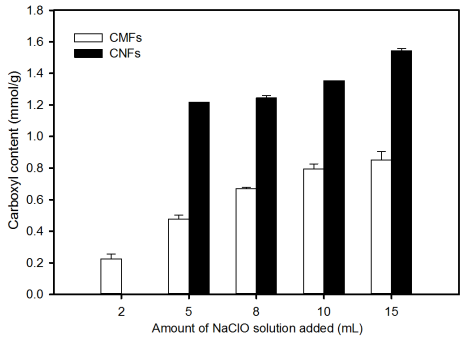

Fig. 5. arboxyl content of CMFs and CNFs at different amounts of NaClO addition.

The maximum carboxyl contents for CMFs and CNFs reached 0.82 mmol/g and 1.58 mmol/g, respectively. Based on the study of Okita *et al*. (2010), the maximum carboxyl content that can be introduced on a cellulose surface in the TEMPO/NaBr/NaClO system is 1.7 mmol/g. Our result was quite close to the maximum threshold.

As described earlier, all CNFs obtained with different amounts of NaClO generated higher carboxyl contents than the CMFs. Qualitative analysis using ATR-IR spectroscopy was applied to clarify this result from quantitative measurements. The results can be seen in Fig. 6a. The carboxylic group peak appeared at 1731 cm^{-1} (Matuana *et al*., 2001); however, the carboxyl vibration in the sodium form (-COONa) appeared at 1605 cm^{-1} (Fujisawa *et al*., 2011). In Fig. 6a, it appears that the IR absorbance intensity increased with the amount of NaClO added, which indicates that the oxidation reaction occurred rather homogeneously throughout the fiber (Saito *et al*., 2006). To quantify the carboxyl formation, we selected the

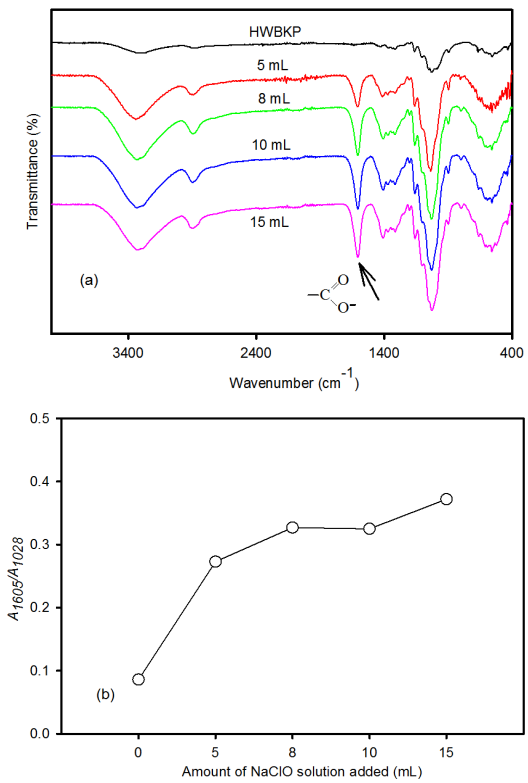

Fig. 6. (a) ATR-IR measurement of CNFs from HWBKP with different amounts of NaClO and (b) the ratio of IR absorbance at 1605 cm^{-1} to that at 1028 cm^{-1} against the amount of NaClO.

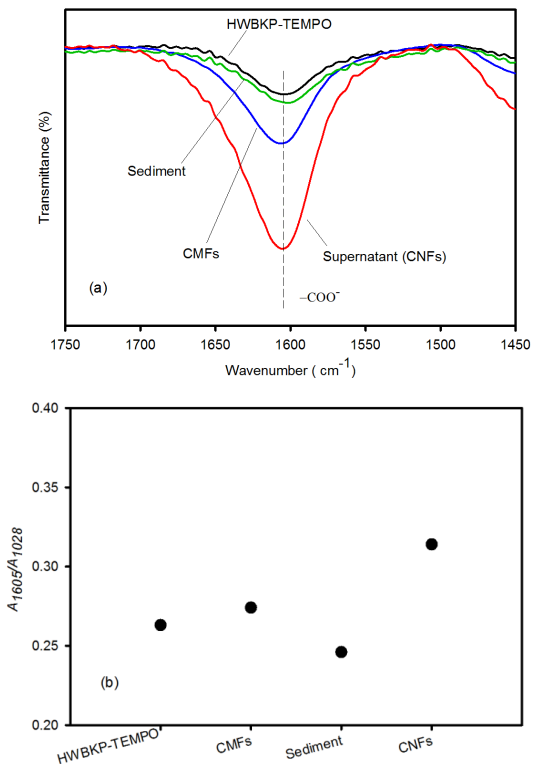

Fig. 7. (a) ATR-IR measurements of different forms of HWBKP after TEMPO-mediated oxidation with 8 mℓ of NaClO and (b) the ratio of IR absorbance at 1605 cm^{-1} to that at 1028 cm^{-1} of different forms of HWBKP after TEMPO-mediated oxidation.

IR peak at 1028 cm^{-1} as a control, because the absorption at 1028 cm^{-1} is assigned to the -CO stretching, which remains unchanged during the reaction. Fig. 6b shows the ratios of the intensities of the normalized absorbances at 1605 cm^{-1} to those at 1028 cm^{-1}, A_{1605}/A_{1028}, evaluated from Fig. 6a (Tomihata and Ikada, 1997; Lasseuguette, 2008; Yang *et al*., 2013). It can be seen that increasing the amount of NaClO increased the carboxyl group ratio. This result was in agreement with the result from the quantitative measurement using conductometric titration (Fig. 5.).

In order to support our findings in earlier discussions (Figs. 4 and 5) that CMFs and CNFs have different carboxyl contents on their surfaces, we also applied ATR-IR spectroscopy to the different forms of HWBKP oxidized by TEMPO, namely HWBKP-TEMPO, CMFs, sediment and CNFs. Fig. 7a shows the IR absorbance of cellulose at around 1605 cm^{-1}. It is clear that the intensity of CNFs was higher than those of sediment and CMFs. This implies that carboxylate group densities are higher on

the surfaces of CNFs than on the surfaces of the others. The same quantitative analysis using the ratio of normalized IR at 1605 cm^{-1} against 1028 cm^{-1} is presented in Fig. 7b. The A_{1605}/A_{1028} ratio for CNFs is the highest, while the sediment form had the lowest ratio.

Overall, from this study, we obtained carboxyl contents of TEMPO-oxidized cellulose through conductometric titration and infrared spectroscopy with several treatments to manipulate its density on the surface of cellulose. Different carboxyl contents on the surfaces of cellulose will influence the viscosity, rheological behavior (Besbes *et al*., 2011), dispersion of the CNFs in solvent (Saito *et al*., 2005; Rodionova *et al*., 2012), dimensions (Benhamou *et al*., 2014), thermal stability (Fukuzumi, *et al*., 2010; Sharma and Varma, 2014), and energy required to isolate CNFs (Tejado *et al*., 2012). On the other hand, it has been reported that the crystallinity does not change owing to increased carboxyl content since it is only a surface modification.

4. CONCLUSION

Conductometric titration and ATR-IR spectroscopy have been used as simple method to quantify the surface carboxyl content of TEMPO-oxidized cellulose. Post-sonication time after the oxidation did not improve carboxyl content. On the other hand, carboxyl content of cellulose could be improved by optimization the addition of NaClO and choosing the raw material for cellulose oxidation. Assisted sonication is the best way to improve carboxyl content of highly crystalline raw material, MCC. From this study it is found that the carboxyl content of CNFs are different from those of their corresponding bulk oxidized cellulose and cellulose macrofibrils (CMFs). The findings of the present work revealed the possibility of managing oxidation efficiency in order to introduce carboxyl groups for further functionalization of cellulose fibrils.

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