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Distribution of carboxylate groups introduced into cotton linters by the TEMPO-mediated oxidation

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Abstract

The 2,2,6,6-tetramethylpiperidine-1-oxy radial (TEMPO)-mediated oxidation was applied to aqueous slurries of cotton linters. The water-insoluble fibrous fractions thus obtained in the yields of more than 78% were characterized by solid-state ^{13}C-NMR, X-ray diffraction and scanning electron microscopic analyses for evaluation of distribution of carboxylate groups formed in the TEMPO-oxidized celluloses. The patterns of solid-state ^{13}C-NMR spectra revealed that the oxidation occurred at the C6 primary hydroxyl groups of cellulose. X-ray diffraction and scanning electron microscopic analyses showed that such C6 oxidation took place at the surfaces of cellulose I crystallites without any oxidation at the C6 of inside cellulose I crystallites. Thus, carboxylate and aldehyde groups introduced into the TEMPO-oxidized celluloses are densely present on the surfaces of cellulose I crystallites. In addition, the obtained results revealed that the shoulder signal due to non-crystalline C6 carbons at about 63 ppm in solid-state ^{13}C-NMR spectra of native celluloses is ascribed to those of surfaces of cellulose I crystallites or those of cellulose microfibrils.

Keywords: TEMPO; Cellulose; Oxidation; NMR; Distribution of carboxyl group

1. Introduction

Chemical modification is an important approach for value-addition of natural polysaccharides. Catalytic oxidation using water-soluble and stable nitroxyl radicals such as 2,2,6,6-tetramethylpyperidine-1-oxy radical (TEMPO) under aqueous conditions has become one of the interesting routes to introduce aldehyde and carboxyl functionalities into polysaccharides (Bragd, van Bekkum, & Besemer, 2004; Chang & Robyt, 1996; de Nooy, Besemer, & van Bekkum, 1995). The most noticeable points of this TEMPO-mediated oxidation of polysaccharides are the followings; highly regioselective oxidation of primary hydroxyl groups in polysaccharides to carboxylate groups can be achieved, and this selective oxidation proceeds under aqueous mild conditions around room temperature at pH 10–11.

In the case of the TEMPO-mediated oxidation of celluloses, which are originally insoluble in water, completely different products are obtained, depending on the celluloses used as the starting materials. When regenerated and mercerized celluloses are used, water-soluble β-1,4-linked polyglucuronic acid sodium salt (cellouronic acid) having the homogeneous chemical structure can be obtained quantitatively as the oxidized products (Isogai & Kato, 1998; Tahiri & Vignon, 2000). In contrast, when native celluloses having the cellulose I crystal structure are adopted, the cellulose slurries at the initial stage maintain their slurry states even by the TEMPO-mediated oxidation with excess reagents for extended reaction. In the latter case, therefore, the TEMPO-oxidized celluloses are obtained as water-insoluble fibers containing small amounts of carboxylate and aldehyde groups in the yields of more than about 80% (Isogai & Kato, 1998; Kitaoka, Isogai, & Onabe, 1999; Saito & Isogai, 2004; Saito, Yanagisawa, & Isogai, in press). This oxidation resistance for native celluloses may be ascribed to their high crystallinities and low accessibilities to the reagents. On the other hand, the TEMPO-mediated oxidation may be one of the interesting surface modification

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methods of native celluloses, where carboxylate and aldehyde functional groups can be effectively introduced into fibrous celluloses under aqueous mild conditions.

In the previous papers, the TEMPO-mediated oxidation was applied to cotton linters under various conditions, and the oxidized products were separated into several fractions by filtration and centrifugation, depending on their sizes and apparent water-solubility, and characterized from several aspects. The major fractions obtained (>ca. 80 mass % of the original cotton linters) were the filter paper-trapped fibers. Carboxylate and aldehyde groups formed in the TEMPO-oxidized cotton linters increased up to about 0.7 and 0.3 mmol/g, respectively, by the oxidation. Crystallinity indices and crystal sizes of cellulose I in the fibrous fractions were nearly unchanged during the oxidation. On the other hand, the apparently water-soluble fractions (the filter paper-passed fractions) in the TEMPO-oxidized cotton linters consisted of colloidal particles having the cellulose I crystal structure and of truly water-soluble cellouronic acid and/or over-oxidized compounds having glucuronic acid and hexeneuronic acid units.

In this paper, the water-insoluble fibrous fractions in the TEMPO-oxidized cotton linters were further analyzed by solid-state ^{13}C-NMR, X-ray diffraction and electron microscopic analyses in order to evaluate the distribution of carboxylate groups formed in the TEMPO-oxidized cotton linters at the fiber cross-section and cellulose microfibril levels. In relation to the obtained results, assignment of the solid-state ^{13}C-NMR signals and the mapping of carboxylate groups present along each microfibril in the TEMPO-oxidized cotton linters were investigated in detail. After submission of this paper, solid-state ^{13}C-NMR and transmission electron microscopic analyses of carboxylated cellulose nanocrystals prepared from cotton linters and microfibrillated cellulose by the TEMPO-mediated oxidation were reported (Montanari, Roumani, Heux, & Vignon, 2005).

2. Materials and methods

2.1. Materials

Cotton linter (Ash-less filter pulp, Advantec Toyo Co., Ltd, Japan) was used as a native cellulose sample. TEMPO, sodium bromide and nine mass % sodium hypochlorite solution and other chemicals were of laboratory grades (Wako Pure Chemicals, Co., Japan), and used without further purification.

2.2. TEMPO-mediated oxidation and fractionation of the oxidized products

Cellulose (5 g) was suspended in water (375 mL) containing TEMPO (0.0125 g) and sodium bromide (0.125 g). The TEMPO-mediated oxidation of the cellulose slurry was started by adding NaClO (4.84 mmol/g cellulose in most cases), and continued at room temperature and pH 10.5 up to 24 h (Saito & Isogai, 2004; Saito et al., in press). After stirring the cellulose slurry for a designed time, the oxidation was quenched by adding aqueous ethanol. The fibrous TEMPO-oxidized product was washed thoroughly with ethanol and then water by filtration. Because cloudy filtrate was obtained by washing the fibrous product on a filter paper with water, the filter paper-trapped fraction and the filter paper-passed fine particles were separated, and the filter paper-passed fine particles were obtained by centrifugation of the filtrate (Saito et al., in press). Each fraction was dried by lyophilization followed by vacuum drying at 30 °C for 48 h.

2.3. Ion exchange of TEMPO-oxidized cellulose

The fibrous fraction (0.3 g) of the TEMPO-oxidized cellulose having sodium carboxylate groups was suspended in water (40 mL) containing calcium chloride (1.5 g). The moles of calcium ion present in the slurry were more than 60 times as much as those of sodium carboxylate groups present in the 0.3 g TEMPO-oxidized cellulose. The slurry was stirred at room temperature for 6 h for ion exchange, and then washed thoroughly with water by filtration. These TEMPO-oxidized celluloses having calcium carboxylate groups were dried by lyophilization followed by vacuum drying at 30 °C for 48 h (Saito & Isogai, in press).

2.4. Analyses

Carboxylate and aldehyde contents in the TEMPO-oxidized celluloses (fibrous fractions) were determined by the electric conductivity titration method (Saito & Isogai, 2004; Saito et al., in press). The carboxyl groups formed by the post $NaClO_2$-oxidation of the TEMPO-oxidized celluloses were regarded as aldehyde groups present in the original TEMPO-oxidized celluloses (Saito et al., in press). The dried samples were converted to pellets using a KBr disk apparatus for IR spectroscopy, and subjected to X-ray diffraction measurement by the reflection method using a Rigaku RINT 2000 with monochromatic Cu Kα radiation at 40 kV and 40 mA. Crystallinity index of cellulose I and the crystal size of the [100] direction for the TEMPO-oxidized celluloses were measured from the X-ray diffraction patterns (Saito & Isogai, 2004; Segal, Creely, Martin, & Conrad, 1959). The crystallinity indices obtained by the X-ray diffraction method were corrected to those obtained by the following solid-state ^{13}C-NMR method. Solid-state ^{13}C-NMR spectra of the TEMPO-oxidized celluloses were obtained on JEOL JNM-EX 400 (100.4 MHz) with cross-polarization and magic angle sample spinning under the following conditions: spinning rate 5 kHz, pulse delay 5 s and contact time 5 ms. Relative signal areas of crystalline C4, non-crystalline C4 and C6 carbons were calculated from the obtained solid-state ^{13}C-NMR spectra on the basis of peak de-convolution method (Kato, Matsuo, & Isogai, 2003). Distribution of calcium ions present in a cross-section of the TEMPO-oxidized cellulose fibers was evaluated by the line analysis mode using an energy-dispersive X-ray (EDX) analyzer (Horiba EMAX-5770X) attached to a field emission-type scanning electron microscope (SEM: Hitachi S-4000) under the reported conditions (Kato, Isogai, & Onabe, 2002).

3. Results and discussion

3.1. Solid-state ^{13}C-NMR analysis of TEMPO-oxidized celluloses

When native celluloses like cotton linters are treated by the TEMPO/NaBr/NaClO system under aqueous conditions, the original fibrous forms of native celluloses are mostly maintained even after the oxidation for 2 h. Table 1 summarizes carboxylate and aldehyde contents, yields and crystal structures of the TEMPO-oxidized celluloses used in this study. As the oxidation time increased, both carboxylate and aldehyde groups in the fibrous fraction increased, while yields slightly and gradually decreased. The crystal structure of cellulose I was maintained even after the oxidation for 24 h. When all carboxylate and aldehyde groups present in the fibrous fractions were regarded to form at the C6 position of cellulose, about 7, 13 and 15% of the C6 primary hydroxyls of cellulose were oxidized by the TEMPO-mediated oxidation at 0.5, 2 and 24 h, respectively.

Table 1
Carboxylate and aldehyde contents of the TEMPO-oxidized celluloses used in this study[a]

Oxidation time	Carboxylate (mmol/g)	Aldehyde (mmol/g)	Yield (%)	Crystal structure
0 h (original cellulose)	0.019	0.003	–	Cellulose I
0.5 h (fibrous fraction)	0.226	0.194	95.6	Cellulose I
2 h (fibrous fraction)	0.604	0.221	90.6	Cellulose I
24 h (fibrous fraction)	0.743	0.284	78.0	Cellulose I
2 h (filter paper-passed fraction)[b,c]	1.590	Not determined	7.0	Low crystalline, Cellulose I
Cellouronic acid Na salt[d]	5.051[e]	0.00[e]	–	Amorphous

[a] Saito and Isogai (2004).
[b] Prepared with 2.42 mmol NaClO per gram of cellulose.
[c] Saito et al. (in press).
[d] Prepared from regenerated cellulose (Isogai & Kato, 1998).
[e] Calculated on the basis of the chemical formula.

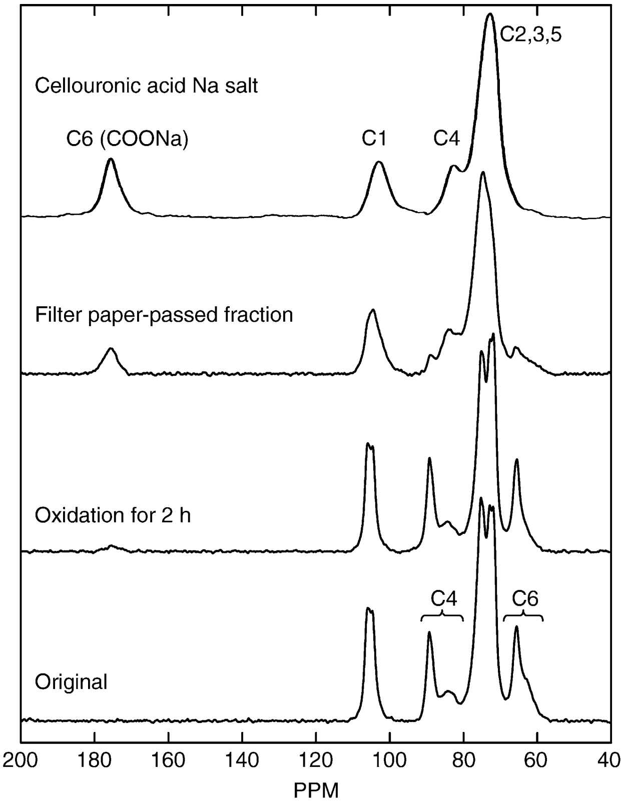

Fig. 1. Solid-state CP-MASS ^{13}C-NMR spectra of the original cellulose, TEMPO-oxidized cellulose, the filter paper-passed fraction of the TEMPO-oxidized cellulose and cellouronic acid sodium salt.

Fig. 1 shows solid-state ^{13}C-NMR spectra of some of the TEMPO-oxidized cellulose samples. The signal due to sodium carboxylate carbons in the products at about 175 ppm increased with increasing the oxidation time. The filter-paper passed fine particle fraction had broad C1, non-crystalline C4 and the C2,3,5 signals, while small signals due to crystalline C4 and C6 of cellulose I remained as well. Signals due to neither aldehyde nor ketone groups were detected in the oxidized products. Probably the amounts of C2- or C3-ketones formed and present in the oxidized products are lower than the detectable level for solid-state ^{13}C-NMR (Röhrling et al., 2002). Aldehyde groups present in the oxidized products (Table 1) are likely to form inter- and/or intra-molecular hemiacetals with hydroxyl groups like dialdehyde cellulose (Kim, Kuga, Wada, Okano, & Kondo, 2000), thus resulting in no signals due to aldehyde groups in the NMR spectra. Cellouronic acid sodium salt had broad C1, C4 and C2,3,5 signals, because this has amorphous X-ray diffraction pattern (Isogai & Kato, 1998).

Extended spectra of the solid-state ^{13}C-NMR from 55 to 95 ppm for the fibrous fractions of the TEMPO-oxidized celluloses prepared at 0–24 h are illustrated in Fig. 2. The crystalline and non-crystalline C4 signals appeared at 87–92 and 81–87 ppm, respectively, although these signals were overlapped to some extent owing to signal broadening under the NMR operation conditions adopted in this study.

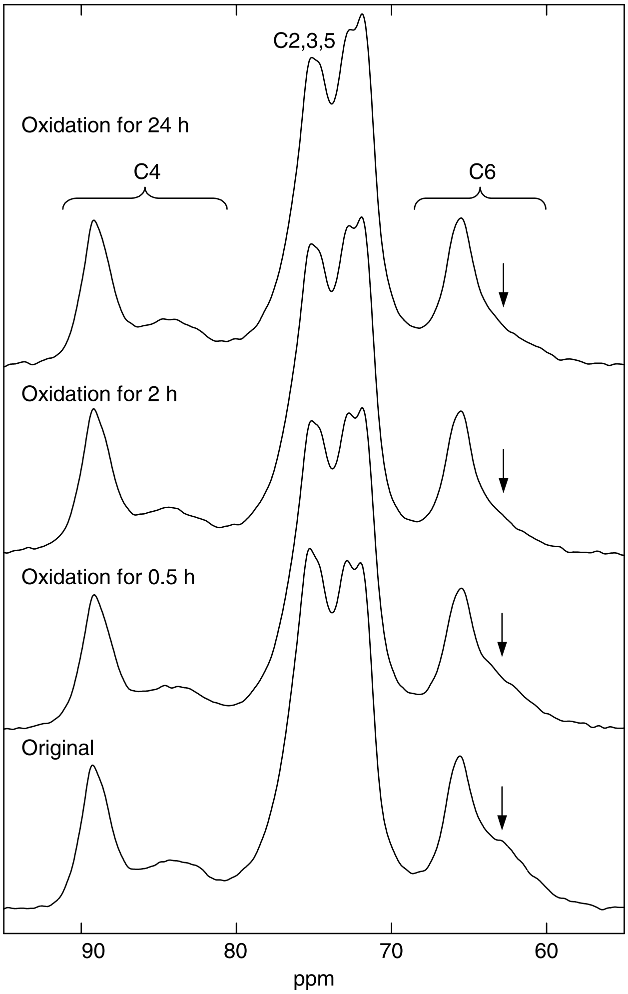

Fig. 2. Extended spectra of solid-state CP-MASS ^{13}C-NMR for the original and TEMPO-oxidized celluloses prepared under various conditions.

The overlapped signals due to C2, C3 and C5 were located at 69–81 ppm. The C6 resonance appeared at 60–69 ppm, and the signal tops for crystalline and non-crystalline C6 carbons were located at 66 and 63 ppm, respectively (Isogai, Usuda, Kato, Uryu, & Atalla, 1989). The shape and ratio of crystalline and non-crystalline C4 signals of the original cellulose were roughly unchanged even after the TEMPO-mediated oxidation for 24 h. The apparently doublet C1 signal of the original cellulose was also nearly unchanged (Fig. 1). These results show that the TEMPO-mediated oxidation of cellulose has nearly no influences on the chemical shift and the pattern of C1 or C4 in solid-state ^{13}C-NMR spectra. On the other hand, the shoulder signal due to non-crystalline C6 carbons at 63 ppm decreased with increasing the TEMPO-oxidation time, while the crystalline C6 signal was unchanged. The shapes and intensities of C2, C3 and C5 signals somewhat changed by the oxidation. These results are consistent with those for carboxylated cellulose nanocrystals reported by Montanari et al. (2005).

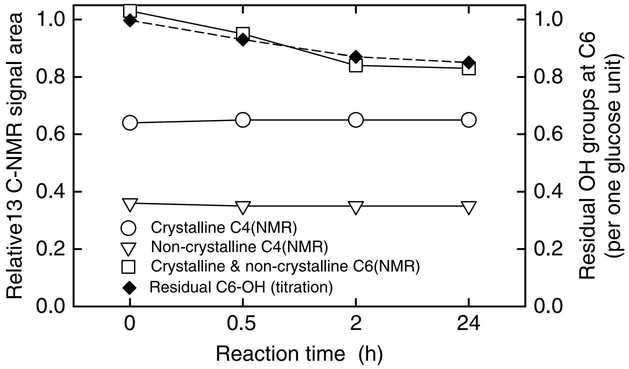

Fig. 3. Relationships between the TEMPO-oxidation time and either relative C4 and C6 signal areas in the solid-state ^{13}C-NMR spectra or residual C6-OH group per one glucose unit calculated from carboxylate and aldehyde contents in the TEMPO-oxidized celluloses.

Fig. 3 depicts changes in relative ^{13}C-NMR signal areas due to crystalline and non-crystalline C4 and C6 of cellulose by the TEMPO-mediated oxidation, which were calculated from the spectra in Fig. 3 and the sum of crystalline and non-crystalline C4 signal areas was adjusted to 1.0. While the signal areas due to both crystalline and non-crystalline C4 carbons were nearly unchanged even after the TEMPO-mediated oxidation of cellulose for 24 h, the C6 signal decreased with increasing the oxidation time. The residual C6 primary hydroxyl groups of the TEMPO-oxidized celluloses, which were calculated from the carboxylate and aldehyde contents in the products by the conductivity titration method (Table 1), are also plotted in Fig. 3. The decreasing pattern of this residual C6 primary hydroxyl groups obtained by the titration method was well consistent with that of the relative C6 signal area obtained from the solid-state ^{13}C-NMR spectra, indicating that the carboxylate and aldehyde groups formed in the TEMPO-oxidized celluloses are mostly present at the C6 position.

Fig. 4 shows the relationships between the TEMPO-oxidation time and either crystallinity or crystal size of cellulose I in the TEMPO-oxidized celluloses. Both these two values were nearly unchanged by the TEMPO-mediated oxidation, showing that carboxylate and aldehyde groups are formed at the C6 position in the non-crystalline region

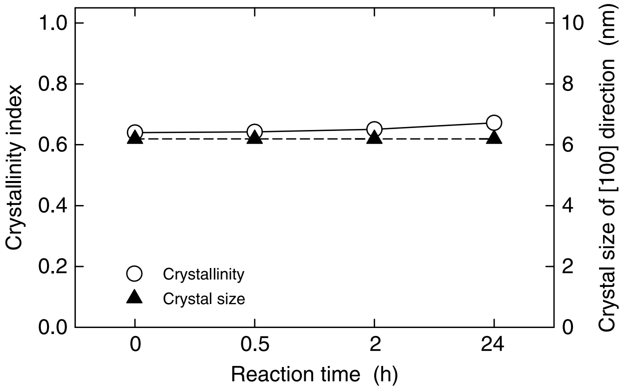

Fig. 4. Relationships between the TEMPO-oxidation time and either crystallinity index or crystal size of cellulose I in the TEMPO-oxidized celluloses.

and/or on crystal surfaces of cellulose I without any oxidation at the C6 primary hydroxyls of inside crystallites of cellulose I. The results obtained in this study show that the shoulder signal at 63 ppm due to the non-crystalline C6 primary hydroxyl groups in the solid-state ^{13}C-NMR spectra is ascribed to those of surfaces of cellulose I crystallites or surfaces of cellulose microfibrils. This assignment of the non-crystalline C6 carbons at 63 ppm is consistent with that reported by Larsson, Hult, Wickholm, Pettersson, and Iversen (1999) and Wickholm, Larsson, and Iversen (1998).

3.2. Distribution of carboxylate and aldehyde groups in TEMPO-oxidized celluloses

Distribution of carboxylate groups formed at the C6 position of cellulose by the TEMPO-mediated oxidation was further studied at cellulose fiber level. It was confirmed beforehand that more than 95% of sodium carboxylate groups in the TEMPO-oxidized celluloses (prepared from cotton linters) were successfully ion-exchanged to the structures like cellulose-COOCa^{+} by treating with an aqueous calcium chloride solution (Saito & Isogai, submitted for publication). Thus, the carboxylate distribution in the TEMPO-oxidized celluloses can be indirectly evaluated by measuring distribution of Ca in the products.

Fig. 5 shows a scanning electron microphotograph (SEM) of a cross-section of the TEMPO-oxidized cellulose fiber containing calcium carboxylate groups. Although the lumen of cotton linters was somewhat collapsed, the EDX line analysis clearly revealed that calcium ions or carboxylate groups in the TEMPO-oxidized cellulose were homogeneously distributed from the fiber surface to the inside fiber. Thus, at the fiber level, the TEMPO-mediated oxidation can be regarded to occur homogeneously at both fiber surfaces and inside fibers. The reagents must easily penetrate the inside cellulose fibers, and the TEMPO-mediated oxidation occurs homogeneously in the cross-section of cellulose fibers without any restrictions.

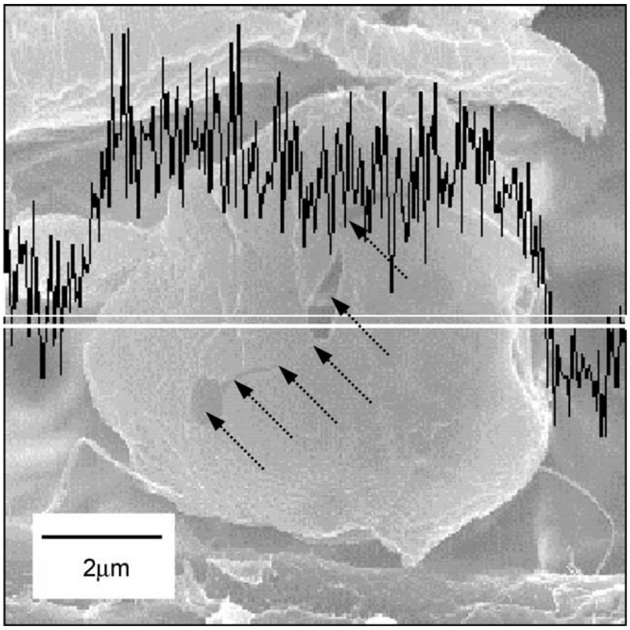

Fig. 5. Distribution of calcium ions determined by the EDX line analysis of a cross-section of TEMPO-oxidized cellulose fibers having calcium carboxylate groups. Arrows show the collapsed lumen.

The sum of carboxylate and aldehyde groups formed in the TEMPO-oxidized celluloses increased up to 15% (i.e. one oxidized glucose unit per 6–7 glucose units in average) of the total C6 primary hydroxyl groups (Table 1). However, because no C6 primary hydroxyl groups of inside cellulose I crystallites are oxidized, carboxylate and aldehyde groups must be present with more densely distributed states on the surfaces of cellulose I crystallites or those of cellulose microfibrils. Although the number of glucose units present at the surfaces of cellulose crystallites or those of cellulose microfibrils depends on the size of these crystallite or microfibril widths and crystallinity index, at least more than 30% of the C6 primary hydroxyl groups of surfaces of cellulose I crystallites or those of cellulose microfibrils in cotton linters are oxidized to either carboxylate or aldehyde group by the TEMPO-mediated oxidation. Nevertheless, these oxidized cellulose molecules neither detached from the cellulose crystallites nor became soluble in the aqueous oxidation medium during the TEMPO-mediated oxidation. As reported in the previous paper (Saito et al., in press), water-soluble fractions in the TEMPO-oxidized cotton linters, which were formed in the yields of about 2%, had carboxylate groups of about 2.1 mmol/g, which corresponded to about 35% of the C6 position in the fractions. There may be a boundary point around 30–35% oxidation of the C6 position to carboxylate groups in each cellulose molecule between solubility and insolubility in the aqueous oxidation medium during the TEMPO-mediated oxidation of native celluloses. Therefore, when the degree of TEMPO-mediated oxidation exceeds the boundary point by the extended TEMPO-oxidation time, the yields of the fibrous fractions of the TEMPO-oxidized celluloses decreased (Table 1).

4. Conclusion

When cotton linters were oxidized by the TEMPO-mediated system under aqueous slurry conditions, carboxylate groups are introduced into the TEMPO-oxidized celluloses up to 0.74 mmol/g and are homogeneously present at the fiber cross-section level from the surface of cellulose fibers to inside fibers. Moreover, solid-state ^{13}C-NMR and X-ray diffraction analyses revealed that the carboxylate and aldehyde groups introduced into the TEMPO-oxidized celluloses were mostly present at the C6 position of only the surfaces of cellulose I crystallites or surfaces of cellulose microfibrils without any oxidation at the C6 primary hydroxyl groups of inside cellulose I crystallites of cotton linters.

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