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# TEMPO-mediated oxidation of native cellulose: Microscopic analysis of fibrous fractions in the oxidized products

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**Abstract**

The 2,2,6,6-tetramethylpiperidine-1-oxy radial (TEMPO)-mediated oxidation was applied to aqueous suspensions of cotton linters, ramie and spruce holocellulose at pH 10.5, and water-insoluble fractions of the TEMPO-oxidized celluloses collected by filtration with water were analyzed by optical and transmission electron microscopy and others. The results showed that both fibrous forms and microfibrillar nature of the original native celluloses were maintained after the TEMPO-mediated oxidation, even though carboxylate and aldehyde groups of 0.67–1.16 and 0.09–0.21 mmol/g, respectively, were introduced into the water-insoluble fractions. Neither crystallinity nor crystal size of cellulose I of the original native celluloses was changed under the conditions adopted in this study. Carboxylate groups in the TEMPO-oxidized ramie were mapped by labeling with lead ions as their counter ions. The transmission electron micrographs indicated that some heterogeneous distribution of carboxylate groups along each cellulose microfibril or each bundle of cellulose microfibrils seemed to be present in the TEMPO-oxidized celluloses.


*Keywords:* Cellulose; Microfibril; TEMPO; Oxidation; Surface; TEM

## 1. Introduction

Catalytic and selective oxidation of primary hydroxyl groups of carbohydrates using water-soluble and stable nitroxyl radicals such as 2,2,6,6-tetramethylpyperidine-1-oxy radical (TEMPO) has opened a new field of polysaccharide chemistry (de Nooy, Besemer, & van Bekkum, 1995). Since the first report by de Nooy et al., applications of this catalytic oxidation to various polysaccharides under aqueous conditions have been widely studied for conversion of polysaccharides to the corresponding polyuronic acids (Bragd, van Bekkum, & Besemer, 2004; Chang & Robyt, 1996; Kato, Kaminaga, Matsuo, & Isogai, 2004).

In the case of the TEMPO-mediated oxidation of celluloses, different products are obtained, depending on the starting materials. When regenerated and mercerized celluloses are used, water-soluble β-1,4-linked polyglucuronic acid sodium salt (cellouronic acid) having a homogeneous chemical structure can be obtained quantitatively as the oxidized products (Isogai & Kato, 1998; Tahili & Vignon, 2000). On the other hand, when native celluloses are adopted, cellulose slurries at the initial stage are maintained even after the TEMPO-mediated oxidation with excess reagents for extended reaction times. However, the TEMPO-mediated oxidation has been recently proposed as one of the most promising methods for surface modifications of native celluloses, where carboxylate and aldehyde functional groups can be effectively introduced into solid native celluloses under aqueous and mild conditions (Gert, Torgashov, Zubets, & Kaputskii, 2005; Kitaoka, Isogai, & Onabe, 1999; Kurosu & Pelton, 2004; Montanari, Roumani, Heux, & Vignon, 2005; Saito & Isogai, 2004).

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In our previous papers, we did structural and chemical analyses on cotton linters subjected to the TEMPO-mediated oxidation under various conditions (Saito & Isogai, 2004; Saito, Yanagisawa, & Isogai, 2005; Saito, Shibata, Isogai, Suguri, & Sumikawa, 2005). Carboxyl and aldehyde groups were introduced into the water-insoluble fractions up to about 0.7 and 0.3 mmol/g, respectively, by the oxidation. The mass recovery ratios were generally higher than 80%. 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 in the non-crystalline region and/or on crystal surfaces of cellulose I without any oxidation at the C6 primary hydroxyls inside cellulose I crystallites.

In this paper, the TEMPO-mediated oxidation was applied to three native cellulosics, i.e., cotton linters, ramie, and spruce holocellulose. Water-insoluble fractions in the oxidized products were analyzed by optical and transmission electron microscopy to evaluate morphological changes. Distribution of carboxylate groups introduced by the TEMPO oxidation was also investigated at cellulose microfibril level.

## 2. Materials and methods

### 2.1. Materials

Three native cellulose samples, i.e., cotton linters (Ash-less filter pulp, Advantec Toyo Co., Japan), ramie (bleached ramie fibers, Teikoku Bouseki Co., Japan) and spruce holocellulose, were used in this study. The holocellulose sample was prepared from 80-mesh wood meal (80-mesh passed) of Norway spruce (*Picea abies*). The wood meal was subjected to repeated treatment with $NaClO_2$ (0.3 g/g sample) at pH 4–5 and 70 °C until the wood meal was mostly delignified, where Klason lignin content was lower than 0.1%. TEMPO, sodium bromide, and 9% sodium hypochlorite solution, and other chemicals and solvents were of laboratory grades (Wako Pure Chemicals, Co., Japan), and used without further purification.

### 2.2. TEMPO-mediated oxidation of cellulose

Cellulose (2 g) was suspended in water (200 mL) containing TEMPO (0.025 g) and sodium bromide (0.25 g). The TEMPO-mediated oxidation of the cellulose slurry was started by adding NaClO (2.42 mmol/g cellulose). The slurry was continuously stirred at room temperature. The pH was maintained to 10.5 by adding 0.5 M NaOH until no more consumption of alkali was observed, indicating that the reaction was completed. The pH was then adjusted to 7 with 0.5 M HCl. The fibrous fraction of the TEMPO-oxidized product was washed thoroughly with water by filtration, and stored at 4 °C without drying.

### 2.3. Transmission electron microscopy

The TEMPO-oxidized and never-dried sample was suspended in water, and this slurry was subjected to ultrasonic treatment for 1–2 min to obtain a sufficiently dispersed suspension. A 10 μl aliquot of the disintegrated cellulose suspension was mounted on a hydrophilically treated carbon-coated grid. Before complete air-drying, 10 μl of 3% uranyl acetate with 1% trehalose was deposited on the sample grid, and it was left standing for 1 min. Then, the excess solution was blotted out with a filter paper and allowed to stand for drying by natural evaporation. In the case of lead ion-containing samples, no such uranyl staining was adopted. The sample grid was observed at 100 kV by means of a JEOL electron microscope (JEM 2000-EXII) equipped with a GATAN image intensifier (model 622-0300). All micrographs were taken at a magnification of 20K on Fuji FG films.

### 2.4. Analyses

Carboxylate and aldehyde contents in the TEMPO-oxidized celluloses were determined by the electric conductivity titration method (Saito & Isogai, 2004). 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. The dried samples were pressed into pellets at ca. 750 MPa. X-ray diffraction was measured on the pellet using Rigaku Rint 2000 in reflection mode. Nickel-filtered Cu Kα radiation of X-ray tube operated at 40 kV and 40 mA were used. Crystallinity index and the crystal size of the [1 0 0] direction of cellulose I for the TEMPO-oxidized celluloses were measured from the $\theta$–$2\theta$ X-ray diffraction profile (Saito & Isogai, 2004; Segal, Creely, Martin, & Conrad, 1959).

## 3. Results and discussion

### 3.1. TEMPO-mediated oxidation of native celluloses

Table 1 shows profiles of the original native celluloses and the corresponding TEMPO-oxidized samples used in this study. Recovery ratios of the oxidized products after washing with water by filtration were more than 90%. Carboxylate and aldehyde contents were different among the three TEMPO-oxidized samples, though the oxidation conditions adopted were the same. Carboxylate and aldehyde contents in the TEMPO-oxidized cotton linters were 0.67 and 0.21 mmol/g, respectively. The sum of carboxylate and aldehyde contents corresponds to approximately one C6-oxidized glucose unit per 7 glucose units in average. In the case of the TEMPO-oxidized ramie and TEMPO-oxidized spruce holocellulose, the sum of carboxylate and aldehyde groups corresponds to approximately one C6-oxidized glucose unit per 5 glucose units in average.

The three native celluloses had various crystallinities and crystal sizes of cellulose I, 59–92% and 3.2–6.2 nm,

Table 1
Profiles of the TEMPO-oxidized celluloses used in this study

| Cellulose sample | Functional group content (mmol/g) | | Recovery ratio (%) | Crystallinity (%) | Crystal size (nm) |
|---|---|---|---|---|---|
| | Carboxylate | Aldehyde | | | |
| Cotton linters | 0.02 | 0.00 | – | 86 | 6.2 |
| TEMPO-oxidized cotton linters | 0.67 | 0.21 | 91 | 86 | 6.2 |
| Ramie | 0.04 | n.d. | – | 92 | 5.6 |
| TEMPO-oxidized ramie | 0.94 | 0.25 | 94 | 92 | 5.8 |
| Spruce holocellulose | 0.04 | n.d. | – | 59 | 3.2 |
| TEMPO-oxidized spruce holocellulose | 1.16 | 0.09 | 96 | 60 | 3.2 |

n.d., not determined.

respectively (Table 1). These values of each native cellulose sample were nearly unchanged even after the TEMPO-mediated oxidation; neither carboxylate nor aldehyde groups are probably formed inside cellulose I crystallites by the oxidation, and thus significant amounts of carboxylate and aldehyde groups are present only on the crystal surfaces and in disordered regions (Montanari et al., 2005; Saito & Isogai, 2004; Saito et al., 2005).

As shown in the scheme of the TEMPO-oxidation of cellulose (Fig. 1), 1 mol carboxylate group is formed by consuming 2 mol NaClO, while 1 mol aldehyde group is formed by 1 mol NaClO. Therefore, the oxidation efficiency can be defines as follows:

$$\text{Oxidation efficiency } (\%) = 100 \times \{2 \times (C_T - C_O) + (A_T - A_O)\}/M_{NaClO}$$

where $M_{NaClO}$ is the quantity of NaClO added (mmol/g), $C_O$ and $C_T$ are carboxylate contents (mmol/g) before and after oxidation, and $A_T$ and $A_O$ are the corresponding aldehyde contents. The calculated values for the TEMPO-mediated oxidation of cotton linters, ramie and spruce holocellulose were 62%, 85%, and 96%, respectively. This order was positively correlated to the recovery ratios of the three TEMPO-oxidized celluloses, and inversely correlated to their crystal sizes (Table 1). The rest of NaClO added might have been consumed for formation of water-soluble fractions and colloidal particles (Saito

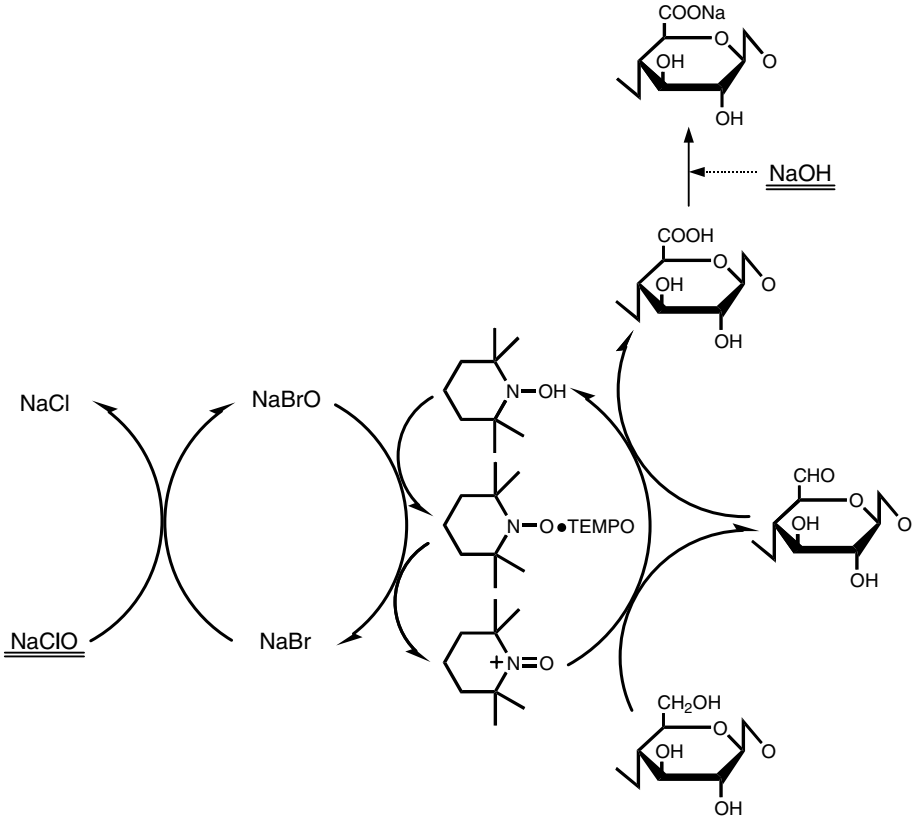


Fig. 1. Scheme of TEMPO-mediated oxidation of cellulose.

et al., 2005) as well as over-oxidation and side reactions, which lead to the decrease in the recovery ratios in Table 1. Thus, the results of Table 1 and the oxidation efficiencies calculated suggest that the larger crystal size, the more side reactions take place on the cellulose during the TEMPO-mediated oxidation, resulting in a decrease in the recovery ratio.

### 3.2. Morphological changes of celluloses during the TEMPO-mediated oxidation

Fig. 2 shows optical microphotographs of cotton linters and ramie before and after the TEMPO-mediated oxidation. In both cellulose samples, no significant changes in fibrous morphology were observed before and after the oxidation, although significant amounts of carboxylate and aldehyde groups were introduced (Table 1). Thus, the original fibrous forms of celluloses can be maintained even after the TEMPO-mediated oxidation, as far as the degree of oxidation is within a certain level (Saito & Isogai, 2004).

Cellulose microfibrils of the TEMPO-oxidized ramie and spruce holocellulose were observed by transmission electron microscopy (TEM) (Fig. 3). The degree of aggregation of cellulose microfibrils is clearly different between the TEMPO-oxidized ramie and spruce holocellulose. In the case of the TEMPO-oxidized ramie, cellulose microfibrils are intimately associated to form aggregates in a similar manner to those for other higher plant celluloses (Hult, Iversen, & Sugiyama, 2003). In contrast, the TEMPO-oxidized spruce holocellulose disintegrated into microfibrils of random orientation. In both cases as shown in Fig. 3, the microfibrillar nature of the original celluloses (Hult et al., 2003) was mostly maintained even after the TEMPO-mediated oxidation. It was reported that excessive TEMPO-oxidation caused formation of short cellulose microfibrils or cellulose micro-crystallites by cleavages of the microfibrils, which were similar to those obtained by strong acid hydrolysis of native celluloses (Montanari et al., 2005). In our case, TEMPO-mediated oxidation conditions were sufficiently mild to mostly maintain the original microfibrillar nature as well as crystallinity and crystal size of cellulose I, even though significant amounts of functional (carboxylate and aldehyde) groups are introduced to the oxidized products.

### 3.3. Distribution of carboxylate groups in the TEMPO-oxidized cellulose

Distribution of carboxylate groups formed at the C6 of ramie cellulose by the TEMPO-mediated oxidation was studied at microfibril level. It was confirmed beforehand that almost all sodium carboxylate groups in the TEMPO-oxidized celluloses were successfully ion-exchanged to the structure of cellulose-$COOPb^{+}$ by treating with lead salt solutions; nearly no structures of $(cellulose\text{-}COO)_2Pb$ are present in the lead ion-treated products (Saito & Isogai, 2005). Thus, distribution of carboxylate groups in the TEMPO-oxidized celluloses can be indirectly evaluated by mapping of lead ions in the products.

In this study, a lead citrate solution adjusted to pH 12 by adding a NaOH solution was used for labeling of the carboxylate groups by ion-exchange. Reynolds (1963) reported that lead ions under alkaline conditions around pH 12 form clusters of $[Pb(OH)_2Pb]^{2+}$ in the highest

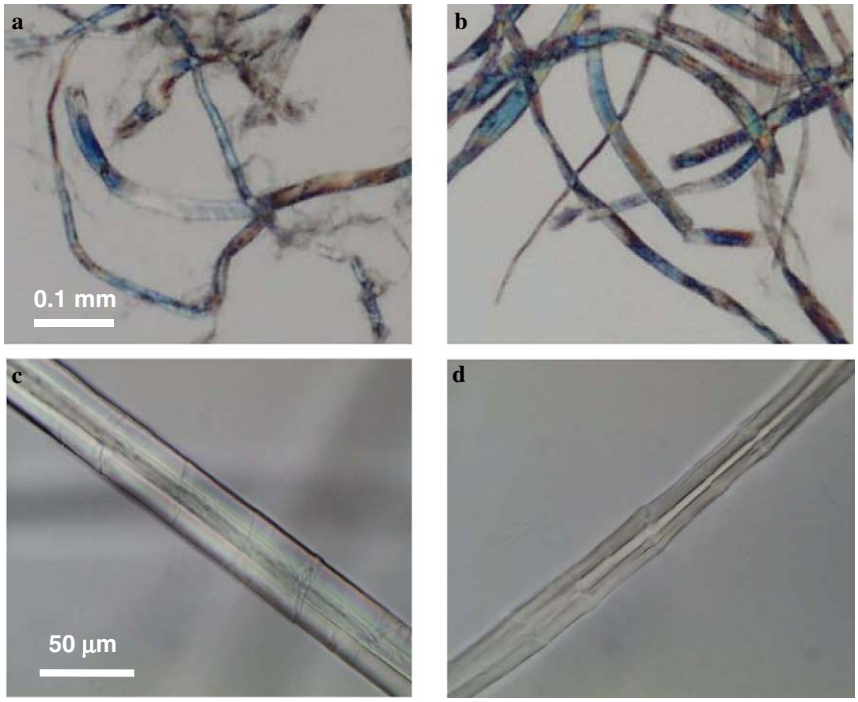


Fig. 2. Optical microphotographs of cotton linters (a), the TEMPO-oxidized cotton linters (b), ramie (c), and the TEMPO-oxidized ramie (d).

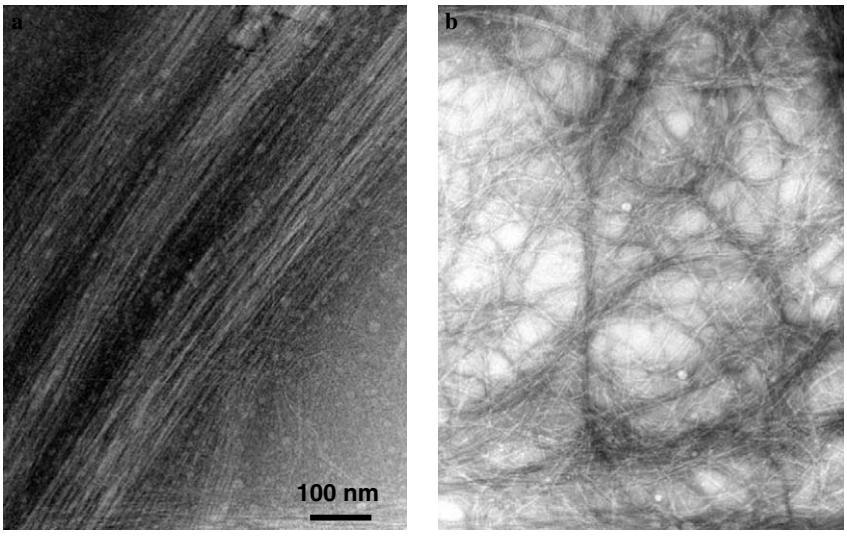


Fig. 3. Transmission electron microphotographs of the TEMPO-oxidized ramie (a) and TEMPO-oxidized spruce holocellulose (b). Observed after negative staining of disintegrated samples with uranyl acetate.

quantity. Therefore, one carboxylate group in the TEMPO-oxidized cellulose may be possible to catch two lead ions at once by treating with the lead citrate solution at pH 12. In fact, as shown in Table 2, the TEMPO-oxidized ramie treated with the lead citrate solution at pH 12, contained lead ions more than those treated with other lead salt solutions. Some of the structures of cellulose-COO-$Pb(OH)_2Pb^+$ once formed may be converted to cellulose-$COOPb^+$ by removal of $Pb(OH)_2$ during washing process of the lead ion-treated samples, thus the value of 1.20 mmol/g for the lead ion content was lower than the theoretical value ($0.94 \times 2 = 1.88$ mmol/g, see Table 1).

Three ramie samples, the original ramie, TEMPO-oxidized ramie, and TEMPO-oxidized and then $NaClO_2$-treated ramie, after the treatment with the lead citrate solution were subjected to the TEM observation without any staining treatments (Fig. 4). Each cellulose microfibril in the TEMPO-oxidized ramie samples (Figs. 4b and c) was clearly observed, compared with that in the original ramie (Fig. 4a). This is because carboxylate groups form salts with heavy and electron-opaque lead ions, and are present on the cellulose microfibril surfaces with high densities in the two oxidized samples. The fact that the microfibrillar nature was maintained in the TEMPO-oxidized ramie was consistent with the results of solid-state $^{13}C$ NMR and X-ray diffraction analyses of the TEMPO-oxidized cotton linters (Saito et al., 2005); carboxylate and aldehyde groups are introduced on cellulose microfibril surfaces and in disordered regions without any oxidation of inside cellulose I crystallites by the TEMPO-mediated oxidation of native celluloses.

In Figs. 4b and c, some localization of relatively dark part due to more lead ions were observed along each cellulose microfibril or each bundle of microfibrils, which are pointed with arrows in these figures. These lead-rich parts may come from some imperfect areas of cellulose crystallites such as twists or kinks present along each cellulose microfibril. The presence of these imperfect areas in each cellulose microfibril has already been reported from TEM observations (Hanley, Revol, Godbout, & Gray, 1997; Willison & Abeysekera, 1985) and small angle neutron-scattering analysis (Nishiyama et al., 2003) of higher-plant celluloses. However, it has been unknown until now whether these imperfect areas are present in the original cellulose microfibrils or formed artificially during chemical treatments, isolation procedures, drying process, ultrasonic irradiations, and others. Novel sample preparation procedures, being devoid of sample damage as possible, together with the carboxyl groups-visualization shown here may provide a direct evidence to answer if the disordered regions are distributed evenly along the length of a microfibril in nature.

Table 2
Lead ion content in the TEMPO-oxidized ramie[a] treated with three lead salt solutions

| Lead salt | Lead ion content (mmol/g cellulose)[b] |
|---|---|
| Lead citrate[c] | 1.20 |
| Lead acetate | 1.01 |
| Lead nitrate | 1.00 |

[a] Carboxylate content of the TEMPO-oxidized ramie was 0.94 mmol/g (see Table 1).
[b] Determined by X-ray fluorescence analysis (Saito & Isogai, 2005).
[c] Adjusted to pH 12 by adding a NaOH solution.

## 4. Conclusions

When native celluloses like cotton linters, ramie, and spruce holocellulose are treated by the TEMPO/NaBr/NaClO system under aqueous conditions, both crystallinity and crystal size of cellulose I of the original native celluloses are mostly unchanged. It was confirmed from TEM

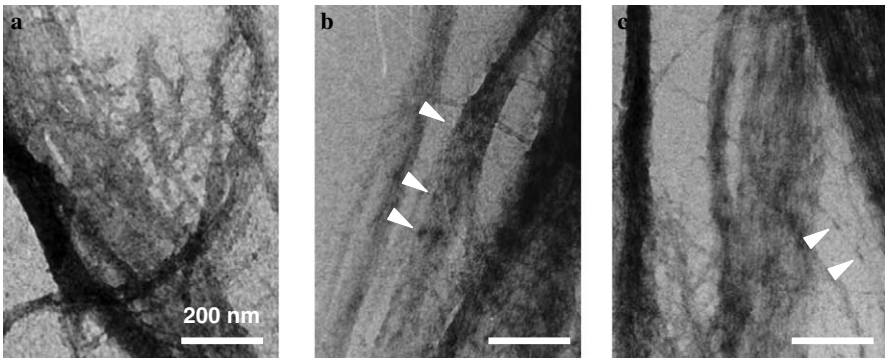


Fig. 4. Transmission electron microphotographs of ramie (a), the TEMPO-oxidized ramie (b), and the TEMPO-oxidized and then $NaClO_2$-treated ramie (c). All carboxylate groups in these cellulose samples had the lead salt structure. Observed without any staining treatments after disintegration of the cellulose samples.

observations that the microfibrillar nature as well as the original fibrous forms of the native celluloses was mostly maintained, even though significant amounts of functional groups (carboxylate and aldehyde groups) were introduced by the TEMPO-mediated oxidation. These results show that carboxylate and aldehyde groups introduced are present on the surfaces of cellulose microfibrils and in disordered regions with high densities. Introduction of lead ions into the TEMPO-oxidized celluloses as counter ions of carboxylate groups in there and the successive TEM observations also supported the above hypothesis. In addition, some heterogeneous distribution of lead ion-rich or carboxylate group-rich parts seemed to be present along each cellulose microfibril or each bundle of microfibrils in the TEMPO-oxidized celluloses.

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