



Oxidation of regenerated cellulose with NaClO_2 catalyzed by TEMPO and NaClO under acid-neutral conditions

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ARTICLE INFO

Article history:

Received 19 February 2009

Received in revised form 13 April 2009

Accepted 16 April 2009

Available online 3 May 2009

Keywords:

Cellulose

4-Acetamide-TEMPO

Cellouronic acid

Sodium chlorite

SEC-MALLS

ABSTRACT

A new TEMPO-mediated oxidation with catalytic amounts of TEMPO and NaClO , and NaClO_2 as the primary oxidant under aqueous conditions at pH 3.5–6.8 was used to prepare water-soluble β -(1 \rightarrow 4)-linked polyglucuronic acid Na salts (cellouronic acids, CUAs) with high molecular weight in good yield. When regenerated cellulose with original degree of polymerization (DP) of 680 was oxidized by the 4-acetamide-TEMPO/ NaClO / NaClO_2 system at pH 5.8 and 40 °C for 3 days, CUA with weight average DP (DP_w) of 490 was obtained quantitatively. No peaks other than six signals from β -(1 \rightarrow 4)-linked anhydroglucuronic acid units of CUA were detected in the solution-state ^{13}C NMR spectra of the oxidized products. Although the oxidized product prepared under the above conditions contained about 20% unoxidized cellulose particles, the non-CUA fraction was separable from CUAs by filtration or salt precipitation. The DP_w values and yields of CUAs after the filtration or salt precipitation treatment were 250–380 and 45–71%, respectively.

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1. Introduction

TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical) is one of the water-soluble, stable nitroxyl radicals, and has been extensively utilized in catalytic oxidation for selective and effective conversion of alcoholic hydroxyls to carboxyls under aqueous conditions (Bailey & Bobbitt, 2007; Barbier et al., 2006; Bragd, van Bekkum, & Besemer, 2004; Desai & Blackwell, 2003; Goldstein & Samuni, 2007; Rejzek, Mukhopadhyay, Wenzel, Lam, & Field, 2007). de Nooy, Besemer, and van Bekkum (1995) first applied TEMPO-mediated oxidation with a catalytic amount of NaBr , using NaClO as a primary oxidant, to water-soluble polysaccharides at pH 9–11. The corresponding polyuronic acids were obtained quantitatively by oxidation of the C6 primary hydroxyls to carboxylate groups by the TEMPO/ NaBr / NaClO system.

The C6 primary hydroxyls of water-insoluble and structural polysaccharides such as cellulose and chitin can also be oxidized to carboxylate groups by the TEMPO/ NaBr / NaClO system in water at pH 9–11. However, solid-state structures or crystallinity of the starting celluloses and chitins strongly influence the reactivity and structures of the oxidized products (Isogai & Kato, 1998; Kato, Kaminaga, Matsuo, & Isogai, 2004). When regenerated and mercerized (alkali-swollen) celluloses (Isogai & Kato, 1998), liquid NH_3 -treated native celluloses (Da Silva Perez, Montanari, & Vignon, 2003) and ball-milled native celluloses (Isogai, Yanagisawa, & Iso-

gai, 2009) are used, cellulose slurries become clear solutions at room temperature within 2 h as the oxidation proceeds, and water-soluble β -(1 \rightarrow 4)-linked polyglucuronic acid sodium salt (cellouronic acid Na salt, CUA) can be obtained quantitatively as the oxidized product. CUAs are both biodegradable and metabolizable by microorganisms in nature (Kato et al., 2002; Konno, Habu, Maeda, Azuma, & Isogai, 2006; Konno, Isogai, Habu, & Iihashi, 2008), and thus are promising new bio-based polyelectrolytes.

When TEMPO-mediated oxidation is applied to native celluloses, only the C6 primary hydroxyl groups present on the microfibril surfaces are oxidized to carboxylate groups during the oxidation (Saito & Isogai, 2004; Saito, Okita, Nge, Sugiyama, & Isogai, 2006; Saito, Shibata, Isogai, Sguri, & Sumikawa, 2005). TEMPO-oxidized native celluloses with carboxylate group contents exceeding a certain level can be converted to individual cellulose nanofibers 3–10 nm in width and a few microns in length, dispersed in water by gentle mechanical agitation (Saito, Kimura, Nishiyama, & Isogai, 2007; Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006).

In preparation of both CUAs and TEMPO-oxidized cellulose nanofibers, however, substantial decreases in degree of polymerization (DP) occur during the oxidation (Bragd, Besemer, & van Bekkum, 2000; de Nooy et al., 1995; Isogai et al., 2009; Saito & Isogai, 2004; Shibata & Isogai, 2003; Shibata, Yanagisawa, Saito, & Isogai, 2006). CUAs prepared from regenerated and mercerized celluloses with DP_v 220–680 have DP_w values from only 40 to 80 (Shibata et al., 2006). Also in TEMPO-mediated oxidation of native celluloses, the DP values decreased to their LODP values of 200–300 (Saito & Isogai, 2004). The following depolymerization mecha-

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nisms of cellulose and other polysaccharides, occurring together with oxidation, have been proposed. (1) β -Elimination of glycoside bonds at the C6 aldehyde groups formed as intermediate structures during oxidation at pH 9–11 (de Nooy, Besemer, van Bekkum, van Dijk, & Smit, 1996; Potthast, Rosenau, & Kosma, 2006; Röhring et al., 2001), (2) active species such as hydroxyl radicals formed in situ as by-products during the oxidation, in turn attack glycoside bonds of polysaccharides (Shibata & Isogai, 2003). Substantial depolymerization of CUAs is detrimental when they are used as bio-based polyelectrolytes in various fields.

If the TEMPO-mediated oxidation of cellulose can proceed under neutral or weakly acidic conditions, CUAs with higher molecular weights may be obtained by avoiding β -elimination. A new oxidation system with catalytic amounts of TEMPO and NaClO using NaClO₂ as the primary oxidant, *i.e.* a TEMPO/NaClO/NaClO₂ system, was applied to low molecular weight compounds to convert their primary hydroxyls to carboxyls in an acetonitrile–water mixture under neutral conditions (Zhao et al., 1999). Even though times required for the oxidation are longer than those with the TEMPO/NaBr/NaClO system, the corresponding carboxyl compounds can be obtained in higher yields by the new TEMPO-mediated oxidation under neutral conditions. NaClO oxidizes TEMPO to the *N*-oxoammonium ion, which then rapidly oxidizes the primary hydroxyl to aldehyde under acidic or neutral conditions, forming

the hydroxylamine. The aldehyde is oxidized to carboxyl by the primary oxidant NaClO₂, forming NaClO, and the hydroxylamine is oxidized to the *N*-oxoammonium ion again by the NaClO thus generated (Fig. 1). No aldehyde groups remain in the oxidized products, and depolymerization of cellulose chains caused by β -elimination is expected to be avoided.

In the present study, the oxidation system TEMPO/NaClO/NaClO₂ was applied to regenerated cellulose suspended in water at pH 3.5–6.8 under various conditions. Optimum conditions for preparation of CUAs with high molecular weights in high yields were investigated.

2. Materials and methods

2.1. Materials

Commercial regenerated cellulose produced from cotton linters using a cuprammonium system (Bemliese®, viscosity average degree of polymerization DP_v 680; Asahi Chemicals Co., Japan) was used as the starting material. The cellulose fibers were cut into short fibers shorter than 5 mm in length before use. TEMPO, 4-acetamide-TEMPO, NaClO₂, 12% NaClO solution, and other chemicals and solvents were laboratory grade (Wako Pure Chemicals, Japan, or Aldrich, USA), used without further purification.

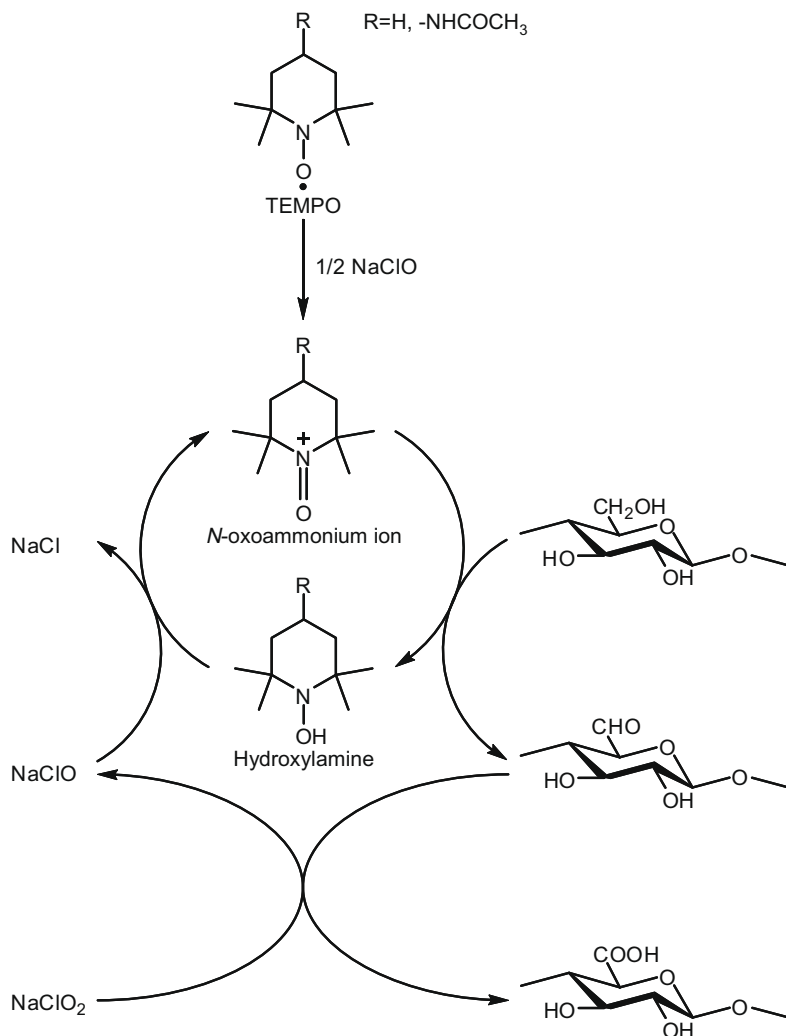


Fig. 1. Oxidation of C6 primary hydroxyl groups of cellulose by TEMPO/NaClO/NaClO₂ system in water at pH 3.5–6.8.

2.2. Oxidation of cellulose

Cellulose (1 g) was placed in an Erlenmeyer flask with a magnetic stirrer bar, and 100 ml of 0.1 M acetate buffer at pH 5.8 containing NaClO₂ (80%, 0.85 g, 7.5 mmol) and 4-acetamide-TEMPO (0.096 g, 0.45 mmol) was added to the flask. The NaClO solution (0.31 ml, 0.5 mmol) was diluted with water by 1%, and added at one step to the suspension. The flask was capped with a universal stopper, and the mixture was stirred at 40 °C for 1 day. Then NaClO₂ (0.85 g, 7.5 mmol) and NaClO (0.31 ml, 0.5 mmol), which was diluted with water by 1%, were further added at one step to the flask. The mixture was stirred at 40 °C for additional 2 days. Constant attention to generation of chlorine gas should always be paid during the addition of NaClO to the mixture (Zhao et al., 1999). The regenerated cellulose fibers were dissolved in the reaction medium in the course of the reaction, and a transparent solution was obtained. Oxidation was quenched by adding an excess of ethanol. The precipitates formed from the solution by ethanol addition were collected, and washed five times with 80% aqueous ethanol by centrifugation. The oxidized products were dissolved again in water, and freeze-dried. The oxidation conditions such as the addition sequence of NaClO₂ and NaClO, pH of the buffer solution, reaction time, temperature and others varied to obtain CUAs with higher molecular weights.

2.3. Removal of fine particles in the oxidized products

The oxidized products, even though apparently dissolved in water, contained some unoxidized cellulose particles, that were detectable by size-exclusion chromatography (SEC) (Saito, Yanagisawa, & Isogai, 2005). Removal of these fractions was attempted by either filtration of 0.1% oxidized product/water solutions three times through 0.1 µm poly(tetrafluoroethylene) (PTFE) disposable membrane (Anotop 25, Whatman, UK) or salt precipitation of 1% oxidized product/water solutions using 1 M NaCl followed by centrifugation at 7740g.

2.4. SEC–MALLS analysis

Molecular weights of the oxidized products were measured using a size-exclusion chromatography (SEC) furnished with a multi-angle laser light-scattering detector (MALLS: DAWN EOS; λ = 685 nm; Wyatt Technologies, USA), using 0.1 M NaCl as eluent. The oxidized product (1 mg) was dissolved in 0.1 M NaCl (1 ml), and the solution filtered using a 0.2 µm PTFE membrane (Millex-LG, Milipore, USA). A polyhydroxymethacrylate gel (SB-806MHQ, 8 mm Ø × 30 cm, Shodex, Japan) was used as the SEC column packing. The MALLS cells and refractive index detector were kept at room temperature and 40 °C, respectively. Details of the SEC–MALLS system and operational conditions have been reported elsewhere (Shibata et al., 2006). The value 0.125 ml/g was used as the specific refractive index increment (dn/dc) of CUA in 0.1 M NaCl (Shibata et al., 2006). Data acquisition and processing was performed with ASTRA software (Wyatt Technologies).

2.5. Other analyses

Carboxyl contents of the oxidized celluloses were determined by conductivity titration from pH 2–3 to pH 11 using 0.04 M NaOH (Saito & Isogai, 2004). Solution-state ¹³C NMR spectra of the oxidized products dissolved in D₂O were recorded using a JEOL AL-PHA-500 spectrometer (JEOL, Japan). 3-Trimethylsilyl-2,2,3,3-d₄-propionic acid sodium salt (Aldrich, USA) was used as an internal standard at 0 ppm. Solid-state ¹³C NMR spectra were obtained using a Bruker MSL spectrometer with magic angle sample spinning at 3 kHz and cross-polarization.

3. Results and discussion

3.1. Effect of oxidation conditions on carboxylate content of the products

The Bemlieste regenerated cellulose is produced from cotton linters with high α-cellulose content and has a high DPv of 680 in comparison with viscose rayon and Tencel, which are produced from wood celluloses and have DPv values of 380. In this study, therefore, Bemlieste was used as a standard sample to select optimum oxidation conditions with NaClO₂ as the primary oxidant, to prepare CUAs with high molecular weight in good yield.

First, the regenerated cellulose was oxidized at 60 °C using 4-acetamide-TEMPO or TEMPO (i.e. 4-H-TEMPO) under various conditions of pH and time (Fig. 2). It was confirmed that these oxidation systems can form significant amounts of carboxylate groups in the cellulose. In all cases, the carboxylate content increased with increasing the oxidation time up to 1 day, and then mostly reached plateau levels. When the oxidation was carried out using 4-acetamide-TEMPO at pH 4.8 and 6.8 for 1–3 days, water-soluble products were obtained quantitatively. The carboxylate contents of those products were in the range 3.7–4.0 mmol/g. Given that the carboxylate groups are formed from the C6 hydroxyl groups, 73–79% of the primary hydroxyls in the cellulose were oxidized to carboxylate groups. Thus, 4-acetamide-TEMPO is more effective than TEMPO in forming carboxylate groups in the cellulose during the oxidation. Based on the literature, 4-acetamide-TEMPO rather than TEMPO seems to be suitable for oxidation of primary hydroxyl groups at pH lower than 8 (Bragd, Besemer, & van Bekkum, 2001). It is not likely that 4-acetamide-TEMPO is unstable at pH 4.8–6.8 under the conditions used in this study.

Fig. 3 shows the relationships between reaction time and carboxylate content of the oxidized products, prepared using the 4-acetamide-TEMPO/NaClO/NaClO₂ system in acetate buffer at pH 4.8 but different temperatures. The oxidized celluloses obtained by reaction at 60 °C for 1–3 days and at 40 °C for 3 days were completely dissolved in the aqueous oxidation medium, while cellulose fibers were still present to some extent in the mixtures after reaction for 3 days at room temperature. When the cellulose was oxidized at 40 °C for 3 days, approximately 84% of the primary hydroxyls in the cellulose were oxidized to carboxylate groups. Complete oxidation of the C6 hydroxyl groups could not be achieved, although the oxidized products were water-soluble.

The results obtained from Figs. 2 and 3 show that oxidation with 4-acetamide-TEMPO at pH 4.8–6.8 at 60 °C for 1 day or 40 °C for 3 days can be regarded as optimum conditions for conver-

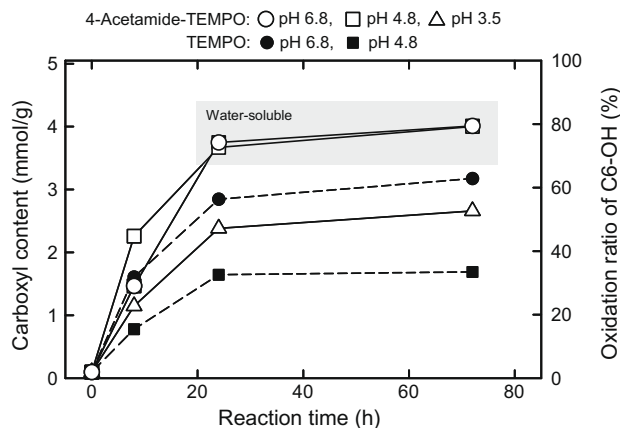


Fig. 2. Carboxyl contents of celluloses oxidized by TEMPO/NaClO/NaClO₂ or 4-acetamide-TEMPO/NaClO/NaClO₂ at different pHs.

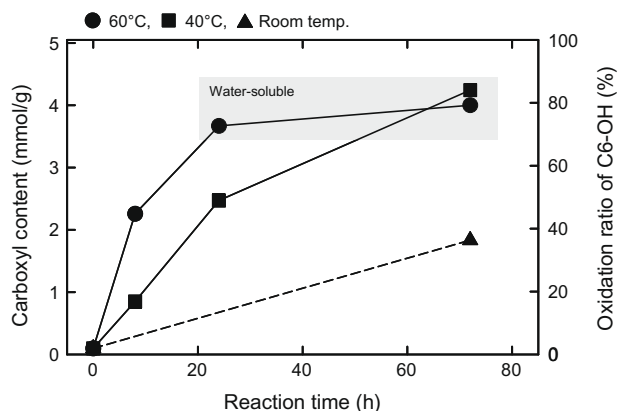


Fig. 3. Carboxyl content of celluloses oxidized by 4-acetamide-TEMPO/NaClO/NaClO₂ at the same pH 4.8 but different temperatures.

sion of regenerated cellulose to water-soluble oxidized products with high carboxylate contents, in yields of at least 90%. Most of the losses of products were caused by handling during isolation and purification, and were independent of the oxidation conditions. However, it is noticeable that 16–27% of the C6 primary hydroxyl groups still remained in the water-soluble oxidized products hence were not oxidized to carboxylate groups.

3.2. Structural characterization of the oxidized products

As described in the previous section, the carboxylate contents of the oxidized products indicate that complete oxidation of the C6 primary hydroxyl groups of the regenerated cellulose could not be achieved (Figs. 2 and 3). The oxidized product with 4.0 mmol/g carboxylate groups, prepared using the 4-acetamide-TEMPO/NaClO/NaClO₂ system for 1 day at 60 °C and pH 4.8, was analyzed by solution-state and solid-state ¹³C NMR (Fig. 4).

Interestingly, the water-soluble and solution-state ¹³C NMR-detectable fraction in the oxidized product had molecular structure

corresponding to almost pure CUA, consisting solely of β-(1 → 4)-linked anhydroglucuronic acid Na salt units. No residual C6 primary hydroxyl groups were detected at about 60 ppm, and no unknown peaks other than the six signals due to the anhydroglucuronic acid unit were present in the spectrum. Generally, CUAs prepared from regenerated celluloses, mercerized celluloses and ball-milled native celluloses by TEMPO-mediated oxidation at pH 10 using NaClO as the primary oxidant have some unknown signals in their solution-state ¹³C NMR spectra. Depolymerization of the oxidized products caused by β-elimination at C6 aldehyde groups formed as intermediate structures during TEMPO-mediated oxidation under alkaline conditions, the presence of small amounts of aldehyde groups, and other functional groups formed by side reactions may lead to such unknown signals in the solution-state ¹³C NMR spectra of CUAs (Iso-gai & Kato, 1998; Potthast et al., 2006; Röhring et al., 2001; Isogai et al., 2009; Shibata & Isogai, 2003).

Because NaClO₂ can selectively oxidize aldehyde groups in water at pH 4–7, the C6 aldehyde groups formed as intermediate structures by the 4-acetamide-TEMPO/NaClO/NaClO₂ system at pH 4.8–6.8 are likely to be converted in situ to carboxylate groups. Moreover, β-elimination is avoidable under such weakly acidic or neutral conditions. These factors might have brought about the formation of highly pure CUA with high molecular weight (as described later) by oxidation of the regenerated cellulose using the 4-acetamide-TEMPO/NaClO/NaClO₂ system. All water-soluble fractions of the oxidized products prepared using 4-acetamide-TEMPO at pH 4.8–6.8 for 1–3 days in Figs. 2 and 3 have the same solution-state ¹³C NMR spectra. Hence, pure CUAs, without any aldehyde groups and/or unknown structures formed by side reactions, can be obtained by TEMPO-mediated oxidation at pH 4.8–6.8 with NaClO₂ as the primary oxidant.

The solid-state ¹³C NMR spectra revealed that C6 primary hydroxyls were still present to some extent in the oxidized product. Peak area calculation of the C6 primary hydroxyls and C6 carboxylate groups from the ¹³C NMR spectrum showed that about 20% of the C6 primary hydroxyl groups were not converted to carboxylate groups. This proportion is in good agreement with that calculated from carboxylate content in Figs. 2 and 3. These results suggest that some unoxidized cellulose, which could not be detected by solution-state ¹³C NMR but were detectable by solid-state ¹³C NMR, are present as fine solid particles, not water-soluble products, in the oxidized products (Saito, Shibata, et al., 2005; Saito, Yanagisawa, et al., 2005). Thus, the oxidized product, even though it was apparently dissolved in water, consisted of almost pure CUA molecules as the major component and some unoxidized fine solid particles.

3.3. Molecular weights of the oxidized products

The oxidized products were further studied by SEC to determine their molecular weights, and four representative SEC elution patterns are shown in Fig. 5. The oxidized cellulose prepared by the 4-acetamide-TEMPO/NaClO/NaClO₂ system in 0.1 M acetate buffer at pH 5.8 for 3 days at 40 °C using the separated addition sequence of the reagents (see Section 2) had the highest molecular weight in those prepared under other various conditions. Each SEC elution pattern of the oxidized products prepared at pH 4.8–6.8, however, consisted of main and minor peaks. The minor peak corresponded to molecular weights higher than those of the main peak, and was attributed to molecular aggregates or incompletely dissolved components. Nano-sized particles of unoxidized cellulose that were present in the oxidized products and dispersed in the 0.1 M NaCl eluent solution are likely to have been eluted in part and separated by the SEC column without being trapped by the pre-filtration with the 0.2 μm PTFE membrane (Saito, Shibata, et al., 2005; Saito, Yanagisawa, et al., 2005).

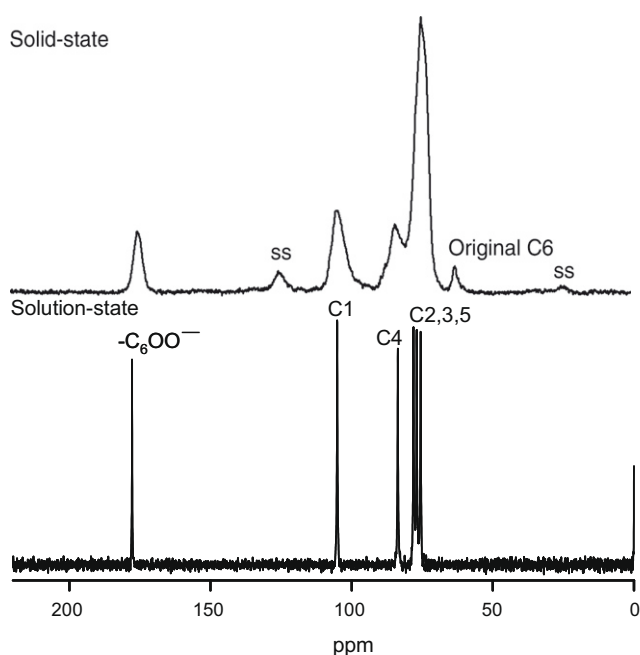


Fig. 4. Solution-state and solid-state ¹³C NMR spectra of 4-acetamide-TEMPO-oxidized cellulose with carboxylate content of 4.0 mmol/g. ss: spinning side band.

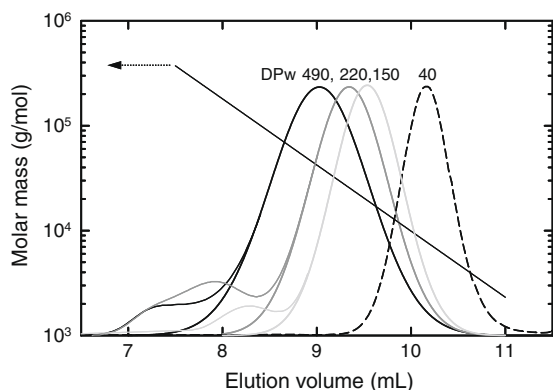


Fig. 5. SEC elution patterns of oxidized celluloses prepared from regenerated cellulose by 4-acetamide-TEMPO/NaClO/NaClO₂ in 0.1 M acetate buffer at pH 5.8 for 3 days at 40 °C (black line) and in 0.2 M acetate buffer at pH 4.8 (dark gray line) or pH 6.8 (light gray line) for 1 day at 60 °C, and by TEMPO/NaBr/NaClO for 2 h at room temperature and pH 10 (dashed line). The molecular weight plots are also depicted.

Assuming that the shapes of the main SEC elution peaks were Gaussians, the small peak due to unoxidized cellulose particles was excluded from MALLS calculation of DPw and DPn from the SEC elution patterns (Fig. 5). Compared with CUA with DPw 40 which was prepared from the same regenerated cellulose using the conventional TEMPO/NaBr/NaClO system at pH 10, the CUAs prepared using the 4-acetamide-TEMPO/NaClO/NaClO₂ system at pH 4.8–6.8 had higher DPw values ranging 220–490. Hence, CUAs with higher DPw values can be obtained by the 4-acetamide-TEMPO-mediated oxidation using NaClO₂ as the primary oxidant. It seems that depolymerization of cellulose chains, caused by β -elimination at the C6 aldehyde groups formed as intermediate structures during the TEMPO/NaBr/NaClO system at pH 10, is avoidable in the new oxidation system at pH 4.8–6.8.

Moreover, the oxidized product prepared using 0.1 M acetate buffer at pH 5.8/40 °C for 3 days by the separated addition sequence of the reagents had DPw higher than for those prepared using 0.2 M buffer at pH 4.8/60 °C or pH 6.8/60 °C for 1 day, even though all the three oxidized products had similar carboxylate contents and the oxidation time at 40 °C was longer. Based on the results of many preliminary experiments (data not shown), the addition sequence of the reagents, oxidation temperature of 40 °C and low buffer concentration are likely to be preferable for preparing CUAs with higher DPw values. It is known that NaClO₂ or some oxidative species formed from NaClO₂ in situ attack glycoside bonds to decrease the molecular weight of cellulose suspended in water especially at high temperatures (Kantouch, Hebeish, & El-Rafie, 1970). Such oxidative cleavage of glycoside bonds of the regenerated cellulose is probably preventable to some extent by adopting the oxidation conditions used to prepare the CUA with DPw 490. The reason for the difference in DPw of the oxidized products prepared between pH 4.8 and 6.8 in Fig. 5 is unknown at present.

In the next series of experiments, removal of the unoxidized cellulose particles present in the oxidized products and dispersed in their aqueous solutions was attempted, by filtration three times through a 0.1 μ m PTFE membrane or salt precipitation with 1 M NaCl followed by centrifugation of the precipitate. It was revealed from Fig. 6 that SEC elution patterns showed that the fraction due to unoxidized cellulose particles was mostly removed by the filtration treatment and completely eliminated by the salt precipitation treatment. A proportion of the CUA molecules with high DP was, however, simultaneously lost, and the yields and DPw values of CUAs decreased to some extent. Nevertheless, almost pure CUAs

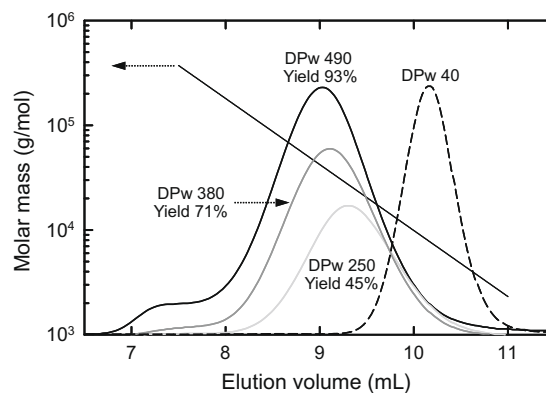


Fig. 6. SEC elution pattern of oxidized cellulose prepared from regenerated cellulose by 4-acetamide-TEMPO/NaClO/NaClO₂ in 0.1 M acetate buffer at pH 5.8 for 3 days at 40 °C (black line), and those after removal of fine particles fraction by the filtration (dark gray line) or salt precipitation (light gray line). The SEC elution pattern of CUA prepared by the TEMPO/NaBr/NaClO system at pH 10 (dash line) is also depicted as a reference.

with much higher DPw values than those of CUAs prepared by the TEMPO/NaBr/NaClO system at pH 9–11 can be obtained by the 4-acetamide-TEMPO/NaClO/NaClO₂ system at pH 4.8–5.8 and the following removal treatment of the unoxidized cellulose particles.

4. Conclusions

A new aqueous TEMPO-mediated oxidation system using NaClO₂ as the primary oxidant was developed to prepare water-soluble CUAs with high DP in good yield from regenerated cellulose. A CUA with high purity and the highest DPw of 490 was obtained by oxidation of Bemliese regenerated cellulose by the 4-acetamide-TEMPO/NaClO/NaClO₂ system in 0.1 M acetate buffer at pH 5.8 and 40 °C for 3 days using the separate addition sequence of the reagents. Even though the oxidized product contained about 20% unoxidized cellulose particles, this fraction was removable by filtration or salt precipitation associated with some reductions of yield and DP. No peaks other than six signals due to β -(1 \rightarrow 4)-linked anhydroglucuronic acid units of CUA were detected in the solution-state ¹³C NMR spectra of the oxidized products.

Acknowledgements

This research was supported in part by the Japan Society for the Promotion of Science (JSPS), Grant-in-Aid for JSPS fellows (Grant No. 18380102). Dr. Yutaka Yoshida of University of Tokyo, Japan, and Dr. Laurent Huex of CERMAV, CNRS, France kindly recorded the solid-state ¹³C NMR spectra.

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