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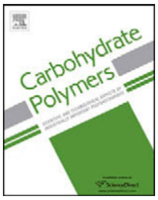

Analysis of carboxylate groups in oxidized never-dried cellulose II catalyzed by TEMPO and 4-acetamide-TEMPO

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ABSTRACT

In this report, never-dried regenerated celluloses, i.e. cellulose II, from their NaOH/urea/H_2O solutions were subjected to TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) or 4-acetamide-TEMPO-mediated oxidation. Water-soluble and -insoluble fractions of carboxyl cellulose (COC) were obtained and their DO were determined to be between 0.08 and 0.85. Apart from conductometric titration, complexometric titration with EDTA was used to analyze the DO of COC. The conductometric titration can be used for lowly or highly oxidized celluloses, while the complexometric titration with EDTA is only adequate for COC with DO lower than 0.6. FT Raman spectroscopy represents characteristic bands of carboxyl and carboxylate groups. It demonstrates the feasibility of establishing novel analyzing methods for DO of COC with high correlation coefficients of up to 0.9867. Moreover, long oxidation duration, high temperature and high amounts of oxidizing agents are important for the synthesis of COC with high DO and yields.

1. Introduction

Polysaccharides containing carboxyl groups have been found in nature or synthesized from natural glucans, e.g. cellulose and pullulan (De Nooy, Besemer, Van Bekkum, Van Dijk, & Smit, 1996; De Ruiter, Josso, Colquhoun, Voragen, & Rombouts, 1992). After a regioselective conversion of primary hydroxyl groups of cellulose into carboxyl groups, β-(1→4)-linked polyglucuronic acid, i.e. cellouronic acid, could be obtained. This conversion can be realized through 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) or TEMPO-derivatives-mediated oxidation (Habibi & Vignon, 2008; Hirota, Furihata, Saito, Kawada, & Isogai, 2010; Hirota, Tamura, Saito, & Isogai, 2009; Montanari, Roumani, Heux, & Vignon, 2005; Saito & Isogai, 2004).

During the alkaline TEMPO-catalyzed oxidation of native cellulose with the TEMPO/NaBr/NaClO system at pH 9–11, only the primary hydroxyl groups on the surface of cellulose crystals can be oxidized. As the result, cellulose nanofibers with a width of 3–5 nm were obtained (Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006). On the other side, only minor amount of final product was obtained as the sodium salt of cellouronic acid (Saito et al., 2006; Zhang et al., 2010). Furthermore, strong decrease in degrees of polymerisation (DP) occurred to cellulose chains during the alkaline TEMPO-mediated oxidation (Saito & Isogai, 2004).

The yield and the average DP of cellouronic acid sodium salt could be significantly raised by using cellulose II as starting materials (Isogai, Yanagisawa & Isogai, 2009). Furthermore, the use of 4-acetamide-TEMPO/NaClO/$NaClO_2$ oxidation system demonstrated another possibility to prepare cellouronic acid with higher average DP and relatively higher yields (Hirota et al., 2009). The 4-acetamide-TEMPO-mediated oxidation could proceed at a pH in the range of 4–7, so that the radicals formed by the application of TEMPO/NaBr/NaClO system at high pH between 9 and 11 and the alkaline peeling of formed glucuronic acids initiating the depolymerisation of cellulose chains can be excluded (Hirota et al., 2009; Saito et al., 2009). In previous studies, dried native or regenerated cellulose was subjected to TEMPO or TEMPO-derivatives-catalyzed oxidations. These celluloses in dried state could have highly crystalline structures, which could hamper the oxidation of primary hydroxyl groups (Hirota et al., 2009; Saito et al., 2009; Saito & Isogai, 2004).

The degree of oxidation (DO) of COC can be determined by various methods including methylene blue adsorption, conductometric titration or quantitative solid-state CP/MAS ^{13}C NMR spectroscopy (Da Silva Perez, Montanari, & Vignon, 2003; Montanari et al., 2005; Saito & Isogai, 2004). Among them, the conductometric titration is the widely applied one. However, these conventional methods normally require high amount of products, long analyzing time and in some cases also the experience of personal.

In this report, never-dried regenerated celluloses, i.e. cellulose II, were oxidized through TEMPO or 4-acetamide-TEMPO-mediated oxidations, in order to prepare COC with various DO under

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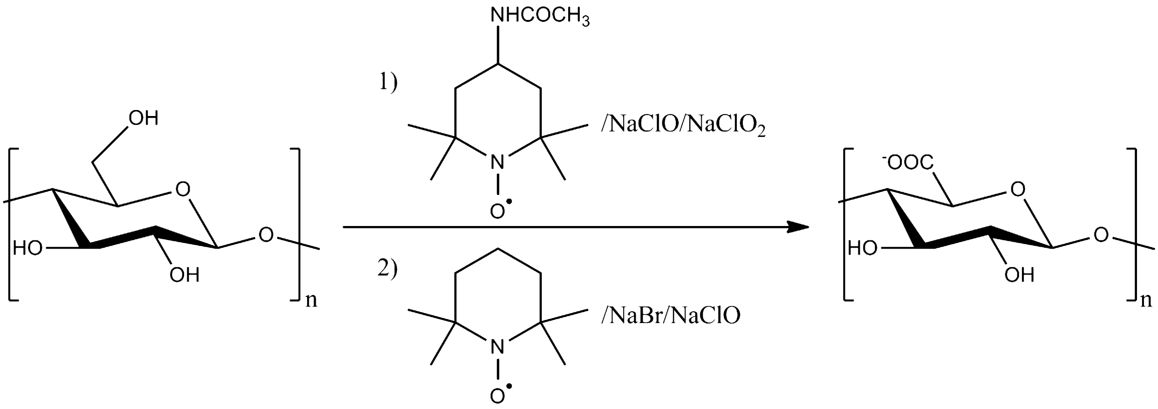


Scheme 1. Schematic representation of TEMPO or 4-acetamide-TEMPO-mediated oxidation of primary hydroxyl groups.

different oxidation conditions. The DO of obtained products were determined via conductometric titration or complexometric titration with EDTA. The introduction of carboxyl groups into cellulose chains were characterised via FT Raman spectroscopy and it showed the feasibility of determining DO based on Raman signals ascribed to carboxylate groups.

2. Experimental

2.1. Materials

Microcrystalline cellulose (MCC) with an average degree of polymerisation (DP) of 276 was received from J. Rettenmaier & Söhne GmbH (Rosenberg, Germany). Pulp V-81 (with 97.0% alpha cellulose, AC) with an average DP of 1180 was purchased from Buckeye Technologies Inc. (Memphis, USA). Cellunier F (CF) with an average DP of 500 was obtained from Rayonier (Jacksonville, USA). 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO) and 4-acetylamino-2,2,6,6-tetramethylpiperidine-1-oxyl (4-acetamide-TEMPO) were obtained from Acros (Geel, Belgium). Demineralised water was used in all experiments. Other chemicals were all of analysis grade and used as received. Dialysis membrane from Spectrum Laboratories Inc. (Rancho Dominquez, USA) has an approximate molecular weight cut off of 500 Da.

2.2. Dissolution and regeneration of cellulose I

MCC, Pulp V-81 or Cellunier F were dissolved in a NaOH/urea/water system as reported before (Zhou, Zhang, Deng, & Wu, 2004). Briefly, cellulose (1 g) was suspended in 80 ml aqueous solution of NaOH (6%)/urea (4%) and this suspension was frozen at −20 °C. After repeated thawing and freezing, a transparent solution could be obtained. The cellulose was then precipitated by adding water to its solution under vigorous stirring with an Ultra-Turrax (Janke & Kunkel GmbH, Staufen, Germany). Thereafter, regenerated cellulose was washed with water until salt-free and separated from water by centrifugation.

2.3. TEMPO-mediated oxidation of regenerated cellulose

TEMPO-mediated oxidation of cellulose was carried out as described before with a few modifications (Scheme 1) (Zhang et al., 2010). Briefly, never-dried regenerated cellulose (1 g) was suspended in 100 ml water at first. The oxidation agent consisting of 0.029 g TEMPO (0.03 mol/mol AGU of cellulose) and 0.318 g NaBr (0.5 mol/mol AGU) in 10 ml water was prepared under continuous stirring until complete dissolution. The oxidation was started after slowly adding 15.7 ml 12% aqueous NaClO solution (5 mol/mol AGU) to the cellulose suspension under stirring. The pH value of the mixture was maintained between 10 and 10.5. After the total addition of the NaClO solution, the pH was kept constant at room temperature (RT) for up to 6 h using 0.5 M aqueous NaOH solution. Thereafter, methanol (5 ml) was added to stop the oxidation and the pH was adjusted to 7.5 with 0.5 M aqueous HCl solution. The mixture was poured into 5 volumes ethanol to precipitate the product. After the elimination of the solvents, the product was mixed with 60 ml water yielding water-soluble and water-insoluble fraction of COC. The water-insoluble fraction and the supernatant were separated by centrifugation. A few water-insoluble fractions appeared as gel-like liquids when suspended in water, but these gel-like liquids could be separated from the aqueous phase. Thus, these COC were also regarded as water-insoluble fractions. The water-insoluble fraction was washed with water until salt-free and then lyophilized. The supernatant was filtrated, dialyzed and lyophilized (Scheme 1).

2.4. 4-Acetamide-TEMPO-mediated oxidation of regenerated cellulose (Scheme 1)

For this oxidation, never-dried regenerated cellulose (1 g) was suspended in 100 ml 2% sodium acetate solution. Then, the oxidation agent consisting of 0.0395 g 4-acetamide-TEMPO (0.03 mol/mol AGU) and 0.628 ml 12% aqueous NaClO solution (0.2 mol/mol AGU) in 10 ml water was added in the cellulose suspension. The pH value of the suspension was adjusted to 6 with diluted acetic acid. Subsequently, $NaClO_2$ (1.4 g, 2.5 mol/mol AGU) was added into the suspension under vigorous stirring and the temperature of the mixture was raised to 60 °C. After being kept at 60 °C for 48 h, the mixture was cooled to RT and its pH value was adjusted to 7.5. Then, the mixture was treated as described above and the products as water-soluble as well as water-insoluble COC were obtained.

2.5. Determination of DO

The DO of COC was determined via two distinct methods:

2.5.1. Conductometric titration

This method was carried out as described before with modifications (Da Silva Perez et al., 2003). Briefly, COC (30–40 mg) was dissolved or suspended in 20 ml 0.01 M aqueous HCl solution and it was titrated with aqueous 0.01 M NaOH solution in portions of 200 μl each time. The conductivity was measured after every addition of NaOH solution and the titration proceeded over the pH range of 2–12. The volume of used NaOH solution was determined

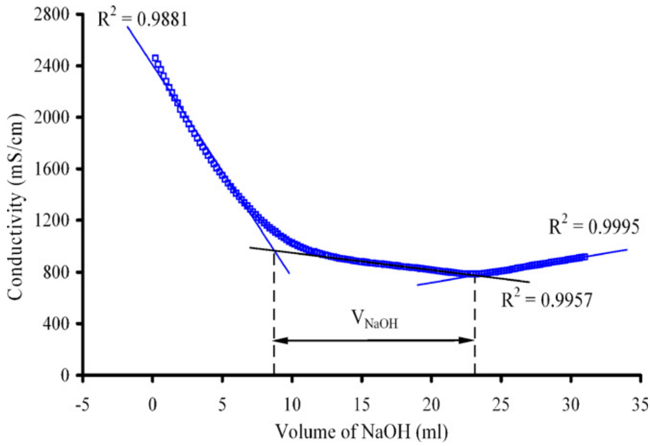


Fig. 1. Conductometric titration curve of water-soluble fraction of COC10.

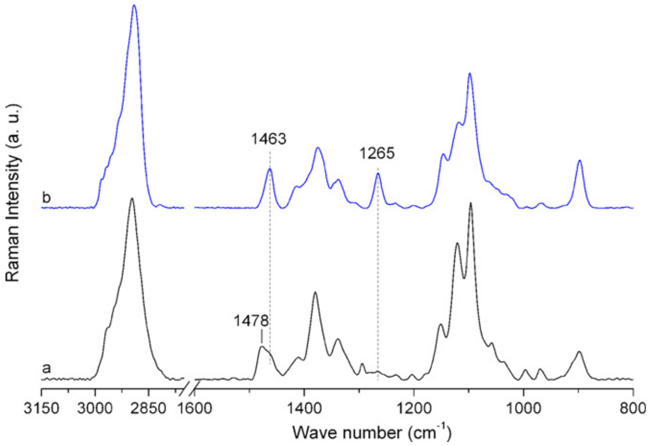


Fig. 2. FT Raman spectra (3150–800 cm^{-1}) of (a) native AC and (b) regenerated AC (from its NaOH/urea/water solution) at RT.

based on the conductometric titration curve (Fig. 1) and the DO was calculated according to:

$$DO = \frac{162 \times V_{NaOH} \times c \times t}{m - V_{NaOH} \times c \times t \times 36.973} \quad (1)$$

where V_{NaOH} is the used volume of aqueous NaOH solution in l, c is its concentration (mol/l) and m is the weight of dried sample in g.

2.5.2. Complexometric titration with EDTA

COC (0.1 g) was dissolved or suspended in 20 ml water at first. Then, 10 ml 1% aqueous $CuSO_4$ solution was added to the solution or suspension. After 30 min vigorous stirring, the formed complex was separated and washed with water until ion-free. Thereafter, the complex was suspended in 40 ml water and mixed with 5 ml 5% aqueous ammonia solution. After the complete dissolution of the complex, a mixture of murexide:NaCl (1:90) was added as indicator for the subsequent titration with 0.01 M aqueous EDTA solution. The titration ends with the colour changing from orange to purple. Based on the amount of EDTA, the DO was calculated according to:

$$DO = \frac{162 \times N_{COONa}}{0.1 - N_{COONa} \times 36.989} \quad (2)$$

$$N_{COONa} = 2 \times V_{EDTA} \times c \times f \quad (3)$$

where N_{COONa} is the molar amount of to be determined carboxylate groups, V_{EDTA} is the used volume of aqueous EDTA solution, c is its concentration and f is the factor of the aqueous EDTA solution.

2.6. Other measurements

The ^{13}C NMR spectra of the products in D_2O were recorded at RT on Bruker DFX 400 spectrometer (Bruker, Etlingen, Germany) with a frequency of 100.13 MHz, 30° pulse length, 0.35 acq. time and a relaxation delay of 3 s. Scans of up to 20,000 were accumulated.

FT Raman spectra of the samples in small aluminium discs were recorded on a Bruker MultiRam spectrometer (Bruker) with Ge diode as detector that is cooled with liquid-nitrogen. A cw-Nd:YAG-laser with an exciting line of 1064 nm was applied as light source for the excitation of Raman scattering. The spectra were recorded over a range of 3500–150 cm^{-1} using an operating spectral resolution of 3 cm^{-1} and a laser power output of 100 mW. A double analysis per 400 scans was carried out for each sample. An average Raman spectrum was formed afterwards and the spectrum was maximal–minimal–normalized as well as analyzed using the operating spectroscopy software OPUS Ver. 6.5 (Bruker).

3. Results and discussion

3.1. Dissolution and regeneration of cellulose

Cellulose I could be dissolved in NaOH/urea/water system and a transparent viscous solution was obtained. The regeneration of cellulose I from its solution leads to the formation of cellulose II. Fig. 2 demonstrates the FT Raman spectra of native pulp cellulose (with 97% alpha cellulose, AC) and regenerated AC. As depicted, the signal at 1478 cm^{-1} with a shoulder at 1456 cm^{-1} was shifted to a single band at 1463 cm^{-1}. Moreover, a new signal emerges at 1265 cm^{-1}. The presence of the bands at 1463 and 1265 cm^{-1} is characteristic for the cellulose allomorph II (Schenzel & Fischer, 2001). In addition, the peak maxima of the signals at 1096 and 1121 cm^{-1} shift slightly to 1098 and 1118 cm^{-1}, respectively. The intensities of these both bands became lower after the regeneration, while the intensity of the band at 897 cm^{-1} increased slightly.

The celluloses used in this report demonstrate distinct origins and molecular weights, but they were all converted into cellulose II after the regeneration from their NaOH/urea/water solutions.

3.2. Oxidation of regenerated cellulose

After the TEMPO or 4-acetamide-TEMPO-mediated oxidation of regenerated cellulose (cellulose II) in never-dried state, carboxylate groups were introduced into cellulose chains. Despite diverse starting celluloses and distinct combinations of the amounts of oxidizing agents, both water-soluble and -insoluble fractions were obtained as COC after the oxidation (Tables 1 and 2). The yields of water-soluble fractions after the TEMPO-mediated oxidation were maximal 20% and the majority of COC was found in water-insoluble fractions.

After the 4-acetamide-TEMPO-mediated oxidation at low temperatures of RT or 40 °C for up to 240 h, no water-soluble fraction was obtained. Also the oxidation at 60 °C for up to 24 h led to the preparation of water-soluble COC with yields less than 9%, as shown for COC9, 11 and 13 (Table 2). Only after prolonged oxidations at 60 °C, e.g. of 48 h, the water-soluble fractions with high yields could be obtained. Moreover, the water-soluble fraction of COC17 had a yield of 91.1% after a 96 h oxidation using high amounts of oxidizing agents (Table 2).

Fig. 3 depicts the ^{13}C NMR spectrum of water-soluble fraction of COC12. It is visible that the signal at 60 ppm ascribed to C6 was very weak, while a strong signal appears at 175 ppm. This signal is attributed to the carbons of carboxyl groups at C6 position (Hirota et al., 2010). Therefore, the carboxyl groups

Table 1
Oxidation of regenerated cellulose with TEMPO/NaBr/NaClO at pH 10–10.5 in water.

Samples	Starting cellulose	Molar ratio[a]			*T* (°C)/t (h)[b]	DO[c]	
		TEMPO	NaBr	NaClO		Water-soluble fraction	Water-insoluble fraction
COC1	AC	0.015	0.25	9	0/4	–	0.27
COC2	AC	0.03	0.5	5	0/2.25	0.68	0.23
COC3	AC	0.05	1.5	10	0/3	0.74	0.36
COC4	AC	0.017	0.26	13.3	22/6	0.85	0.08
COC5	MCC	0.025	0.75	10	22/5	0.75	0.25
COC6	MCC	0.03	0.5	10	22/2.75	0.66	0.2

[a] Molar ratio in mol per mol AGU of cellulose.
[b] *T* (°C)/t (h): oxidation temperature in °C and oxidation duration in hours.
[c] DO of water-insoluble fractions of COC1, 2, 4, 5 and 6 were determined via complexometric titration with EDTA; DO of water-soluble fraction of COC4 was determined via conductometric titration; and the other COC were determined via FT Raman spectroscopy.

Table 2
Oxidation of regenerated cellulose with 4-acetamide-TEMPO/NaClO/$NaClO_2$ at pH 6 and 60 °C in water.

Samples[a]	Starting cellulose	Molar ratio[b]			Oxidation duration (h)	Water-soluble fraction		Water-insoluble fraction	
		4-Acetamide-TEMPO	NaClO	$NaClO_2$		DO[c]	Yield	DO[c]	Yield
COC7	CF	0.025	0.2	2	240	–	0	0.34	100
COC8	CF	0.025	0.2	2	88	–	0	0.36	100
COC9	CF	0.03	0.2	3	24	0.55	<9	0.38	>91
COC10	CF	0.03	0.2	2.5	40	0.72	n.d.	0.44	n.d.
COC11	CF	0.05	0.3	3	22	0.68	<9	0.5	>91
COC12	CF	0.05	0.3	3.5	48	0.59	69	0.42	31
COC13	AC	0.03	0.2	2.5	24	0.7	9	0.42	91
COC14	AC	0.03	0.2	2.5	48	0.62	35.8	0.41	64.2
COC15	AC	0.03	0.2	2.5	72	0.71	42.1	0.5	57.9
COC16	AC	0.03	0.2	2.5	120	0.79	48.6	0.47	51.4
COC17	AC	0.05	0.3	3.5	96	0.84	91.1	0.56	8.9

[a] COC7 and 8 was prepared at RT and 40 °C, respectively.
[b] Molar ratio in mol per mol AGU of cellulose.
[c] DO of water-insoluble fractions of COC7–16 were determined via complexometric titration with EDTA; DO of water-soluble fractions of COC10, 12 and 15–17 were determined via conductometric titration; and the other COC were determined via FT Raman spectroscopy. n.d., not determined.

were regioselectively introduced into cellulose chains during the TEMPO or 4-acetamide-TEMPO-mediated oxidation. Besides, it was reported that low amounts of aldehyde groups were also formed during the TEMPO-mediated oxidation of cellulose (Saito & Isogai, 2004). In contrast, the 4-acetamide-TEMPO-mediated oxidation of cellulose using the $NaClO_2$ as oxidizing agent converted primary hydroxyl groups only into carboxyl groups (Hirota et al., 2009; Saito et al., 2009).

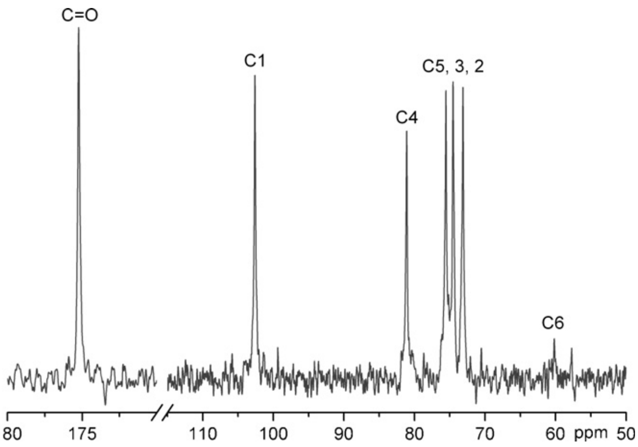


Fig. 3. ^{13}C NMR spectrum (180–50 ppm) of water-soluble fraction of COC12 in D_2O at RT.

3.3. Determination of DO

3.3.1. Conductometric and complexometric titration

The DO of oxidized celluloses can be determined via various methods, e.g. conductometric titration and methylene blue adsorption (Da Silva Perez et al., 2003; Isogai et al., 2009; Saito & Isogai, 2004). The conductometric titration is the most widely used method for the determination of DO. Moreover, the carboxylate groups could interact with Cu^{2+} cations and uptake the latter from their aqueous solution (Deans & Dixon, 1992). The Cu^{2+} cations could be titrated by complexometric titration with EDTA through forming water-insoluble complex between Cu^{2+} cations and EDTA (Dean, 1999).

Using the conductometric titration and complexometric titration with EDTA, the DO of a few obtained COC were determined and the results were listed in Table 3. It is notable that the DO lower than 0.6 could be analyzed with good consistence via both methods. However, the complexometric titration with EDTA could not deliver satisfying DO for the COC with a DO higher than 0.6. This is

Table 3
DO of COC determined by conductometric titration and complexometric titration with EDTA.

Samples	DO (complexometric titration with EDTA)	DO (conductometric titration)
COC2 (insoluble fraction)	0.23	0.23
COC15 (insoluble fraction)	0.5	0.5
COC12 (soluble fraction)	0.56	0.59
COC15 (soluble fraction)	0.65	0.71
COC16 (soluble fraction)	0.7	0.79
COC17 (soluble fraction)	0.7	0.84

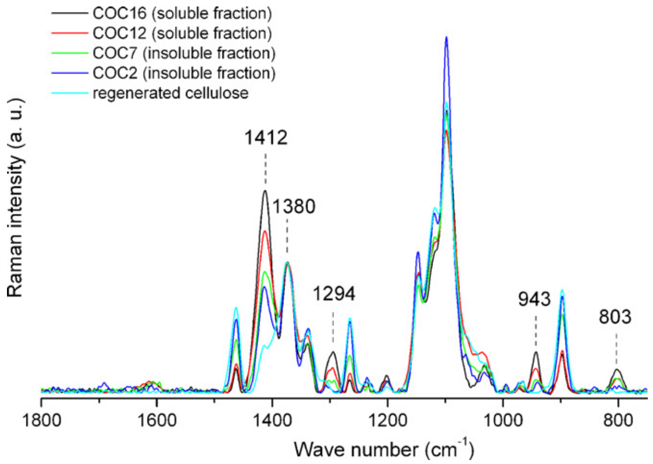


Fig. 4. FT Raman spectra (1800–750 cm^{-1}) of regenerated cellulose and four COC with distinct DO. The spectra were normalized on the peak at 1380 cm^{-1}.

possibly due to the limited accessibility of carboxylate groups for the complexation with Cu^{2+} cations. Consequently, a lower DO was determined by complexometric titration with EDTA in comparison to the conductometric titration.

Hence, the DO lower than 0.6 can be determined via both methods, while only conductometric titration provides reasonable values for COC with DO higher than 0.6. In particular, water-soluble COC should be analyzed via conductometric titration, because they normally exhibit high DO.

3.3.2. FT Raman spectroscopy

Fig. 4 depicts the FT Raman spectra of regenerated cellulose and obtained COC exhibiting distinct DO. Compared to regenerated cellulose, COC display a few modifications of Raman signals. Apart from the emergence of new bands and modifications of a few peaks, most of the bands ascribed to vibrations of cellulose backbone between 1500 and 800 cm^{-1} are still visible (Schenzel & Fischer, 2001).

The intensities of the peaks at 1463 and 897 cm^{-1} decrease with higher DO. Because the peak at 1463 cm^{-1} is attributed to stretching vibrations of CH_2 groups, its intensity should be reduced along with the conversion of CH_2OH into COO^- groups.

Moreover, new bands around 1610, at 1412, 1294, 943 and 803 cm^{-1} emerge within the FT Raman spectra of COC. These bands are attributed to vibrations of carboxylate groups and their intensities increase with rising DO of COC. Within the FT Raman spectrum of the insoluble fraction of COC2, a very small signal is notable at 1691 cm^{-1}, which is possibly attributable to the vibrations of aldehyde groups (Socrates, 2001). As reported before, COC contained aldehyde groups at C6 position in very low contents after the TEMPO-mediated oxidation of cellulose at pH 9–11 (Saito & Isogai, 2004).

As shown in Fig. 5, COC with COOH groups shows a new band at 1738 cm^{-1} in comparison to COC with COONa groups. This new band is ascribed to stretching vibrations of COOH groups (Saito et al., 2006; Socrates, 2001). On the other side, the intensity of the band at 1412 cm^{-1} within the FT Raman spectrum of COC containing COOH groups decreases. Furthermore, the bands at 1610, 1294 and 943 cm^{-1} disappear, while the positions of other bands stay constant. Hence, the bands at 1610, 1412, 1294 and 943 cm^{-1} should be attributed to vibrations of carboxylate groups (as sodium salt).

According to Figs. 4 and 5, the intensities of the bands ascribed to the vibrations of carboxylate groups increase with rising DO. Therefore, it is possible to establish novel analyzing method for DO based on the FT Raman spectra of obtained COC. Based on intensities or areas of Raman bands at 1412 or 1294 cm^{-1} attributed to carboxylate groups, Fits for the DO of COC could be generated. After plotting the Fits against DO of COC, calibration curves with high correlation coefficients of up to 0.9867 were obtained (Fig. 6). Thus, novel method for determining DO of COC can be established based on the characteristic Raman signals ascribed to vibrations of carboxylate groups. This method is rapid and non-destructive in comparison to other conventional methods, e.g. conductometric titration and solid-state CP/MAS ^{13}C NMR spectroscopy. In particular, this method requires only little amount of sample compared to other methods, which demonstrates an advantage for the COC obtained in low yields.

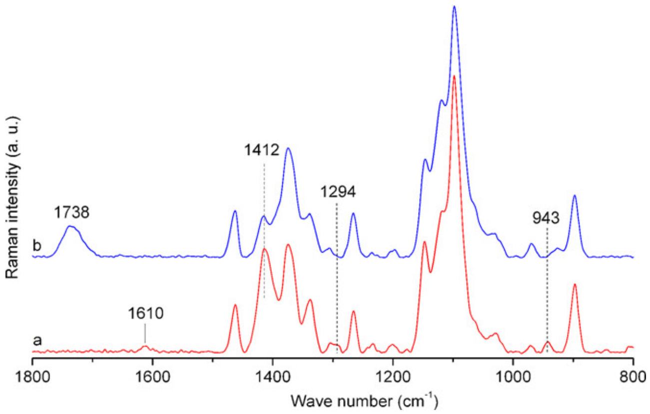


Fig. 5. FT Raman spectra (1800–800 cm^{-1}) of COC9 (insoluble fraction) with (a) carboxylate (COONa) groups and (b) carboxyl (COOH) groups at RT. The spectra were normalized on the peak at 1380 cm^{-1}.

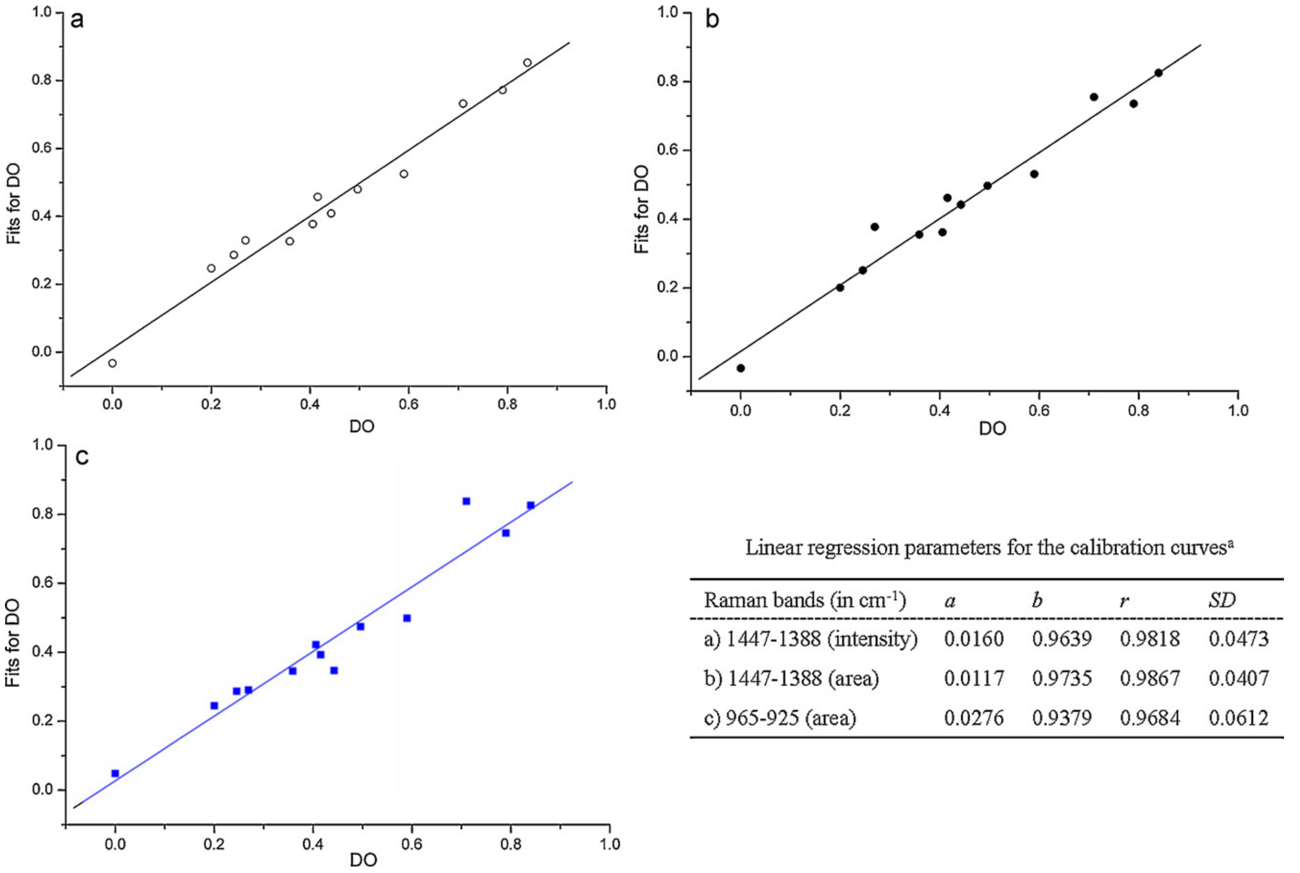
3.4. Effect of oxidation parameters on DO and yields of COC

After the oxidation, both water-soluble and -insoluble COC fractions exhibiting distinct DO were obtained (Da Silva Perez et al., 2003; Hirota et al., 2010; Isogai et al., 2009). Generally, the water-soluble COC show higher DO than water-insoluble COC (Tables 1 and 2).

According to Tables 1 and 2, the water-soluble COC normally have DO higher than 0.5, while the water-insoluble COC have DO lower than 0.6. The TEMPO-catalyzed oxidation needed only short oxidation durations of a few hours for the synthesis of water-soluble COC with high DO, while 4-acetamide-TEMPO-mediated oxidation required much longer oxidation durations. However, the yields of water-soluble fractions after the 4-acetamide-TEMPO-mediated oxidation are significantly higher.

Besides, the water-insoluble fraction of COC17 exhibits a DO of 0.56, which is even as high as the DO of some water-soluble fractions, e.g. COC9 and 12 having DO of 0.55 and 0.59, respectively. The COC17 was prepared from AC having an average DP of 1180, while COC9 and 12 were from CF with an average DP of 500. As reported before, the strong depolymerisation of cellulose chains could be avoided to some extent by the use of 4-acetamide-TEMPO/NaClO/$NaClO_2$ system at a pH lower than 7 (Hirota et al., 2009; Saito et al., 2009). After the oxidation, the water-insoluble fraction of COC17 may have a higher DP than the water-soluble fractions of COC9 and 12. Thus, their different water-solubilities may lie in their distinct molecular weights.

After the TEMPO-mediated oxidation, the water-soluble COC exhibits DO higher than 0.6, while the water-insoluble COC fraction demonstrates low DO of maximal 0.36. By using different combinations of the amounts of oxidizing agents, the DO of water-soluble fraction could be raised to 0.85. The highest one of 0.85 was



Linear regression parameters for the calibration curves^a

Raman bands (in cm^{-1})	*a*	*b*	*r*	*SD*
a) 1447-1388 (intensity)	0.0160	0.9639	0.9818	0.0473
b) 1447-1388 (area)	0.0117	0.9735	0.9867	0.0407
c) 965-925 (area)	0.0276	0.9379	0.9684	0.0612

Fig. 6. Calibration curves obtained after plotting Fits for DO against DO of COC using the intensities or areas of the bands at 1412 or 1294 cm^{-1} as analyzing parameters. ^{a} $Y = a + bX$; where Y is the Fit for DO obtained by Quant-Analysis function of used spectroscopic program based on the corresponding Raman bands, X is DO, a is the Y-intercept, b is the slope, r is the correlation coefficient, SD is the standard deviation, the significance level p is <0.0001 and the sample volume n is 13 for all three calibrations.

obtained after the oxidation using low amount of TEMPO and high amount of NaClO at RT. As previously revealed, higher amounts of NaClO could increase the amounts of carboxyl groups and reduce the amounts of aldehyde groups in COC (Saito & Isogai, 2004; Saito, Shibata, Isogai, Suguri, & Sumikawa, 2005). Therefore, the high amount of NaClO (13.3 mol per mol AGU of cellulose) promoted the TEMPO-mediated oxidation of cellulose. However, during the TEMPO-mediated oxidation of cellulose at pH 9–11, strong depolymerisation of cellulose chains takes place. The molecular weights of prepared COC were highly reduced in comparison to that of starting cellulose (Hirota et al., 2010; Saito & Isogai, 2004).

After the 4-acetamide-TEMPO-mediated oxidation at RT or 40 °C for up to 240 h, no water-soluble COC fraction was obtained. Only water-insoluble fractions of COC7 and 8 with DO of 0.34 or 0.36 were received. These COC formed gel-like liquids when dispersed in water. At 60 °C, water-soluble COC fraction with DO up to 0.7 was prepared after oxidation durations of at least 22 h. The DO and yield of water-soluble fractions increased with longer oxidation durations at 60 °C. Comparing COC13, 14, 15 and 16 which were prepared after different oxidation durations under other equal conditions, the yield of water-soluble COC-fraction increased with prolonged oxidation duration. The DO of these water-soluble COC rises also with longer oxidation duration, except the COC13 with the lowest yield on water-soluble fraction. Furthermore, the yield of water-insoluble COC-fractions decreased with rising oxidation duration and their DO lie between 0.4 and 0.5. After 120 h oxidation, water-soluble COC16 with a DO of 0.79 contributed to 48.6% of final products. Thus, much longer oxidation durations are necessary for the preparation of water-soluble COC with high yields by 4-acetamide-TEMPO-catalyzed oxidation.

Moreover, using higher amounts of 4-acetamide-TEMPO/NaClO/NaClO$_2$ after long oxidation duration of 96 h, water-soluble COC17 was obtained with the highest yield of 91.1%. Besides, water-soluble COC17 shows a high DO of 0.84. Thus, high amounts of oxidizing agents could promote the oxidation of primary hydroxyl groups of cellulose, leading to water-soluble COC with high yields. In particular, a higher amount of catalyst, 4-acetamide-TEMPO, significantly accelerated the oxidation of cellulose comparing the DO and yields of water-soluble COC16 and 17.

Hence, long oxidation durations (e.g. more than 48 h), high oxidation temperature (e.g. 60 °C) and also high amounts of oxidizing agents are important for the preparation of water-soluble COC with high DO and yields through 4-acetamide-TEMPO-catalyzed oxidation.

4. Conclusion

In this report, regenerated cellulose (cellulose II) in never-dried state was oxidized through TEMPO or 4-acetamide-TEMPO-catalyzed oxidation. After the oxidation, water-soluble as well as -insoluble fractions were obtained as COC. Water-soluble COC exhibited DO higher than 0.5 and water-insoluble COC showed DO lower than 0.6. Water-soluble COC with high DO of 0.84 and high yield of 91.1% could be prepared after 4-acetamide-TEMPO-mediated oxidation using high amounts of oxidizing agents at 60 °C for 96 h. The DO of COC could be determined via conductometric titration or complexometric titration with EDTA. The conductometric titration can be used for COC with low or high DO, while the complexometric titration with EDTA is only adequate for COC with DO lower than 0.6. FT Raman spectroscopy presents characteristic signals ascribed to the vibrations of carboxylate groups. Based on the intensities or areas of FT Raman signals at 1412 or 1294 cm^{-1}, novel rapid and non-destructive analyzing methods for DO with high correlation coefficients of up to 0.9867 were established.

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