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Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

EPR spectroscopy applied to the study of the TEMPO mediated oxidation of nanocellulose

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ARTICLE INFO

Article history: Received 7 May 2015 Received in revised form 24 September 2015 Accepted 25 September 2015 Available online 30 September 2015

Keywords: Cellulose nanocrystals TEMPO Conductimetry ESR EPR

ABSTRACT

Two different methods of pH control were used in the synthesis of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) oxidized cellulose nanocrystals (CNC) and the reaction kinetics and degree of oxidation were investigated. In method I the media pH was controlled by addition of NaOH solution. The effect of the oxidant concentration (sodium hypochloride, NaClO) on the final degree of oxidation and crystallinity of the samples was investigated. Conditions for obtaining an optimum balance between high crystallinity and degree of oxidation were selected from those results. In method II, pH was fixed by using a buffer solution. Electron spin resonance (ESR) spectroscopy offered direct information of the decay of TEMPO concentration under these conditions. The kinetics of the reaction was determined, finding a direct correlation between these results and those corresponding to the decay of the NaClO concentration and the advance of the CNC degree of oxidation. Differences found between the two methods were analyzed.

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1. Introduction

Among the organic polymers, cellulose, a carbohydrate polymer obtained from higher plants, tunicate, bacteria, and algae, is the most ubiquitous and abundant bioresource, which makes it very attractive from the view point of sustainable development.

Native cellulose is formed by β -(1,4)-D glucopyranose polymer chains that aggregate in crystals as a results of multiple intra and inter H bonding between the anhydro glucose units (AGU). Further aggregation into higher structures results in cellulose fibers of microscopic size. To fully benefit of the mechanical properties of crystalline cellulose, researchers have worked on the deconstruction of this hierarchical structure. Mechanical, enzymatic and chemical processes (or combination of those) have been applied to separate the cellulose nanofibrils that form microscopic bundles. Fibrils have lateral size in the order of nanometers and length in the order of microns and they contain crystalline and amorphous cellulosic regions. Further acidic attack degrades the amorphous regions to obtain highly crystalline rod (or needle)-like nanoparticles called cellulose nanocrystals, CNC.

tunicin, bacterial cellulose, etc.) often by strong acid hydrolysis. Acid treatments using concentrated H₂SO₄ solutions, produce stable suspensions of highly crystalline CNC (loelovich, 2012a, 2012b). Perhaps, one of the most common strategies for introducing carboxyl groups on the CNC surface is the oxidation of the superficial hydroxyls. This reaction can be carried out under mild conditions, at room temperature, using water as solvent, a primary oxidant, and catalytic amounts of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), as first reported by de Nooy et al. more than two decades ago (De Nooy, Besemer, & Van Bekkum, 1995; Zhang, Fischer, Geissler, & Brendler, 2012). This oxidation has become very common in recent years, as an interesting method for introducing carboxyl groups at the C6 positions of the AGU. The advantages of TEMPO-mediated oxidation are not only the mild conditions used, but also the high reaction yield, the high selectivity for the oxida-

tion of only the primary OH groups (Bailey, Bobbitt, & Wiberg, 2007;

De Nooy et al., 1995), and the modest requirements of the catalytic

Because of their dimensions (5–20 nm in lateral size, and hundreds of nm in length) and crystalline structure. CNC have high

mechanical properties, reaching very high values of the Young's

modulus. While cellulosic plant fibers have tensile modulus \sim 20 GPa, neat cellulose nanocrystals have modulus \sim 50–100 GPa,

closer to the theoretical value of crystalline cellulose \sim 150–200 GPa

(Habibi, Lucia, & Rojas, 2010). CNC are produced from vari-

ous biological sources (e.g., bleached wood pulp, cotton, manila,

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Fig. 1. Scheme of the reaction mechanism of the TEMPO-mediated oxidation.

Adapted from Da Silva Perez et al. (2003).

process that only ask for inexpensive NaClO, NaBr and NaOH, as well as catalytic quantities of TEMPO (Shinoda, Saito, Okita, & Isogai, 2012).

The mechanism of the reaction is fairly well understood: the oxidizing species TEMPO+, is mainly generated by the primary oxidant NaBrO, formed by the reaction between NaClO and NaBr, as briefly outlined in Fig. 1.

The oxoammonium ion, is then reduced to its hydroxylamine form (TEMPOH), and TEMPO is recycled in the system by TEM-POH oxidation or its comproportionation reaction with TEMPO+, reaction (1):

$$TEMPOH + TEMPO^+ \rightarrow 2TEMPO^{\bullet} + H^+$$
(1)

According to the literature (Gehmayr, Potthast, & Sixta, 2012; Isogai, Saito, & Fukuzumi, 2011; Okita, Saito, & Isogai, 2010; Saito, Shibata, Isogai, Suguri, & Sumikawa, 2005; Saito, Hirota, Tamura, & Isogai, 2010; Shinoda et al., 2012), the amount of functional groups introduced in the samples of TEMPO-oxidized cellulose has been determined by various different conventional analytical and instrumental techniques. These techniques provided an estimate of the overall content of carboxylic groups in the sample.

The use of TEMPO as a catalyst for the oxidation of cellulose is mainly based on the studies of the kinetics and mechanism of alcohols by oxoammonium salts in homogeneous systems (Bailey et al., 2007; Bragd, Besemer, & Van Bekkum, 2000). However, little is known about the actual conditions that warrant a good yield of oxidation under practical operational conditions.

In this article, we particularly analyze the effect of varying the concentration of the main oxidant on the kinetics, the degree of oxidation, and the crystallinity of the samples keeping a fixed ratio between cellulose and catalyst (2 wt.%). We also provide for the first time, a quantitative analysis of TEMPO evolution and consumption by ESR spectroscopy, also called electron paramagnetic resonance (EPR) spectroscopy.

2. Materials and methods

2.1. Materials

Microcrystalline cellulose powder was purchased from Sigma–Aldrich. Cellulose acid hydrolysis was carried out using sulfuric acid (98% w/v, Anedra). The dialysis of the crystals suspension was performed using Spectra/Por Standard RC dialysis tubing and bidistilled water. NaClO (as commercial bleach), NaBr (Timper, p.a.), a pH 10 buffer based on borates (Biopack) and 2,2,6,6-tetramethylpiperidine-1-oxyl, TEMPO (Aldrich, 98%) were used in the oxidation experiments. KI (Biopack, 98%) and $Na_2S_2O_3$ (Anedra) were used in the conductimetric titration.

2.2. Methods

2.2.1. Acid hydrolysis of microcrystalline cellulose (MCC)

Cellulose nanocrystals were obtained by acid hydrolysis, using a modified procedure of a previously reported technique (Habibi, Chanzy, & Vignon, 2006; loelovich, 2012b). Briefly, in order to obtain a homogeneous suspension, 30 g of MCC were added in 175 g of distilled water and dispersed by mechanical stirring, while the whole system was immersed in an ice bath. Afterwards, 325 g of concentrated sulfuric acid were slowly added (1 h) keeping the temperature below 20° C. Then, the mixture was thermostatized at 44° C and vigorously stirred during 2 h, to be further diluted with distilled water (1:4). The suspension was then dialyzed against distilled water for five days and then, against bidistilled water for two days. The nanocrystals obtained were filtered through a polyester cloth with pore size of 18 μ m. Concentrated suspensions were obtained by evaporation at *T* < 60° C with rotary evaporator.

2.2.2. Oxidation mediated with TEMPO

In order to obtain the oxidized nanocrystals, a 2.896% w/v suspension of CNC (10 mL, 1.788 mmol of AGU) was mixed with 10 ml of a TEMPO solution and 10 ml of a NaBr solution, both containing 0.3576 mmol (3.1 and 36.8 mg, respectively). Then, different samples were prepared by adding different volumes (2.3, 4.6, 9.2, 23 and 37 ml) of a solution of NaClO (14.416 g/l) to the previous mixture (Table 1). As shown in the scheme of reaction (Fig. 1), the product of the reaction is a carboxyl group, which results in the reduction of the pH as the reaction advances. Since the reaction proceeds in alkaline medium, the pH has to be maintained between 10 and 10.5. In this work, two methods were used to achieve this goal:

Method I consisted in maintaining the pH approximately constant, by adding aliquots of a NaOH solution (0.5 N) as required. In

Table 1	
Oxidation (conditions.

Sample	NaClO (mmol)	AGU:NaClO molar ratio
T1	0.447	1:0.25
T2	0.894	1:0.5
T3	1.788	1:1
T4	4.470	1:2.5
T5	7.152	1:4

the beginning of the reaction, the addition of aliquots was more frequent and the frequency decreased as the reaction advanced.

In experiments performed according to *method II*, a pH 10 buffer was used as solvent for NaClO, NaBr and TEMPO solutions, instead of distillated water, to maintain the pH approximately constant through all the reaction course. As it will be discussed in Section 3.2 this procedure allows the continuous monitoring of the TEMPO concentration.

The reaction carried out using method I was considered finished when the pH remained constant without requiring new additions of NaOH (for 1 min). Then, the mixture was taken to neutrality at pH 7 by adding HCl (0.5 N). Each sample was centrifuged to separate the solids (oxidized cellulose crystals) from the solution and it was washed twice with distilled water to eliminate the inorganic salts. Finally, it was freeze-dried.

2.2.3. Characterization

To determine the success of the oxidation, the dried samples were analyzed by FTIR (Bruker IFS 25 spectrophotometer) using transmission mode for pellets prepared by mixing minimum amounts of the samples in KBr powder, with a resolution of 4 cm^{-1} and averaging 64 scans.

To determine the crystallinity index, X-ray diffractograms of the samples were obtained with a X PANalytical X'Pert PRO (Cu(α) lamp), scanning from 2 to 60° at a scan velocity of 0.016°/s. Crystalline peaks 1–10, 110, 102, 200 and 004 appear at 2 θ : 14.6°, 16.8°, 20.2°, 22.7° and 34.7°, respectively (French, 2014; Park, Baker, Himmel, Parilla, & Johnson, 2010; Zhou, Fu, Zheng, & Zhan, 2012). The crystallinity index (CI) was calculated using the ratio of the area under the crystalline peaks to the total area in the range 10–36° (Marcovich, Reboredo, & Aranguren, 2001).

The degree of oxidation, DO, was calculated from conductimetric titrations. It is defined as the quantity of oxidized primary carbons divided by the total amount of glucose units (AGU). To obtain this value, the sample (0.1 g) was suspended in 50 ml of HCl 0.01 N and titrated with a solution of NaOH 0.1 N.

$$DO = \frac{162 \cdot V \cdot c}{m - V \cdot c \cdot 36} \tag{2}$$

In this equation, V is the volume of NaOH used (in liters), c is the concentration of the NaOH and m is the weighed mass of the sample (in grams) (Zhang et al., 2012).

2.2.4. ESR study

TEMPO is a stable free radical which can be readily quantified by EPR spectroscopy. Its spectrum consists on a triplet with a line splitting of 1.71 mT ascribed to the nitrogen hyperfine coupling constant. Electron paramagnetic resonance experiments were performed at 298 K with a Bruker ELEXSYS E500T spectrometer, operating at X band. Typical data acquisition parameters were used in the experiments: data points, 2048; central field, 360 mT; sweep width, 20 mT; scans 1–10; microwave power, 20.5 mW; modulation frequency, 100 kHz; sweep time, 60 s; modulation amplitude, 2 Gpp. Amplitude signals were transformed in radical concentrations by comparing the area under the EPR absorption spectrum with that of appropriate standards through a calibration curve recorded at the same microwave power (Brusa and Grela, 2003).

To correlate the evolution of TEMPO concentration with the DO, a sample ([NaClO] = 111.75 mM; [TEMPO] = 0.894 mM; [NaBr] = 8.94 mM) was prepared according to method II to maintain the pH approximately constant. The reaction was performed inside a quartz tube placed in the ESR cavity and the TEMPO concentration was directly monitored. Parallel experiments under the same conditions were performed to obtain the DO and the amount of NaClO consumed. The titration of the remaining NaClO was done by iodimetry, with the addition of an excess of KI used to stop the



Fig. 2. CNC crystals freeze-dried and resuspended in water. (a) Before oxidation at 1% w/v and (b) oxidized (T4) at 5% w/v. In the first case, part of the crystals precipitate, but in the second case, the suspension is stable for at least one month.

reaction. This excess was necessary to avoid the decomposition of NaClO that would occur at neutral pH.

3. Results and discussion

3.1. Optimization of oxidation conditions

The oxidation reactions were performed using Method I and the conditions of Table 1 in order to find the highest possible degree of oxidation, while still maintaining the crystallinity index of the sample. It is interesting to highlight the qualitative change of the behavior of the CNC before and after the oxidation. When a sample of untreated CNC is freeze-dried, resuspension in water to obtain a stable suspension becomes extremely difficult, even at low concentrations (1% w/v), as shown in Fig. 2(a), where part of the CNC is precipitated. However, once oxidized, the affinity of the treated CNC with water increases due to the appearance of the carboxyl groups, so that highly concentrated suspensions (5% w/v) can be obtained from previously freeze dried cellulose, and they are stable for several weeks as shown in Fig. 2(b). This is true for samples T3 and T4.

Fig. 3 compares the FTIR spectrum of CNC before oxidation with the spectra of the oxidized recovered solids corresponding



Fig. 3. FTIR spectra of (a) CNC before oxidation, (b) T1, (c) T4 and (d) the supernatant recovered from T5 (T5 soluble fraction).



Fig. 4. Diffraction patterns of (a) CNC before oxidation, (b) T4 and (c) T5.

to samples T1 and T4 and the supernatant recovered from the centrifugation of T5 (soluble fraction). The spectra corresponding to T2 and T3, not shown for simplicity, are similar to the spectra of T1 and T4, respectively.

The peak at $1730 \,\mathrm{cm}^{-1}$ that appears in the spectrum of T4 corresponds to the carboxyl absorption in its acid form (H-bonded carboxyl). Since at the end of the oxidation reaction, HCl is added until reaching pH 7, protonation of the carboxylate group occurs, which leads to the appearance of the 1730 cm⁻¹ band (Azzam, Heux, Putaux, & Jean, 2010; Jin, Edler, Marken, & Scott, 2014). This peak is absent in the spectra of the not oxidized CNC, but also in the solid recovered by centrifugation of the samples T1, T2 and T5 (solid phase) (the last two not shown). In the cases of T1 and T2, the conversion is too low, so that the generated carboxyl groups can not to be identified by FTIR. On the other hand, in the case of the T5 sample, the reaction conditions are so strong, that not only the surficial OHs are oxidized, but the CNC bonds are broken, and the oxidant has access to all the cellulose chains so the crystallinity is lost and thus cellulose becomes dissolved in the reaction medium. The solid separated by centrifugation, is not oxidized or the degree of oxidation is too low to be detected by FTIR. However, the supernatant contains the dissolved cellulose, which is the oxidized product of the reaction, and its FTIR spectrum corresponds to that of oxidized cellulose, as proven by the presence of the peak at 1730 cm⁻¹ in the spectrum (d) of Fig. 3. Other peaks are characteristic of the COOH group, as the broad one between 1440 and 1375 cm⁻¹ due to a combination of the C–O stretching and O–H deformation vibrations, although it appears overlapped to other characteristic bands of CNC and can be overlooked because of its weak nature (Socrates, 2004). The FTIR spectrum of T4, shows a decrease of the band at 1050–1015 cm⁻¹ corresponding to the absorption of primary alcohols, while there is an increase at 955–915 cm⁻¹, due to OH from the carboxylic acid (Schwanninger, Rodrigues, Pereira, & Hinterstoisser, 2004).

X-ray diffraction patterns for the original CNC and the oxidized CNC were obtained (T3, T4 and T5 solids) and are shown in Fig. 4. For simplicity, T3 is omitted, since its pattern is similar to that of T4. The calculated crystallinity indexes of the samples are reported in Table 2.

The destruction of the crystals in the conditions of T5, already commented in the discussion of the FTIR results, is in agreement with the diffractogram, showing essentially the amorphous halo.

Table 2Cristalinity indexes of the samples.

Sample	% CI	
CNC	65.5	
T3	69.9	
T4	78.8	

Table 3	
Crystal size of CNC, T3 and T4.	
	-

Sample	Crystal size (Å)
CNC	46.28
T3	40.31
T4	40.74

There is a shift of the maximum of the 200 peak, from 22.7° for the CNC to 23.2° and 23° for the T3 and T4 samples, respectively. This indicates that the distance between these planes has decreased during the reaction probably due to stronger inter-chain hydrogen bonding (Cullity & Stock, 2001). Also, the peaks are broader in the oxidized samples, suggesting a decrease on the size of the crystals, which can be calculated using Scherrer Eq. (3)

$$d = \frac{0.9\lambda}{\cos(\theta) \cdot \beta} \tag{3}$$

where β is the line broadening at half the maximum intensity, λ the X-ray wavelength (1.54 Å) and θ the Bragg angle (Cullity & Stock, 2001). The results obtained are shown in Table 3.

The degree of oxidation can be determined by conductimetric titration and using Eq. (2). The curves obtained show three zones (Fig. 5). The first one, before point "a", with an important decrease of conductivity, corresponds to the neutralization of the HCl. In the second zone, between points "a" and "b", the conductivity remains theoretically constant due to the neutralization of the oxidized cellulose (this zone is not detected when performing the titration on not oxidized CNC (Araki, Wada, Kuga, & Okano, 1998; Gu, Catchmark, Kaiser, & Archibald, 2013; Zhang et al., 2012)). The different behavior observed in these two regions is related to the fact that HCl is a strong acid, while oxidized cellulose is weak. Finally, in the third zone, after point "b", the conductivity increases markedly because of the excess of NaOH added. The volume used to titrate the oxidized cellulose can be calculated from the volumes that correspond to the limits of the three regions of the curve, $V_b - V_a$. The



Fig. 5. Conductimetric titration of the oxidized CNC. The symbols correspond to the experimental values and the straight lines to the best least squares regression.



Fig. 6. EPR spectra of TEMPO before and after the oxidation.

degree of oxidation calculated by this method is 26.60% and 19.83% for the samples T4 and T3, respectively (1.603 and 1.202 mmol of COOH per gram of CNC). Both results are in the range of values reported in the literature (Da Silva Perez, Montanari, & Vignon, 2003).

From the results shown above, we concluded that the conditions used for T4 sample are the best for obtaining a high degree of oxidation and crystallinity. Thus, in the following experiments we used these reaction conditions and investigate the efficiency of the catalyzed reaction following the evolution of the TEMPO concentration by EPR spectroscopy.

3.2. Effective reaction-chain length using method I

An ideal catalyst should have an infinite turnover number. However, side reactions and practical limitations often hamper this condition. Thus, controlling the effective reaction-chain length under operational conditions is of main importance for planning an industrial process. We define *reaction-chain length* as the number of times each molecule of TEMPO reacts with cellulose producing the oxidized CNC. For this purpose two different experiments were performed.

The first one consists on determining the concentration of TEMPO before and after the oxidation process by EPR (Fig. 6). Concentrations of TEMPO were determined as discussed in the experimental section by calculating the area below the absorption curve. By this procedure we evaluate that 0.37 mM of TEMPO are depleted during the oxidation reaction. It is interesting to compare this value with the amount of [COOH] equivalents formed, which can be readily obtained as the product between DO and the initial [AGU] (11.18 mM). This means that each TEMPO molecule produces at least 30 carboxyl groups, indicating a considerable reaction-chain length.

3.3. ESR study of the oxidation using method II

It is interesting to notice that, when using method I (the one commonly reported in the literature) although desirable, the evolution of TEMPO during the course of the reaction cannot be followed, because the pH needs to be continuously adjusted by the addition of NaOH accordingly to method I as described in Section 2.2.4. pH abrupt variations affect the concentration of TEMPO due to the reaction between TEMPO and hydroxyl ions.

$$2\text{TEMPO}^+ + 2\text{OH}^- \rightarrow \text{H}_2\text{O}_2 + 2\text{TEMPO}^{\bullet}$$
(4)



Fig. 7. Experimental (black squared dots) and fitted [TEMPO] decay (red continuous curve) and experimental (blue circular dots) and fitted %DO (green dashed curve) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

As an alternative, the reaction was carried out under buffered conditions using method II, to ensure a constant pH. Thus, we performed an experiment using the same conditions as for sample T4, ([TEMPO] = 0.894 mM, [NaClO] = 111.75 mM, [NaBr] = 8.94 mM) but, this time all solutions were prepared in the presence of a buffer instead of distillated water, as explained in the experimental section. The results obtained are shown in Fig. 7. The values of [TEMPO] were obtained by directly monitoring the spectra of a sample reacting inside the EPR cavity. The results could be fitted with an exponential decay function,

$$C = A \cdot \exp\left(-\frac{t}{t_0}\right) \tag{5}$$

with $t_0 = 202.4 \, \text{s}$.

Parallel experiments allowed us to quantify the degree of oxidation as already explained, by conductimetric titration, as shown in Fig. 7.

It is interesting to observe that the advance in the DO can be nicely reproduced using Eq. (6),

$$DO = DO_f \left[1 - \exp\left(-\frac{t}{t_0}\right) \right]$$
(6)

and the same phenomenological constant derived previously $(t_0 = 202.4 \text{ s})$, confirming the EPR results and the correlation between the two observations. This is shown in the green dashed line in Fig. 7.

Also, a similar equation can describe the decay of the NaClO concentration, once again, using the same phenomenological constant.

It is apparent that DO% diminishes from 26.60% (1.603 mmol of COOH per gram of CNC) in pure water to 10% (0.612 mmol of COOH per gram of CNC) in the presence of the buffer. The difference is assigned to the adsorption of the borates present in the buffer. As shown in Fig. 8, the FTIR spectra of the CNC, the cellulose oxidized by method I and that oxidized in presence of the buffer (method II), show some differences. The peaks at 1359 and 1400 cm⁻¹ are assigned to B–O stretching (Socrates, 2004; Tamayo, Rubio, Rodríguez, Rubio, & Oteo, 2009).

The oxidized sample obtained in presence of the buffer was washed several times with distilled water before obtaining the spectra, so the borates that remain in the samples should be



Fig. 8. FTIR spectra of not oxidized CNC (a) and oxidized CNC using method I (b) and method II (c).

strongly adsorbed, probably in the same way that the sulfates that are attached to the CNC after the acid hydrolysis. The content of borates is probably high enough to diminish the activity of the hydroxyl groups, because the borate groups are presumably adsorbed onto or near them, generating some steric impediment for further reaction.

4. Conclusion

The reaction conditions of the TEMPO mediated oxidation of CNC were optimized, and thus concentration of reagents and time of reaction were selected to achieve nanocrystals with high crystallinity and high degree of oxidation (conditions used to obtain the sample T4). ESR spectroscopy proved to be a useful analytical tool that allowed quantifying for the first time the catalytic activity of TEMPO, resulting in a significant reaction-chain length (30 and 8 oxidation steps per TEMPO consumed, for method I and II, respectively). The measurement of the efficiency of this catalyst is important for the green chemistry involved, allowing the use of a minimum quantity of reactants. The use of buffered conditions allows, for the first time, the continuous measurement of the TEMPO concentration. The results of the study confirm that the rate of decay of the TEMPO concentration can be related to the decay of the NaClO concentration and to the advance of the degree of oxidation (DO) of the cellulose nanocrystals, showing that the phenomenological kinetics of the reaction corresponds to a first order model. The experimental kinetic constant, is the same for the modeling of the changes of the concentrations of NaClO and TEMPO, as well as for the increase of %DO.

Acknowledgments

The authors thank for the financial support to National Research Council of Republic Argentina (CONICET), the Science and Technology National Promotion Agency (ANPCyT) and the National University of Mar del Plata (UNMdP).

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