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Rate-limiting Steps in Bromide-free TEMPO-mediated Oxidation of Cellulose – Quantification of the N-Oxoammonium Cation by Iodometric Titration and UV-Vis Spectroscopy

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ABSTRACT

A iodometric titration method was introduced to study the conversion of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) to the corresponding N-oxoammonium cation (TEMPO⁺) by hypochlorite in the absence and presence of bromide ion. The validity of the titration was verified with UV-Vis spectroscopy combined with a multivariate curve resolution (MCR) algorithm to calculate the concentrations and spectral signatures of the pure components (i.e. TEMPO, Cl(+1) and TEMPO⁺).

37 The formation of the oxoammonium cation was successfully followed during the activation of
38 TEMPO by HOCl and HOBr. It was found that HOBr is a more effective activator for TEMPO than
39 HOCl is. Moreover, the importance of a separate activation step for TEMPO with bromide-free
40 TEMPO oxidations could be identified with this titration method. The content of TEMPO⁺ was also
41 monitored during the TEMPO-mediated oxidation of a cellulosic pulp by hypochlorite in the absence
42 and presence of bromide. It was found that the oxidation of the alcoholic groups by TEMPO⁺ was
43 generally the rate-determining step and much slower than the regeneration of TEMPO⁺ through
44 oxidation of the hydroxylamine by HOCl and HOBr. However, at high pH the latter reaction became
45 rate-limiting.

46

47 **Keywords:** birch pulp, iodometric titration, multivariate curve resolution, oxoammonium cation,
48 TEMPO-mediated oxidation, UV-Vis spectrometry

49 1. Introduction

50 The chemistry of catalytic oxidation of alcohols with oxoammonium ion in aqueous media was
51 reported decades ago [1-4]. The fast and selective oxidation of primary alcohols to carboxylates via
52 aldehydes by oxoammonium ion was introduced later [5]. More recently, this method was applied
53 to the oxidation of cellulose [6] and to the preparation of nanofibrillated cellulose (NFC) [7, 8].
54 Altogether, this oxidation has gained a vast research interest [9-21]. The TEMPO-mediated oxidation
55 reduces drastically the energy consumption during the pulp disintegration to NFC, which is a
56 transparent gel consisting of individual cellulose microfibrils and microfibril bundles. The energy
57 consumption of the nanocellulose production via a TEMPO-mediated oxidation can be reduced from
58 700-1400 MJ kg⁻¹ (microfibrillated cellulose (MFC) process with a high pressure homogenizer
59 treatment) to less than 7 MJ kg⁻¹ (TEMPO-oxidized NFC) [22]. NFC can be utilized, for example, as a
60 reinforcing agent in composites [22, 23]. Other applications of NCF include gas-barrier films,
61 electronics, cosmetics, and flame-resistant materials [22].

62 The most widely studied TEMPO-oxidation process is based on the use of the NaBr/TEMPO/NaOCl
63 system. The pH level during this oxidation is typically 10 or higher [10, 12, 13, 19, 22]. The amount
64 of bromide is commonly higher than the amount of TEMPO (mass ratio 10:1) during the oxidation
65 [7]. Moreover, some TEMPO-oxidation methods without the application of bromide have been
66 reported in the literature [20, 24]. For example, acid-neutral conditions (pH 3.5-6.8) have been
67 applied to oxidize regenerated cellulose applying a TEMPO/NaOCl/NaClO₂ procedure without the
68 use of bromide [24]. With this low pH range, high carboxylate contents for the oxidized pulps (4
69 mmol COOH / g) have been reported after an extended reaction time of almost 80 h. In addition, an
70 electro-mediated oxidation has been used to oxidize the primary hydroxyl groups of cellulose to
71 carboxylates without the addition of either NaOCl or NaBr. In this oxidation, TEMPO or a TEMPO

72 derivative is used with an amperometric system for the oxidation of the pulp. However, achieving a
 73 carboxylate content of 1 mmol g⁻¹ of pulp requires a 48 hours oxidation, which indicates an
 74 extremely low reaction rate [20]. As a conclusion, the TEMPO-mediated oxidations without bromide
 75 are slower compared to the ones that apply bromide. However, a process without bromide would
 76 be of interest since its presence in the waste water streams is highly undesired [11].

77

78 **Scheme 1.** Modified activation mechanism of TEMPO radical by HOX [11].

79 The role of bromide as a radical TEMPO activator has not yet been clearly elaborated. The lack of an
 80 analysis method for TEMPO⁺ impedes the study of the catalytic cycle of the TEMPO-mediated
 81 oxidation. On one hand, it has been proposed that the primary oxidant, e.g. NaOBr when NaOCl is
 82 present (Scheme 1), oxidizes the hydroxyl amine to oxidized TEMPO via a radical intermediate [12,
 83 25]. On the other hand, NaOCl has been proposed to activate TEMPO at pH 10 prior to the
 84 NaOBr/TEMPO oxidation without a radical TEMPO intermediate [22]. Both NaClO₂ and NaOCl have
 85 been proposed as TEMPO activators during the TEMPO oxidation under the low pH conditions (pH
 86 3.5-6.8) [21, 24, 26]. HOBr has been proposed to be the activator for TEMPO during the
 87 NaBr/TEMPO/NaOCl oxidation at pH 10.8 where the formation of HOBr in the presence of ClO⁻ and
 88 Br⁻ promotes the conversion of TEMPO to TEMPO⁺ in a reaction which is similar to the one shown
 89 in Scheme 1 [14]. A similar reaction was proposed earlier by de Nooy et al. [9].

90 The pK_a values of HOCl (7.5) and HOBr (8.7) define the applicable pH level during the TEMPO
 91 activation. At pH > pK_a the hypohalous acids exist increasingly as hypohalites that are inactive in the
 92 catalytic oxidation. Accordingly, the NaBr/TEMPO/NaOCl oxidations take place under higher pH
 93 level than the NaOCl/TEMPO oxidations [11]. The complexity of the system and the number of the
 94 reactions involved are substantial when both HOCl and HOBr are present in the solution [27].
 95 Interestingly, the NaBr/TEMPO/NaOCl (pH 10) oxidation of starch without a separate TEMPO
 96 activation step is reported to be three times faster than the NaOCl/TEMPO oxidation [11]. However,
 97 the reaction rates of the initial conversion of TEMPO to TEMPO⁺ and its catalytic reaction with starch
 98 were not studied separately [11].

99 Several reaction paths have been proposed for the TEMPO-mediated oxidation of the primary and
 100 secondary hydroxyl groups. However, some of the elementary reactions, e.g. the conversion of the
 101 hydroxylamine (TEMPOH) to TEMPO⁺ and the initial conversion of TEMPO to TEMPO⁺, are lacking
 102 plausible reaction mechanisms. Scheme 2 summarizes the most commonly proposed reaction
 103 routes and mechanisms published so far. The conversion of TEMPO to TEMPO⁺ (A→C) has been
 104 quite often left without explanation in the early studies [4, 5]. Nevertheless, some routes for this
 105 conversion have been proposed, even though the reaction mechanisms have not been addressed.

TEMPO⁺ has been proposed to form during a reaction between CuCl₂ and TEMPO [3]. Similarly, the formation of TEMPO⁺ during a reaction between Br(+1) and TEMPO [14, 18, 25, 28] as well as between Cl(+1) and TEMPO [11, 16, 24, 26, 29] or Cl₂ and TEMPO [30] have been reported in numerous studies. In addition, the conversion of TEMPO to TEMPO⁺ has been proposed to take place during a reaction with NaClO₂ under neutral and acidic conditions [26] and during a reaction between ClO₂ and TEMPO via a transition complex intermediate [31, 32]. Despite the vast research interest on the TEMPO-mediated oxidation, the reaction mechanisms for the formation of TEMPO⁺ still remain unrevealed.

114

Scheme 2. Proposed reaction routes for TEMPO-mediated oxidation of primary and secondary alcohols.

The TEMPO-mediated oxidation of alcoholic groups has been suggested to proceed via an A→C→F→H route under alkaline conditions and via an A→C→E→H route under acidic conditions,

118

119 where the formation of a reactive complex (E, F) is generated by a nucleophilic attack of an
120 alcoholate anion on the nitrogen atom of TEMPO⁺ [5, 10, 12, 22, 33, 34]. Additionally, an
121 A→B→D→H route has been proposed to occur under alkaline conditions, where the formation of
122 a reactive complex (D) is generated by a nucleophilic attack of an alcoholate anion on the oxygen
123 atom of TEMPO [34]. Moreover, hydroxylamine has been proposed to shift to the protonated
124 hydroxylamine H→G under low pH [12, 35]. Furthermore, the conversion of TEMPOH to TEMPO⁺
125 (H→C) is proposed to take place under alkaline conditions with [7, 12, 36] and without [11, 22, 26,
126 29, 37-39] the radical intermediate by a reaction with X(+1) or by applying an amperometric system.

127 For the present, only few elementary reaction rate constants for the TEMPO-mediated oxidation
128 process have been determined. Some kinetic studies have been published with primary and
129 secondary alcohols. The equilibrium constant (K) for the formation of a complex (F) with MeO⁻ and
130 TEMPO⁺ ($1.3 \times 10^{13} \text{ M}^{-1}$) is reported to be 10^6 times larger than that for the formation of the
131 corresponding *i*-PrO⁻ complex ($1.1 \times 10^7 \text{ M}^{-1}$) [34]. In addition, the rate constants for the reactions
132 between aldehydes / alcohols and TEMPO⁺ under alkaline conditions have been studied [9]. Those
133 reactions were followed by monitoring the consumption of hypochlorite [9]. However, measuring
134 hypochlorite consumption does not allow differentiating between the reaction of TEMPO⁺ with the
135 alcohol and the conversion of TEMPOH to TEMPO⁺. Thus specific quantification of TEMPO⁺ would
136 be useful for the determination of the rate constants for the individual reactions in the catalytic
137 cycle. In addition, it is obvious that the mechanism of the conversion of TEMPO or TEMPOH to
138 TEMPO⁺ is not clearly defined due to the rival reaction routes which have been proposed in the
139 earlier studies. Therefore, a method for the determination of TEMPO⁺ would be a useful tool within
140 this field of research.

141 In this study, we introduce a iodometric titration method to quantitatively monitor TEMPO⁺ during
142 TEMPO-mediated oxidation of alcohols. The validity of the iodometric titration method is verified
143 with UV-Vis absorption spectroscopy by fitting with the spectra of the pure components, i.e. TEMPO,
144 Cl(+1) and TEMPO⁺, as calculated by a multivariate curve resolution algorithm (MCR). Moreover, the
145 method with UV-Vis spectroscopy and MCR algorithm can be applied separately to quantify both
146 TEMPO⁺ and TEMPO. The iodometric titration method [1, 2, 30, 40] is applied to study a separate
147 activation step of TEMPO prior to the TEMPO-mediated oxidation. Moreover, the role of HOX (e.g.
148 HOCl and HOBr) as an activator of TEMPO is studied by the titration method. Finally, the TEMPO-
149 mediated oxidation with a separate activation step of TEMPO and the titration method to determine
150 TEMPO⁺ are applied during an oxidation of a cellulosic pulp, a process commonly applied for the
151 preparation of NFC.

152 2. Experimental

153 2.1. Materials

154 Industrially dried fully-bleached birch kraft pulp (Finland), fully-bleached eucalyptus kraft pulp
155 (Brazil) and eucalyptus prehydrolysis kraft pulp (Brazil) were used as the raw materials for the
156 TEMPO-mediated oxidation of cellulose. Xylitol (Sigma Aldrich (St Louis)) was used as the raw
157 material for the TEMPO-mediated oxidation of alcohol. TEMPO (Sigma Aldrich (St Louis)) was used
158 as a catalyst. A 13 % NaOCl solution (Merck (Darmstadt, Germany)) was the primary oxidant in the
159 TEMPO-oxidations. 22 g of ortho-boric acid (VWR (Leuven, Belgium)) and 1.8 g of NaOH pellets (VWR
160 (Leuven, Belgium)) were diluted to 2000 ml of distilled water to prepare a borate buffer (pH 8.3) in
161 situ. 1 M NaOH (Merck (Darmstadt, Germany)), 1 M HCl (Merck (Darmstadt, Germany)), Büchi
162 reactor (volume 1.6 dm³), and Metrohm 718 Stat Titrino titrator with pH adjustment were applied
163 during the pulp oxidations. UV-Vis absorption spectra were measured with a Shimadzu UV-2550
164 spectrophotometer (Shimadzu Corporation (Kyoto, Japan)). Ion-exchanged water was used in pulp
165 washings.

166 2.2. Activation of TEMPO by NaOCl

167 NaOCl and TEMPO were mixed in a buffer solution (pH 8.3) at room temperature. The consumption
168 of Cl(+1) and the formation of TEMPO⁺ were monitored by iodometric titration. In parallel
169 experiments absorption spectra of the samples withdrawn were measured in the UV-visible region
170 with a Shimadzu UV-2550 spectrophotometer. Reference spectra of the buffer, 2 mM TEMPO in the
171 buffer and 4 mM NaOCl were also measured. All solutions were diluted with the buffer solution in
172 a 1:5 ratio prior to the measurements. The concentrations of Cl(+1), TEMPO and TEMPO⁺ were
173 obtained through mathematical analysis of the spectra.

174

175

176 2.3. Oxidations of the cellulose pulps and xylitol

177 All oxidations were carried out in a Büchi glass reactor (1.6 dm³) at 25 °C. The chemical dosages and
178 the consistency of the pulp suspension were varied while its volume was 1.2 dm³ in all experiments.
179 Radical TEMPO was mixed with a stoichiometric excess of NaOCl in water. The pH level of the
180 solution was adjusted to 7.5 with sulfuric acid. The solution was mixed in a closed vessel until TEMPO
181 was completely dissolved and converted to TEMPO⁺ by HOCl. The pulp and the activated TEMPO
182 solution were mixed and NaOCl was added to the closed reactor by a pump. After the addition of
183 NaOCl, pH was kept constant at the target level by adding 1 M NaOH with an automatic titrator. The
184 oxidation rate was followed by iodometric titration until all oxidant was consumed.

185 Xylitol was oxidized correspondingly excluding the adjustment of oxidation solution pH with borate
186 buffer to 8.3. Automatic titrator was not used for pH adjustment after NaOCl addition due to
187 buffered conditions.

188 2.4. Conversion of residual aldehydes to carboxylates with chlorous acid

189 TEMPO-oxidized pulp suspension was acidified to pH 3 with HCl and NaClO₂ Was added (10 mM
190 initial concentration). The suspension was mixed in the Büchi reactor for 2h at 50 °C. Finally, the
191 pulp was washed with pure water. CED-viscosity of the pulp was analyzed according to the standard
192 method SCAN-CM 15.99 prior to the calculation of DP [41].

193 2.5. Analyzing of carboxylate and aldehyde contents of pulps

194 The carboxylate content of the pulps were determined by conductometric titration (SCAN-CM
195 65:02) using Methrohm 751 GPD Titrino automatic titrator and Tiamo 1.2.1. software. The aldehyde
196 contents of the pulps were calculated as the difference in their carboxylate contents after and
197 before the post-oxidation with HClO₂.

198

199 2.6. Iodometric titration of Cl(+1), Br(+1)) and TEMPO⁺

200 The applied titration sequence is based on the method of Wartiovaara [42] excluding the analysis
201 of TEMPO⁺. Wartiovaara describes a three point titration of ClO₂ and hypochlorite at pH 8.3, further
202 titration of chlorite at pH below 2, and finally, the titration of chlorate at pH below 1. Only the
203 alkaline (pH 8.3) titrations to determine the formed TEMPO⁺ and Cl(+1) were conducted. The
204 liberated iodine was titrated against Na₂S₂O₃ using starch as an indicator. The titration of iodine with
205 sodium thiosulfate is based on the following reaction (Eq 1):

207 First, 25 ml of a borate buffer (pH 8.3) was added to two sample containers. Then, 0.5 ml of DMSO,
208 which can be used as a masking agent for HOCl and HOBr, was added to one of the sample
209 containers [43-47]. A known amount of the sample solutions together with an excess amount of KI
210 was added to both of the sample containers. When bromine was present, the solution required
211 stabilization for a few minutes prior to the addition of KI, since HOBr is trapped by DMSO more
212 slowly than HOCl. All samples were titrated against Na₂S₂O₃ using starch as an indicator. The
213 following reactions (Eq. 2 and Eq. 3) occur in the mildly alkaline medium:

216 The DMSO containing sample includes only the reaction product (iodine) with TEMPO⁺, since HOCl
217 (or HOBr) is trapped with DMSO. Iodide reduces TEMPO⁺ to TEMPO radical [1, 2, 30, 40]. Thus the
218 thiosulphate consumption corresponds stoichiometrically to the amount of TEMPO⁺ in the sample
219 (Eq. 1 and Eq. 3).

220 2.8. UV-Vis absorption spectroscopy of TEMPO/NaOCl/buffer solutions

NaOCl/TEMPO mixtures in a buffer solution (pH 8.3) were prepared and measured with a Shimadzu UV-2550 spectrophotometer correspondingly to the ones which were applied in the titrations. 2 mM TEMPO and 4 mM NaOCl solutions in the buffer media and the buffer solution without any added chemicals were measured as reference samples. All solutions were diluted with a ratio of 1/5 by the buffer solution prior to the measurements.

2.9. Mathematical reconstruction of pure spectral components from UV-Vis data

First, the range of linear response in the UV-Vis measurements was identified by looking at the singularities in their loading factors from Principal Component Analysis (PCA) [48]; the range was assumed to be TEMPO, TEMPO⁺, HOCl/OCl mixture (which is referred to as Cl(+1)) and a constant background consisting of a baseline and absorption by the buffer. A number of varying components equal to three was also confirmed by PCA: the first three factors explain over 99% of data variance (for data at 200-400 nm). The background, derived from the spectrum of a buffer-only solution, was subtracted from all spectra. The millimolar spectra of pure TEMPO and Cl(+1) were taken from spectra of the respective substance in buffer, after subtracting the background and dividing for the concentration. The spectrum of TEMPO⁺ was reconstructed by an algorithm, inspired by the Multivariate Curve Resolution-Alternate Least Squares method [49], which recursively alternates least-squares fitting of the bilinear model $\mathbf{D} = \mathbf{CS} + \mathbf{E}$ and the application of constraints to the obtained solution. $\mathbf{D}$ is the $n \times w$ matrix of UV-Vis measurements (n = number of measurements, w = number of wavechannels), $\mathbf{C}$ is the $n \times p$ matrix of concentrations in mmol/l (p = number of independent components, here equal to 3), $\mathbf{S}$ is the $p \times w$ matrix of spectra of pure components and $\mathbf{E}$ accounts for noise. The constraints included non-negativity of absorbances and concentrations, stoichiometric balances and monotonic decrease of reactants; they were applied by substituting any absorption or concentration that violated these constraints with the closest admissible value. The initial estimate for the spectrum of 1 mM TEMPO⁺ was taken as the spectrum from the latest point (332 minutes) in the reaction mixture, subtracted by the background, divided by 1.8 (i.e. 90% of the initial 1 mM TEMPO) and again subtracted by the spectrum of 1 mM Cl(+1). The algorithm was iterated until convergence and the final spectra were used to obtain the concentrations of the corresponding substances by linear least-squares fit. All calculations were performed using MATLAB® version 8.2 R2014a (The Mathworks (USA)).

3. Results and discussion

3.1. Iodometric titration of TEMPO⁺ and Cl(+1)

The reaction of TEMPO and NaOCl was studied in a pH 8.3 buffer solution because at this pH the content of the undissociated HOCl (pKa 7.5), that is the reactive species, is still relatively high (~14 %). The reaction was monitored as a function of time by iodometric titrations with and without DMSO, which is used as a masking agent for HOCl [43-46] (S1). The formation of a reaction product was detected when DMSO was applied. This compound disappeared when an aliphatic alcohol, n-

257 propanol, was added in the reaction mixture of TEMPO and NaOCl. The slow formation under the
 258 low reactant concentrations and the disappearance with the added alcohol promotes an
 259 assumption that the component is the oxidized form of TEMPO, namely TEMPO⁺ which is reported
 260 to react stoichiometrically with iodine as described by Eq. 3 [1, 2, 30, 40]. TEMPO⁺ is converted to
 261 its reduced form, a hydroxylamine (TEMPOH), during the reaction with alcohols (Scheme 2). Primary
 262 aliphatic alcohols can be oxidized with TEMPO to the corresponding aldehydes in few minutes [5].
 263 The oxidation of the alcohols with TEMPO is clearly faster with added NaBr [11]. The co-catalysis by
 264 bromide could be due to the faster activation of TEMPO to its oxidized form.

265 The thiosulphate consumption with the added DMSO was converted to the concentration of
 266 TEMPO⁺ according to Eqs. 1 and 3 (Fig. 3). The concentration of Cl(+1) was obtained from the
 267 difference in the thiosulphate consumption with and without the added DMSO by applying Eqs. 1
 268 and 2. TEMPO⁺ was clearly formed in parallel with the consumption of Cl(+1).

269

270

271 **Fig. 1.** The correlation between the consumed Cl(+1) and the formed TEMPO⁺ in a reaction of 2 mM
 272 TEMPO and 4 mM NaOCl in a borate buffer (pH 8.3) at room temperature. The concentrations of
 273 TEMPO⁺ formed and Cl(+1) consumed were obtained by iodometric titration. Corresponding
 274 correlation with reaction times added is shown in supplementary material.

275 The correlation between the consumed Cl(+1) and the TEMPO⁺ formed is further illustrated in Fig.
 276 1. The stoichiometry of the activation reaction with HOCl and TEMPO is proposed to be 1:2 [11].
 277 The observed results correlate with the 1:2 stoichiometry except for the longest reaction times. This
 278 deviation could be explained with the formation of ClO₃⁻ which was detected after several hours of
 279 reaction time. The chlorate formation correlates with the self-decomposition of HOCl [27], which is
 280 more pronounced at long reaction times and especially at high Cl(+1) concentrations.

281 3.2. UV-Vis absorption spectroscopy of TEMPO, TEMPO⁺ and Cl(+1)

282 The conversion of TEMPO and Cl(+1) and the formation of TEMPO⁺ were monitored also by UV-Vis
 283 absorption spectroscopy (Fig. 3). The spectra of the reaction solutions and the corresponding
 284 spectra of Cl(+1) in the borate buffer are shown in the supplementary material. Mathematical fitting
 285 was used instead of direct observation of the absorption maxima shifting due to the overlapping of
 286 the spectra of TEMPO, TEMPO⁺, and the Cl(+1). The mathematically reconstructed spectra of
 287 TEMPO, TEMPO⁺, and Cl(+1), shown in Fig. 2, are based on the measurements illustrated in the
 288 supplementary material.

289
 290 **Fig. 2.** Mathematically reconstructed spectra of TEMPO, TEMPO⁺, and Cl(+1) from monitoring the
 291 reaction of 2 mM TEMPO and 4 mM NaOCl in a borate buffer (pH 8.3) by UV-Vis spectroscopy at
 292 room temperature.

293 The formation rate of TEMPO⁺ in 2 mM TEMPO solution, which is typically applied in the TEMPO-
 294 mediated oxidation, and the formation rate in a more concentrated TEMPO solution are compared
 295 in Fig. 4. The concentration ratio of NaOCl and TEMPO and the concentration of NaOCl clearly
 296 influence on how fast TEMPO⁺ is formed. In the plateau point of TEMPO⁺ formation the consumption
 297 of thiosulfate (iodometric titration in the presence of DMSO) is almost equal to the amount of
 298 TEMPO added. The results demonstrate the benefit of a separate activation step of TEMPO prior to
 299 its application as a bromide-free oxidation catalyst e.g. for the oxidation of cellulosic pulps. A
 300 separate activation of TEMPO by NaOCl at pH 7-8 can be accomplished within an half of hour when
 301 20-40 mM TEMPO and excess of NaOCl are used (results not shown).

302

303 **Fig. 3** Disappearance of Cl(+1) and TEMPO and formation of TEMPO⁺ in a reaction mixture of 2 mM
 304 TEMPO and 4 mM NaOCl in a borate buffer (pH 8.3) at room temperature. The graph compares the
 305 results obtained by iodometric titration and UV-Vis spectroscopy combined with MCR.

306

307 **Fig. 4.** Effect of the concentrations of TEMPO and NaOCl on the conversion to TEMPO⁺ in a borate
 308 buffer (pH 8.3) as a function of time, analyzed by iodometric titration.

309 3.3. Iodometric titration of TEMPO⁺ in the presence of added NaBr

310 The use of DMSO as a masking agent for HOCl has been reported widely [43-46]. DMSO can be
 311 applied as a masking agent for HOBr correspondingly [47]. However, the reaction rate between
 312 HOBr and DMSO is slower than the reaction rate between HOCl and DMSO. The required time for
 313 removing all HOBr in the mixture of NaBr and NaOCl by DMSO was determined by varying the
 314 incubation time prior to the addition of KI and starch (Fig. 5). The trapping of HOBr by DMSO

315 appeared to follow the first order exponential decay, thus, the following equation was fitted to the
316 titration data:

317
$$[\text{HOBr}] = [\text{HOBr}]_0 \cdot \exp(-kt)$$
 (Eq. 4)

318 where the rate constant k is 0.10 s^{-1} . Thus a delay of 2 min between the addition of DMSO and the
319 addition of KI was considered appropriate to enable the titration of TEMPO^+ regardless of the
320 amount of $\text{Br}(+1)$ present in the sample solution.

321
322 **Fig. 5.** The decomposition of HOBr, formed in 4 mM NaBr and 4 mM NaOCl in a borate buffer (pH
323 8.3) at RT, by DMSO as monitored by iodometric titration.

324 The iodometric titration with and without added DMSO was applied for monitoring the
325 NaBr/TEMPO/NaOCl reaction system similarly as presented in supplementary data (S1) for the
326 TEMPO/NaOCl system (S2). The formation rate of TEMPO^+ was high despite the 10 times lower
327 concentration level compared to the bromide-free system (S1 and Fig. 3). The use of bromide as an
328 accelerating compound of TEMPO-mediated oxidation is well known and reported [10, 12]. Our
329 experiments verify one of the important effects of bromide as a co-catalyst is the faster conversion
330 of TEMPO to TEMPO^+ .

331 The correlation between the consumption of $\text{Cl}(+1)/\text{Br}(+1)$ and formation of TEMPO^+ (Fig. 6) was
332 similar to the one detected with the TEMPO/NaOCl system (S1 and Fig. 3). In both cases the
333 hypohalous acids were consumed and TEMPO^+ formed in the theoretical 1:2 ratio.

334

335 **Fig. 6.** Disappearance of Cl(+1)/Br(+1) and formation of TEMPO⁺ in a reaction mixture of 0.2 mM
 336 TEMPO, 0.2 mM NaBr, and 0.2 mM NaOCl in a borate buffer (pH 8.3) at room temperature.

337 3.4. Monitoring of the TEMPO-mediated oxidation of xylitol

338 The iodometric titration of Cl(+1) and TEMPO⁺ was applied to study the catalytic oxidation of xylitol
 339 with preactivated TEMPO (2 mM) at pH 8.3 in buffered conditions [Fig. 7]. Interestingly, 30 mM
 340 NaOCl was consumed during reaction with 7.5 mM xylitol. The observed 4:1 stoichiometry
 341 corresponds to the expected conversion of xylitol into xylaric acid. The concentration of TEMPO⁺
 342 remained at a constant level until all hypochlorite was consumed after which also TEMPO⁺
 343 disappeared.

344

345 **Fig.7.** Disappearance of Cl(+1) and TEMPO⁺ during TEMPO (0.4 mM) catalyzed oxidation of a 7.5 mM
 346 xylitol with 30 mM NaOCl at room temperature. pH was buffered with borate buffer to 8.3.

347 3.5. Monitoring of the TEMPO-mediated oxidation of bleached pulps by iodometric titration

348 The iodometric titration of $\text{Cl}(+1)$ and TEMPO^+ was applied to study the catalytic oxidation of a
349 bleached birch kraft pulp with preactivated TEMPO (2 mM) at pH 9 that has been reported to be the
350 optimum pH for the bromide-free oxidation [11] (Fig. 8 A). During the course of the oxidation TEMPO
351 existed mostly in the form of TEMPO^+ , the concentration of which stayed at a constant level (1.7
352 mM) until all $\text{Cl}(+1)$ was consumed (Fig. 8 A). Similar observations were made with bleached
353 eucalyptus kraft and eucalyptus prehydrolysis kraft pulps (supplementary material). Then TEMPO^+
354 disappeared at a rate equal to the final rate of conversion of $\text{Cl}(+1)$. These observations indicate that
355 the oxidation of TEMPOH to TEMPO^+ is much faster than its oxidative reaction with the primary
356 alcohol groups in cellulose. A similar conclusion was reported earlier by Bragd et al. [11] who found
357 that the rate of TEMPO catalyzed oxidation of methyl α -D-glucopyranoside was unaffected by the
358 concentration of $\text{Cl}(+1)$. However, when pH of the reaction mixture was adjusted from 9 to 10.5 (Fig.
359 8 B) during the reaction, TEMPO^+ almost disappeared ($< 0.2 \text{ mM TEMPO}^+$) after which the reaction
360 continued with a much lower rate. Thus, at the high pH reoxidation of TEMPOH became the rate-
361 limiting reaction, probably due to the low content of HOCl at the high alkalinity.

362 Under identical conditions more carboxylate and aldehyde groups (0.92 and 0.21 mmol/g,
363 respectively) were formed in the eucalyptus prehydrolysis kraft pulp in comparison with the normal
364 eucalyptus kraft pulp (0.75 and 0.13 mmol/g, respectively) (supplementary material). The enhanced
365 oxidation of the prehydrolysis kraft pulp can be explained by its lower xylan content. Similar effect
366 of the xylan content on the oxidation of cellulosic pulps has been reported earlier [29].

367 When a small amount of NaBr was used as a co-catalyst, the concentration of TEMPO^+ stayed close
368 to the concentration of the added TEMPO even though the oxidation was carried out at pH 10 (Fig.
369 8 C). The bromide-assisted oxidation has been reported to be fastest at pH 10 [11]. Our results verify
370 that the NaBr addition that leads to formation of HOBr, promotes the reoxidation of TEMPOH at
371 least at high pH levels. In part, this can be understood by the lower degree of dissociation of HOBr
372 (pK_a 8.7) in comparison with HOCl (pK_a 7.5). These examples demonstrate the utility of the
373 iodometric titration in unravelling the rate-determining steps in the TEMPO catalyzed oxidation of
374 cellulosic pulps.

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379 **Fig. 8.** Disappearance of Cl(+1) and TEMPO⁺ during TEMPO (2 mM) catalyzed oxidation of a bleached
380 birch kraft pulp (4 % consistency) with NaOCl at room temperature. [A] oxidation with preactivated
381 TEMPO and 57 mM NaOCl at pH 9, [B] repetition of A oxidation except pH adjustment (9 → 10.5)
382 with NaOH after 12 minutes reaction time (dash line). [C] Disappearance of Cl(+1)/Br(+1) and
383 TEMPO⁺ during TEMPO (2 mM) and NaBr (0.4 mM) catalyzed oxidation of a bleached birch kraft pulp
384 (3.3 % consistency) with NaOCl (97 mM) at pH 10 at room temperature. TEMPO was preactivated
385 prior to the oxidation.

386 4. Conclusions

387 The quantification of TEMPO⁺ by iodometric titration is a useful tool for studying the chemistry of
388 the TEMPO-mediated oxidation of primary alcohols. Similar and complementary information can be
389 obtained by UV-Vis spectroscopy combined with multivariate curve resolution, which can be applied
390 for simultaneous quantification of Cl(+1)/Br(+1), TEMPO⁺ and TEMPO. In general, conversion of
391 TEMPO to TEMPO⁺ is the slowest step in bromide-free oxidation of the alcohols by Cl(+1). The use
392 of bromide accelerates this conversion that can also be accomplished through a separate
393 pretreatment of TEMPO with an excess of Cl(+1) in neutral conditions. The rate of the preactivation
394 depends on the concentrations of TEMPO and Cl(+1) and is high enough to be applied in potential
395 industrial processes such as catalytic oxidation of cellulosic pulps. TEMPO⁺ was the dominant form
396 of TEMPO in the catalytic oxidation of a birch kraft pulp under optimal conditions which confirms
397 the earlier observation on the oxidation of the hydroxymethyl groups being the rate-determining
398 step in the catalytic process.

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482   **Supplementary data**

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484   **Fig. S1.** Iodometric titration of a reaction mixture of 2 mM TEMPO and 4 mM NaOCl in a borate buffer (pH
 485   8.3) at room temperature. A product, which was not trapped by DMSO, was formed as a function of reaction
 486   time.

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489   **Fig. S2.** Iodometric titration of a reaction mixture of 0.2 mM TEMPO, 0.2 mM NaBr and 0.2 mM NaOCl in a
 490   borate buffer (pH 8.3) at room temperature. A 2 min delay was applied between the additions of DMSO and
 491   KI to remove HOBr before the TEMPO⁺ titration.

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494 **Fig. S3.** UV-Vis spectra of borate buffer solution (pH 8.3), 2 mM TEMPO in buffer solution and 4 mM NaOCl
 495 in buffer solution. TEMPO and NaOCl solutions were diluted (1/5) with buffer solution just before UV-Vis
 496 measurement.

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503 **Fig. S4.** UV-Vis spectra of mixtures (2 mM TEMPO and 4 mM NaOCl) in borate buffer solution (pH 8.3) as a
 504 function of time. TEMPO/NaOCl solutions were diluted (1/5) with buffer solution just before UV-Vis
 505 measurement.

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508 **Fig. S5.** The correlation between the consumed Cl(+1) and the formed TEMPO⁺ including the reaction times.
 509 2 mM TEMPO and 4 mM NaOCl were diluted with a borate buffer (pH 8.3) at room temperature. The
 510 measured TEMPO⁺ and the consumed Cl(+1) are based on the iodometric titrations.

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513 **Fig. S6.** Disappearance of Cl(+1) and TEMPO⁺ during TEMPO (2 mM) catalyzed oxidation of [A] a fully bleached
 514 eucalyptus kraft pulp with preactivated TEMPO, 98 mM NaOCl, 4 % pulp consistency at room temperature.
 515 A eucalyptus prehydrolysis kraft pulp [B] was oxidized under similar conditions.

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520 **Table S1.** TEMPO-mediated oxidation of bleached birch kraft pulp (8A-C), bleached eucalyptus kraft pulp (S6
521 A) and bleached eucalyptus prehydrolysis kraft pulp (S6B). Catalyst addition was 2 mM and oxidations were
522 applied at room temperature.

523	Oxidation	Fig. 8 A and B	Fig. 8 C	Fig. S6 A	Fig. S6 B
524	Catalyst	TEMPO	TEMPO	TEMPO	TEMPO
525	Preactivation	HOCl	-	HOCl	HOCl
526	Primary oxidant	HOCl	HOBr	HOCl	HOCl
527	Oxidation pH	9	10	9	9
528	Pulp consistency (%)	4	3.3	4	4
529	Reaction time, min	37	180	51	50
530	NaClO addition	1.4	3.0	2.4	2.4
531	(mmol NaClO / g pulp)				
532	Carboxylate content	0.57	0.99	0.75	0.92
533	(mmol COOH / g pulp)				
534	Aldehyde content	0.09	0.02	0.13	0.21
535	(mmol COOH / g pulp)				
536	DP after residual aldehyde	1860	1450	936	612
537	conversion to carboxylates				