Oxidation of Reducing End Groups in Celluloses According to Different Protocols

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Abstract

The recently established fluorescence-based CCOA method provides carbonyl group profiles, reporting both aldehyde and keto functionalities [1]. To assess the state of oxidation even better, a differentiation between aldehydes – mainly present as reducing end groups (REGs) – and keto groups would be very attractive. Our approach to distinguish those groups in cellulosics was based on the oxidation of the reducing end to the corresponding acids, leaving behind only keto groups for CCOA profiling. Hence, the overall carbonyl profile was monitored before oxidation, and a keto profile was obtained after the oxidation step. Two procedures for the oxidation of REGs have been tested: the formazane method [2] and the oxidation by silver ions [3]. Both approaches have been thoroughly evaluated to address the degree of conversion and the effect on the molecular weight.

Introduction

The main causes for the formation of carbonyl groups in cellulosics are isolation, processing, and purification procedures. In general, acidic pulping processes introduce more carbonyl groups than alkaline ones. Both, pulping of wood and especially bleaching of the resulting pulp – which mainly utilizes oxidative procedures – are responsible for generation and further conversion of oxidized groups in cellulose. Such functionalities represent sites of pronounced chemical instability, they must be considered as “hot spots” along the polymer chain, where subsequent chemical side reactions, such as chain cleavage, cross-linking or condensation, are likely to occur. It is therefore not surprising that these oxidized functions – despite their relatively low concentration in the μmol/g range – are crucial factors in governing properties and behavior of pulp samples, such as dissolution, chemical stability, bleachability, reactivity in derivatization reaction, aging behavior, and yellowing behavior and so on.

The CCOA method provides a good means to measure carbonyl groups in relation to the molecular weight, so-called carbonyl profiles. However, the CCOA procedure does not distinguish between keto and aldehyde groups; it moreover reports the overall amount of both functional groups. This includes the naturally occurring reducing end groups in cellulose, which are aldehydes masked as hemiacetals. To report additionally oxidized sites in cellulose a differentiation from the reducing ends is necessary. Different approaches are theoretically conceivable: a selective derivatization of either aldehyde and keto groups, a mathematical calculation of the reducing end groups and its subtraction from the molecular weight distribution data, or a selective “masking” of either of the two oxidized species by oxidation or reduction [1,2]. An elegant approach would employ a fluorophore, of which the wavelength depended strongly on the type of carbonyl it is attached to. So far, such a differentiation of keto and aldehyde groups by labeling is not available. The mathematical calculation of reducing end groups from the number average molecular weight data are theoretically possible, and would directly result in a distribution of keto and aldehyde groups relative to the molecular weight. Further chemical manipulation of the
cellulose is not necessary here. However, the calculation approach also has considerable drawbacks: the error of the measurement of Mn is rather large and depends highly on the data evaluation of the GPC measurements, and so would the calculated REG content. In addition, pulp samples and also aged papers contain oxidized reducing end groups, since the REG is a primary site of oxidative attack. Such groups would finally be responsible for further inaccuracy of the data. The third approach consist in the conversion of one carbonyl species into an inert or “masked” form, which is not reported by the labeling procedure. We employed the latter approach, in particular the oxidation of aldehydes (REGs) to the corresponding acids, in our studies. After the oxidation, labeling reports exclusively the keto groups in the sample. Drawbacks are further chemical modification of the material, and the prerequisite of a quantitative reaction. In this paper, we wish to report investigations toward selective oxidation of reducing end groups in order to obtain keto group profiles which can be correlated to the overall carbonyl (keto + aldehyde) profiles and allow a differentiation of carbonyl structures in cellulosics.

**Experimental**

Chemicals were obtained from commercial sources and were of the highest purity available. Pulps from different origins and sources were used. The CCOA labeling was performed as described earlier [3-5]. Different pulps were used: hardwood magnesium bisulfite A, Hardwood sulfite B, Softwood sulfite, Eucalyptus Kraft pulp, cotton linters.

**General analytics.**

Gel permeation chromatography (GPC) measurements used the following components: online degasser, Dionex DG-2410; Kontron 420 pump, pulse damper; autosampler, HP 1100 column oven, Gynkotek STH 585, fluorescence detector TSP FL2000; multiple-angle laser light scattering (MALLS) detector, Wyatt Dawn DSP with argon ion laser \(\lambda_0 = 488 \text{ nm}\); refractive index (RI) detector, Shodex RI-71; Data evaluation was performed with standard Chromeleon and Astra software. The pH value of the solutions in DMAc/LiCl was determined by pouring a defined amount into 100 ml of deionized water.

**GPC method**

The following parameters were used in the GPC measurements: flow, 1.00 ml min\(^{-1}\); columns, four PL gel mixedA ALS, 20µm, 7.5 x 300 mm; fluorescence detection, \(?_{ex} = 290 \text{ nm}, \ ?_{em} = 340 \text{ nm}\); injection volume, 100 µl; run time, 45 min. DMAc/LiCl (0.9% w/v), filtered through a 0.02 µm filter, was used as the eluant.

**Silver ion oxidation.**

Diamminsilver solution \([\text{Ag(NH}_3)_2]^+\) was prepared by dissolving 1 g of AgNO\(_3\) in 7.5 ml water and adding 0.8 ml NH\(_3\) conc. The initial brown precipitate was dissolved by further addition of conc. NH\(_3\). The solution was then diluted with water to a volume of 75 ml. Air-dried pulp (50 mg) was disintegrated in a lab mixer. Excess water was removed and 8 ml of diamminsilver solution \([\text{Ag(NH}_3)_2]^+\) were added. Temperature and reaction time were varied (12; 24; 48 and 168 hours, r.t., 40° C; 80° C and 100° C). The oxidized pulp was thoroughly washed with H\(_2\)O and 0.1 M EDTA (pH = 10), and excess liquid was filtered off. Subsequently, CCOA labeling was performed: 4 ml of a solution of 1.25 mg CCOA in 4 ml of zinc acetate buffer (pH = 4) were added, and the suspension was shaken in a water bath at 40° C for 7d.

**TTC oxidation.**

Air- dried pulp (50 mg) was disintegrated in a lab mixer, and washed with H\(_2\)O and EtOH. DMAc (6 ml) was added, and the suspension was shaken overnight at r.t. Excess DMAc was filtered off and 4 ml of DMAc/LiCl (9 %) were added. After dissolution of the cellulose, the solution was diluted with DMAc (6 ml). Three stock solutions were prepared: solution A: 0.01 M TTC in DMAc/LiCl (3.6 %) (0.067 g TTC dissolved in 20 ml of DMAc/LiCl (3.6 %)), solution B: 2 M \(t\)-BuNH\(_2\) in DMAc/LiCl (3.6 %) (2.102 mL \(t\)-BuNH\(_2\) in 10 ml of DMAc/LiCl (3.6 %)), solution C: DMAc/LiCl (3.6 %). 1 ml
of 0.5 % cellulose solution in DMAc/LiCl (3.6 \%) and 2 ml of solution A were stirred in a 5 ml vial. 1 ml of solution B was added, and the vial is heated at 75° C for 10 min in a water bath and immediately cooled to r.t. The cellulose was reprecipitated in 150 ml of water/ethanol (v/v = 50:50), and labelled according to the CCOA procedure.

Results and Discussion

Oxidation with silver ions.

The silver ion oxidation converts the reducing ends to the corresponding acids according to the equations in Figure 1. This procedure was initially applied to label reducing ends in cellulose for visualization in microscopy [6, 7].

\[
\text{Ag}^+ + 2 \text{NH}_3 \rightarrow [\text{Ag(NH}_3\text{)}_2]^+
\]

\[
\text{Cell - C} \xrightarrow{H} \text{O} + 2[\text{Ag(NH}_3\text{)}_2]^+ + 2\text{OH}^- 
\]

\[
\text{Cell - C} \xrightarrow{OH} \text{O} + 2\text{Ag}^0 + 2\text{NH}_3 + \text{H}_2\text{O}
\]

Figure 1. Oxidation of reducing ends of cellulose by diamminsilver cations.

Table 1. Removal of carbonyls and reduction of the molecular weight by oxidation with silver complex.

<table>
<thead>
<tr>
<th>Pulp</th>
<th>Carbonyl content in µmol/g</th>
<th>Decrease of carbonyls ΔC=O</th>
<th>Reduction of Mw in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hw Sulfite A</td>
<td>18.1</td>
<td>8.3 µmol/g</td>
<td>38.1</td>
</tr>
<tr>
<td>Hw Sulfite B</td>
<td>9.4</td>
<td>4.2 µmol/g</td>
<td>35.1</td>
</tr>
<tr>
<td>Sw Sulfite</td>
<td>12.3</td>
<td>3.3 µmol/g</td>
<td>47.0</td>
</tr>
<tr>
<td>Hw Kraft</td>
<td>5.5</td>
<td>1.5 µmol/g</td>
<td>32.7</td>
</tr>
<tr>
<td>Cotton Linters</td>
<td>3.9</td>
<td>0.8 µmol/g</td>
<td>11.5</td>
</tr>
</tbody>
</table>

For most pulps, oxidation with complex silver ions caused the expected decrease in carbonyl group contents. Only cotton linters (CL) and eucalyptus kraft pulp (Hw Kraft pulp), which originally contained a low number of carbonyls, did not respond with a significant reduction in carbonyl groups. The decrease of carbonyl groups (ΔC=O) due to the silver oxidation at 100° C for 10 min is shown in Table1.

From Figure 2 and Figure 3 it was evident that the oxidation with the silverdiammin complex required elevated temperatures in order to significantly decrease the number of reducing end groups. While the aldehyde groups of the hardwood sulfite pulp were strongly consumed at the beginning of the treatment, the functional groups of the cotton linters were rather inert in this phase. Thus, pulps of different origin and provenience behaved quite differently during this oxidation step.

In order to complete, the oxidation with silver ions must be carried out under alkaline conditions (pH 10), which in most cases imposes a degradation of the cellulose sample due to beta-elimination processes at keto and aldehyde groups along the cellulose chains.
Figure 2. Effect of time and temperature on the oxidative removal of carbonyl groups in different pulps by oxidation with diamminsilver complex.

Figure 3. Reaction kinetics for Hw sulfite A pulp (left) and cotton linters (right) oxidation by diamminsilver complex at r.t. and 40°C.

The extent of the observed degradation again highly depended on the respective pulp sample and ranged between 3 – 47 % of the original Mw (Figure 4). While sulfite and kraft pulps showed a larger reduction in Mw (33 – 47 %), cotton linters was only degraded in range of 3 – 11 %.

In addition, a different dissolution behavior of the samples in DMAC/LiCl (9 %) was observed after oxidative treatment. A high temperature treatment (T > 60° C) with silver ions, which is more effective in reducing the carbonyl content, caused decreased solubility of the cellulose sample in DMAC/LiCl (9 %). Cotton Linters even became completely insoluble under these conditions. Only if the time of treatment at temperatures above 60° C was less then 2 hours, cellulose samples treated in DMAC/LiCl (9 %) could still be dissolved.
Oxidation with TTC.

Oxidation with TTC (2,3,5-triphenyltetrazolium chloride) has already been known for a long time in pulp analytics to determine the reducing end groups in paper materials [8]. Upon oxidation of the aldehyde groups in pulp to the corresponding acids, purple-colored triphenylformazane is formed, see the equations in Figure 5.

According to the original procedure, a suspension of pulp in aqueous alkaline solution (KOH) had been used. Strlic and Pihlar [9] considerably improved the method by employing the cellulose in homogenous solution in DMAc/LiCl in the presence of tert-butylamine. In both cases the formation of formazane is followed spectrophotometrically at 546 nm or 524 nm, respectively. In our approach, we utilized TTC oxidation in homogeneous solution and followed the changes in the carbonyl content by standard CCOA analysis. This required a re-precipitation of the pulp sample in order to remove excess oxidant and byproducts prior to CCOA labeling, but afforded total carbonyl (keto) content and profiles (Figure 6).
This procedure was applied to different pulp samples. For most pulp samples a degradation of cellulose, as seen by a decrease in the molecular weight, was observed, which was severe in some cases (sulfite pulps). This reduction in molecular weight has to be considered when applying the simple spectroscopical method: novel REGs are formed during cellulose degradation, which add to the originally present ones. This might eventually lead to incorrect data of the measured reducing end groups, if the values are not corrected for sample degradation. From our previous investigations [10], we learned that a mere heating of pulp samples (also sulfite pulps) in DMAc/LiCl for 10 min at 75°C did not result in a strong degradation. Hence, other compounds in the mixture must have triggered the cellulose degradation. Therefore, we investigated different auxiliary bases, such as pyridine, tert-butylamine (tert-BuNH₂), N-methylmorpholine (NMM), dicyclohexylethylamine (DCHEA), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and morpholine. All bases had a negative effect on the molecular weight, but not all of the auxiliaries were able to promote the oxidation with TTC. Especially DBU and morpholine as auxiliary bases in the oxidation with TTC did not result in a significant reduction of the amount of carbonyl groups (cf. Figure 7). tert-Butylamine, morpholine and Hünig base, in contrast, had a pronounced effect.

![Figure 6](image-url)

**Figure 6.** General approach for the oxidation of cellulosics with TTC in combination with CCOA analysis.

![Figure 7](image-url)

**Figure 7.** Effect of different auxiliary bases on the molecular weight (left) and on the oxidative removal of aldehyde groups (right) by oxidation with TTC.
Figure 8 depicts the effect of auxiliary bases on different pulp samples. It was evident that sulfite pulps were rather sensitive to the oxidative treatment in DMAc/LiCl independent of the base used. Hardwood kraft pulp and especially ancient rag paper showed much less degradation under the conditions applied. For such pulp samples, the TTC procedure appeared suitable to monitor keto group profiles with a tolerably small simultaneous degradation of the cellulose. Such a keto group profile, a distribution of keto groups relative to the molecular weight distribution, is given in Figure 9 for a eucalyptus sulfite pulp and a rag paper. In general, the total amount of keto groups was rather low. According to the TTC method, approx. 10% of the carbonyl groups were attributed to keto functionalities. Figure 9 nicely demonstrates that oxidation to keto groups predominated in the bulk region (DP 200-2000) in both pulps. However, the keto group distribution differed significantly as expected (Figure 10).

**Summary**

Two chemical approaches have been tested to oxidize reducing end groups in cellulose to obtain keto group profiles after removing aldehyde groups oxidatively. The oxidation with diamminsilver complex gave only an insufficient reduction in the carbonyl content under mild conditions, but caused a severe degradation of cellulose under more drastic settings.

Oxidation with TTC under homogeneous conditions in DMAc/LiCl resulted in the expected reduction in carbonyl groups. However, also here a reduction in the molecular weight was observed, for some industrial pulps a drastic DP loss was found. There was no unambiguous correlation of MW reduction with the type of auxiliary base applied (neither base strength nor nucleophilicity). In case of rag paper and hardwood kraft pulp, keto group profiles were obtained, the loss in DP being tolerable in these cases.
Figure 9. Carbonyl profile, keto profile and molecular weight distribution of a hardwood sulfite pulp (upper) and a rag paper from 1825 (lower).

Figure 10. Numerical evaluation of the keto group distribution after TTC oxidation of rag paper (left) and hardwood kraft pulp (right).
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References


