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Analysis of Oxidized Functionalities in Cellulose

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Abstract This review gives an overview on oxidized functionalities in celluloses, i.e., carbonyl and carboxyl groups, with regard to their chemical structure, the different ways of introduction, and their analytics. Starting from different processes introducing oxidative modifications into celluloses a survey on the chemical nature of these functionalities is given and analytical approaches towards their determination are discussed. Special emphasis is placed on recent developments which combine group-selective fluorescence labeling with multi-detector GPC analysis to provide carbonyl and carboxyl group profiles according to the CCOA and FDAM method, respectively. Examples of monitoring the oxidation state of celluloses and its changes during processing stages are given, for example bleaching, aging, dissolution or irradiation procedures.

Keywords CCOA method · Cellulose · Carbonyl groups · Carboxyl groups · FDAM method · Fluorescence labeling · GPC

Abbreviations

ADAM	9-Anthryldiazomethane
AGU	Anhydroglucose unit
CCOA	Carbazole-9-carboxylic acid [2-(2-aminooxy-ethoxy)ethoxy]amide
DAM-MC	4-Diazomethyl-7-methoxycoumarine
Dansyl	5-(Dimethylamino)-1-naphthalenesulfonic acid
DMAc	*N*,*N*-Dimethylacetamide
DP	Degree of polymerization
DS	Degree of substitution
DS_{CO}	Degree of substitution of carbonyl groups
DS_{COOH}	Degree of substitution of carboxyl groups
ECF	Elemental chlorine free
FAD	Flavin adenine dinucleotide
FDAM	9*H*-Fluoren-2-yl-diazomethane
GPC	Gel permeation chromatography
HexA	Hexenuronic acid
Lyocell	Fiber production process based on direct dissolution of cellulose in NMMO
MALLS	Multi-angle laser light scattering
M_n	Number average molecular weight
M_w	Weight average molecular weight
MW	Molecular weight
MWD	Molecular weight distribution
NMM	*N*-Methylmorpholine
NMMO	*N*-Methylmorpholine *N*-oxide
P	Peroxide bleaching stage
PDAM	1-Pyrenyl-diazomethane
PHK	Prehydrolysis kraft pulp
PDI	Polydispersity index
REG	Reducing end group(s)
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl

TCF	Totally chlorine free
TMP	Thermo-mechanical pulp
TOF-SIMS	Time-of-flight secondary ion mass spectrometry
TTC	2,3,5-Triphenyltetrazolium chloride
Z	Ozone bleaching stage

1 Oxidized Groups in Cellulose

1.1 A Survey of Oxidative Modifications

Cellulose as synthesized by nature can be considered a quite perfect molecule: anhydroglucose units (AGU) are connected by β-1,4-glycosidic linkages resulting in a homopolymer with three hydroxyl groups per AGU and a terminal aldehyde masked as hemiacetal at the reducing end. While such cellulose synthesized in vitro or in vivo represents the ideal polymer molecule in terms of chemical purity, processing steps, such as isolation and purification, as well as natural conditions, such as exposure to environmental stress and aging, are factors for the introduction of additional oxidized functionalities.

Due to the 3 hydroxyl groups available for oxidation within one anhydroglucose unit and due to the polymeric character of the cellulose a great variety of structural modifications and combinations is possible. As with other types of chemical changes at the cellulose molecule also in this case the oxidation can affect different structural levels differently. Depending on the oxidative stress imposed on the cellulose, the individual hydroxyls within the AGU and within the polymer chain are involved to varying extent and may respond to further treatment and reactions in a specific way. Despite their low concentration in the μmol/g range, oxidative functionalities are one of the prime factors to determine macroscopic properties and chemical behavior of cellulosic materials (Fig. 1).

The main causes for the formation of carbonyl and carboxyl groups in cellulose are isolation and purification procedures besides natural aging. This applies in particular to cellulosic pulps from wood, which has undergone a number of processing steps to be freed from lignin, hemicelluloses, and extractives.

Although we are far away from being able to fully analyze an oxidized cellulose with all these different possibilities of oxidative modifications with regard to their type and exact location, cellulose chemists have taken major steps towards developing methodology to address those problems. Major obstacles on the way to a comprehensive analysis of oxidized functionalities are certainly the low concentration of these structures in cellulosic materials, the characterization of their position within the molecular weight distribu-

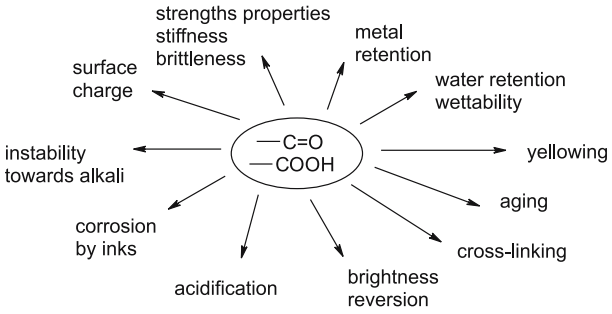


Fig. 1 Possible effects of carbonyl and carboxyl groups on macroscopic properties of cellulose

tion (MWD), and the problems generally faced in analytics of celluloses, such as limited solubility, the polymeric character, morphological structure and limited accessibility.

This review summarizes the generation, nature and determination of oxidized structures in celluloses as well as recent developments in their analytics.

1.1.1 Pulping

The first process to bring about oxidative changes onto cellulose is pulping. In acidic sulfite pulping, generally a larger number of carbonyl groups is introduced as compared to prehydrolysis kraft pulping. In celluloses from kraft pulping procedures, most carbonyls actually originate from the hemicellulose fraction. The alkaline conditions of kraft pulping favor a more narrow distribution of the molecular weight as well as the dissolution of low molecular weight material, which has a high relative carbonyl content. During sulfite pulping, hydrolysis of cellulose and hence the generation of more reducing end groups (REG) is promoted. Aldonic acids as final oxidation products have been isolated from sulfite cooking liquors [1]. If paper pulps are considered the situation is mainly influenced by the content of hemicelluloses, which per se contain a larger number of carbonyls due to the smaller molecular weight and a higher number of carboxyl groups due to the side chain uronic acids. While the oxidation of reducing ends of dissolved monomers and oligomers from hemicellulose and cellulose during sulfite pulping is rather pronounced, the oxidation of the reducing end of the non-dissolved remaining cellulose was found to be rather small. However, pulping with bisulfite, having a higher process pH, favors the oxidation of the REG to the corresponding aldonic acids [2]. After alkaline pulping, metasaccharinic and gluconic acid residues are typical carboxyl groups in cellulose [3]. Metasaccharinic acid is the final product of the stopping reaction after alkali-induced peeling and rearrange-

ment reactions. Under alkaline conditions, gluconic acid carboxyl groups are crucial with regard to cellulose stabilization, since they hamper degradation by the peeling process.

1.1.2 Bleaching

Bleaching of pulp represents the main origin of additional carbonyl groups in cellulose. While pulping proceeds in the absence of strong oxidants, oxidizing agents are deliberately used in bleaching to achieve the desired brightness effect.

Chlorine dioxide (D stage) is considered rather selective for lignin moieties. Cellulose integrity is not affected to a large extent [4]. Chlorine dioxide primarily reacts with the reducing end groups of carbohydrates converting them to carboxylic acids. However, depending on the pH other chlorine-containing species are formed, e.g., hypochlorous acid and chlorate. In that respect, also effects of those oxidants have to be considered. Hypochlorite formerly used directly as H stage is being phased out mainly due to environmental concerns. If HOCl is present in a pH range between 2 and 8, considerable amounts of carbonyls are introduced into cellulose, whereas under alkaline conditions chiefly carboxyl groups are formed. An H stage in all pH ranges is accompanied by a decrease in the molecular weight, which depends linearly on the oxidative damage done, i.e., on the carbonyls created, but not on the pH, as proposed by Lewin and Epstein [5]. The oxidation potential and selectivity in hypochlorite stages is strongly governed by the conditions chosen [6, 7], as the active species depend on the pH: in acidic solution the equilibrium between free halogen and hypohalous acid is shifted to the side of free halogen, whereas under alkaline conditions the hypohalous acid is the effective and also more selective species. In the pH range between 2 and 8, where hydroxyl groups are predominantly oxidized to keto and aldehyde groups, the drop in DP is most severe, whereas the oxidative power of hypohalite at a higher pH is merely sufficient to oxidize aldehyde groups to the corresponding acids, leaving the DP largely unaltered.

The oxygen-based bleaching chemicals of TCF bleaching sequences exhibit a lower selectivity towards lignin and residual chromophores so that oxidative damage involves also the carbohydrates. In particular, the heavy metal management during pulping becomes an important issue, since a number of very reactive radical species, such as hydroxyl and hydroperoxyl radicals, are formed in the presence of the bleaching agents and transition metal ions (Fenton and Haber–Weiss cycles). Other sources of radicals are disproportionation reactions under alkaline conditions, which applies especially to hydrogen peroxide, secondary radical formation in homolytic reactions and autoxidation. Gierer [8] and Gratzl [9] state the generation of different oxygen-derived radical species in all TCF sequences as the main cause of the

limited selectivity. According to Chirat and Lachenal the poor selectivity of ozone is mainly due to the oxidant ozone itself [10].

Keto groups are introduced during an ozone (Z) stage [11, 12]. Besides the action of hydroxyl radicals, which can be generated in an ozone stage either by slow decomposition of ozone in water or be triggered in the presence of metal ions, also an ionic pathway by the common 1,3-insertion mechanism of ozone has been proposed [13, 14], leading to the eventual formation of lactones.

Such processes are always accompanied by a DP loss, either by electrophilic attack of ozone, by an ozone-catalyzed cleavage of the glycosidic bond or by attack of secondary radical species [15]. Residual lignin also plays a crucial role in ozone bleaching. Model studies showed that lignin with free phenolic hydroxyl groups accelerated carbohydrate oxidation, probably by activation of oxygen via phenoxyl radicals, whereas etherified phenolic model compounds had a protective effect [16, 17].

During a hydrogen peroxide (P) stage, basically the same applies as for an ozone stage, if radicals are present and their subsequent reactions are allowed to proceed. In their absence, however, a P stage introduces predominantly keto groups, which neither cause a pronounced DP loss nor a decreased brightness. Hence, the keto groups were thought to be generated at position C3. Even though chain scission can occur also in this case, a non-reducing chain and a diketo derivative, subsequently leading to a stable saccharinic acid derivative, are formed [18, 19]. A peroxide stage followed by alkaline extraction (E) significantly reduces the total amount of carbonyl groups, leading to a pronounced brightness stability. One reason for this is also the removal of 2,3-diketo structures by reaction with the hydroperoxide anion (HOO^-), which can add to keto and enone structures as well. The formed acids are then extracted within the E stage.

During an alkaline oxygen stage the formation of keto structures by oxidation of ketols is postulated, finally leading to acids. Sodium permanganate treatments yield a moderate increase in both, carbonyls and carboxyl groups at short reaction times [20].

In ECF and TCF bleaching the same reactions that lead to the formation of carbonyl groups can also account for the generation of carboxyl groups, especially if radical species are involved.

1.1.3 Irradiation

High energy radiation, for instance γ-irradiation or β-irradiation (electron beaming), causes a considerable increase in carbonyl groups, mainly through the action of radical species being generated. Both procedures are accompanied by a DP loss, which can be used to adjust the molecular weight of the cellulose prior to utilization in the viscose process [21]. The number of car-

bonyls introduced by high energy electron beams is directly correlated with the applied dosage [22]. e-Beaming is also considered as a means for cellulose activation, since the radiation can enter also highly ordered regions in cellulose, changing the morphology for subsequent reactions in a favorable way [21]. Also γ-irradiation shows a linear relationship between dose and carbonyl content [23]. The presence of lignin during γ-irradiation does not prevent cellulose degradation [24].

1.1.4 Aging Processes

The presence of oxygen, often also in the presence of light, causes a number of autoxidative reactions to proceed. Accelerated aging linearly increases with the partial pressure of oxygen [25]. Once the process has started, radical reactions come into play that finally result in the formation of hydroperoxyl structures [26, 27], which in turn can activate oxygen to generate very reactive radical species, such as hydroxyl or hydroperoxyl radicals. These radicals react with cellulose under H-atom abstraction, finally leading to formation of carbonyl or carboxyl groups, to chain cleavage, and thus also to a loss in DP and fiber strength. Additional factors triggering oxidation reactions during aging are transition metal ions, especially iron and copper from writing and painting media, chromophores that serve as activators, as well as elevated temperature, air pollutants and light. Light-induced processes have been extensively studied on high-yield pulps containing considerable amounts of lignin [28–30], but have recently also been investigated in the case of fully bleached pulp samples [31].

Natural aging, as experienced in historic documents, drawings and cellulosic fabrics is thought to be caused by two major parallel processes, hydrolytic cleavage [32–34] of the glycosidic bond by acid of various origins and oxidation [35–37], triggered by external factors such as metal ions, air, light, or pollutants. While the hydrolytic pathway is well investigated [35], fewer studies report on the oxidation reactions [38].

Thermo- and photo-oxidative degradation in the context of paper conservation science was lately summarized and investigated [39]. Methylene blue dyeing was used to investigate reactions occurring at wet-dry interfaces [40]. Investigation on different pulps has shown that there is indeed an influence of carbonyl groups during the aging of alkaline pulps [41]. Reactions occurring under alkaline conditions in aged papers as they are deliberately induced during deacidification treatments are increasingly addressed [42].

1.2
Carbonyl Groups in Cellulose

1.2.1
The Reducing End Group (REG)

The reducing end groups in cellulose are the only naturally occurring carbonyl functionalities in this material. Cellulose from *Acetobacter xylinum* [43] contains an amount of carbonyl groups which corresponds approximately to the number of reducing end groups, and can be considered as rather genuine material.

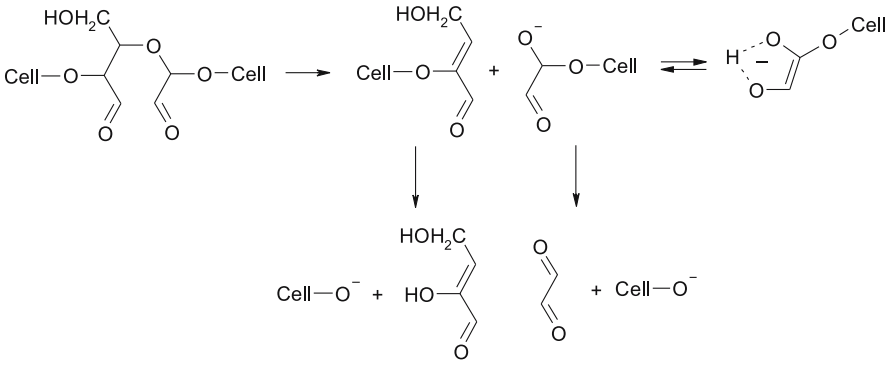
The reducing ends are the starting points for the well-known peeling reaction. The conditions for the peeling to proceed require alkaline media and a free 2-OH group. In case of a substituted hydroxyl at C2, as it occurs for instance in hemicelluloses, the peeling rate is drastically reduced.

Reducing end groups can be readily further oxidized to the corresponding aldonic acids.

The reducing ends are very likely to be present as hemiacetals in pyranose units, but only to a small extent as aldehydes and aldehyde hydrates [44].

1.2.2
2,3-Dialdehyde Structures

Oxidation with periodate under acidic conditions, the *Malaprade* reaction, is mainly used to introduce a large number of aldehydes into cellulose [45]. Whether a similar reaction proceeds also under conditions of natural or accelerated aging conditions has not been clarified, but corresponding processes have been postulated to occur [46]. The oxidized groups introduced are either used to further functionalize the cellulose, e.g., by reaction with



Scheme 1 β-Elimination starting from C2,C3-dialdehyde structures in cellulose [93]

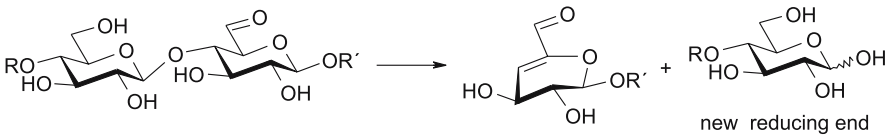
substituted amino functionalities, oxidation to the corresponding acids, or reduction. All of those procedures significantly change the properties of the cellulose.

The oxidation proceeds predominantly at C2 and C3 under aldehyde formation, with concomitant ring opening cleavage between the C2–C3 bond. The periodate oxidation is accompanied by a decrease in crystallinity [47, 48]. Kim et al. [49] investigated the influence of this oxidation in the crystalline domains. Besides the decrease in crystallinity an uneven distribution of aldehyde groups was shown by gold labeling. From this result, it was proposed that the oxidation proceeds highly heterogeneously, forming isolated oxidized domains. The mechanism for β-elimination at 2,3-dialdehyde celluloses under alkaline conditions is shown in Scheme 1.

1.2.3
6-Aldehyde Structures

Celluloses oxidized to an aldehyde at C6 are found for instance as intermediates of the TEMPO oxidation [50]. Depending on the reaction conditions a large number of such groups may survive in the final products, the polyglucuronic acids, and also in partially TEMPO-oxidized pulps materials it is highly likely that a large number of carbonyl groups are present as C6-aldehyde.

Cellulose can be oxidized to different degrees of C6-aldehyde content by photolysis of the 6-azido-6-deoxy derivatives [51]. The β-elimination reaction of the 6-aldehyde (Scheme 2) may lead to terminal double bonds exhibiting a UV absorption ($\lambda_{max} = 250$ nm) [52, 53]. With model C6-aldehyde celluloses, β-elimination started at 30 °C at pH 9; at elevated temperatures (80 °C) β-elimination was detected already at very low pH levels (pH 3.5). Similar results were obtained with HOCl-oxidized pulps, with β-elimination starting at room temperature at pH 8.5 (unpublished results).



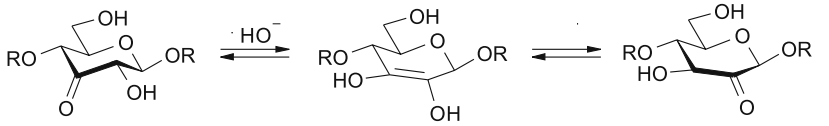
Scheme 2 β-Elimination starting from C6-aldehyde structures in cellulose

1.2.4
Keto Groups

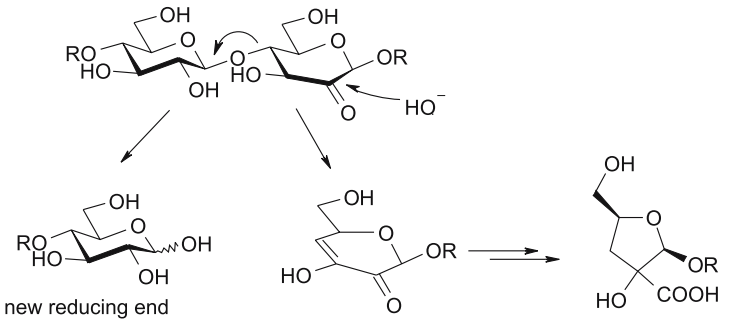
Keto groups can be introduced at position C2 or C3, and they may be present also as 2,3-diketo structures. According to keto-enol tautomerism the po-

sition of such carbonyls may fluctuate (Scheme 3). Their reaction products formed under alkaline conditions differ significantly depending on the original position of the keto group.

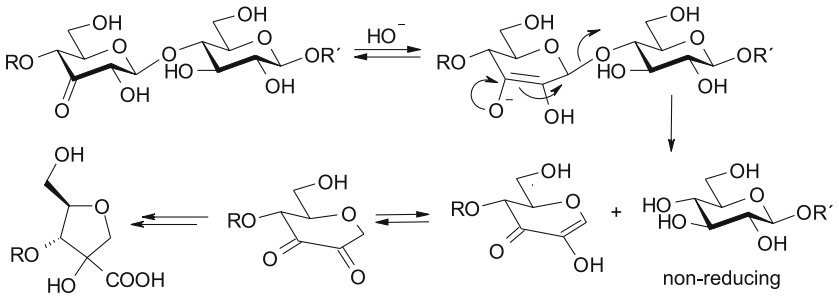
While a keto group at C2 leads to the generation of a new reducing end group (Scheme 4) and this way at least to a starting point for further peeling reactions, a keto at position C3 is considered a more innocent carbonyl function [18]. Also in this case the cellulose chain will be cleaved, however, the resulting fragments contain a novel non-reducing end group and an acid formed by rearrangement (Scheme 5). Both structures can be considered rather stable in subsequent reactions.



Scheme 3 Keto-enol tautomerism at carbonyl moieties in cellulose



Scheme 4 Cleavage of the glycosidic bond by β-elimination starting from C2-keto structures along the cellulose chain



Scheme 5 Cleavage of the glycosidic bond by β-elimination starting from C3-keto structures along the cellulose chain

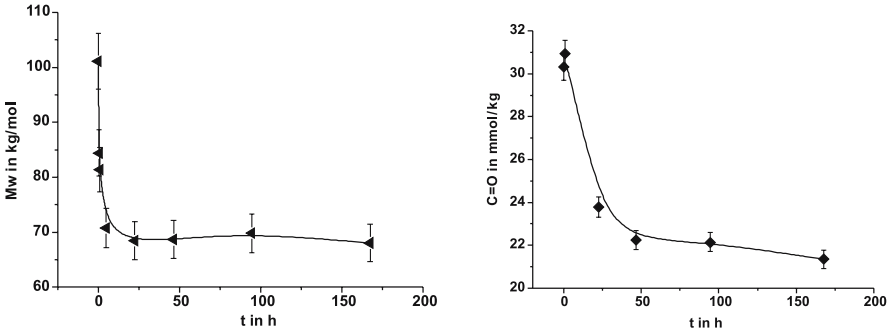


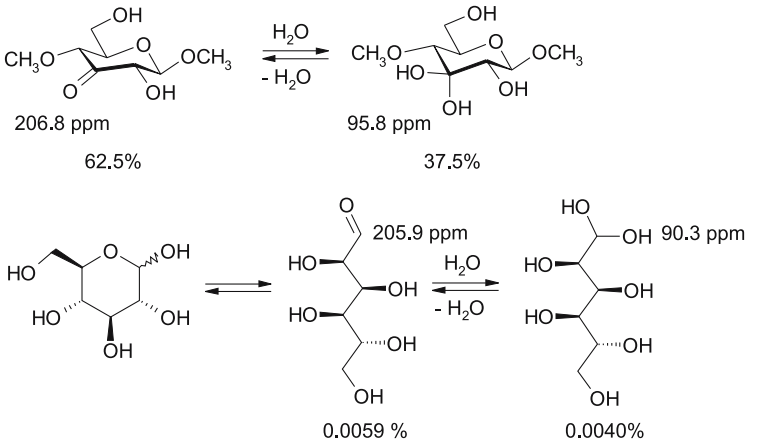
Fig. 2 Effect of alkaline conditions on oxidized cotton linters. *Left*: Changes in M_w (error bars 5%). *Right*: Changes in carbonyl groups (error bars 2%)

How β-elimination reactions and subsequent processes under alkaline conditions affect the carbonyl groups and the M_w of an oxidized cotton linters is shown in Fig. 2. The initial concentration of carbonyls is 30 μmol/g, introduced by hypochlorite treatment at pH 7. The sample was treated with a buffer solution of pH 11 at 40 °C. The M_w suffers a steep decrease within the first 5 hours, the drop in carbonyls proceeds at a significantly lower rate. While the changes in M_w level off after approx. 20 hours of treatment, the carbonyl groups are slightly decreasing further (unpublished results).

1.2.5 The Nature of Carbonyls in Cellulose

Carbonyl groups are not always present in their usual "double-bond" form, but also exist as the tautomeric enols or as hydrates upon addition of water, which was demonstrated by means of differently oxidized carbohydrate model compounds by NMR [102] (Scheme 6). A recent study revealed a much higher percentage of hydrated aldehyde groups compared to the pure acyclic form for all aldohexoses in water [44].

The hydration/(hemi)acetalization state of aldehyde and keto groups in celluloses is largely unknown. Non-conjugated carbonyl structures generally absorb in the near UV, around 270–280 nm, for aldehyde and keto groups. Even though high degrees of oxidation can be obtained, e.g., by periodate oxidation, the carbonyl groups can hardly be detected by spectroscopic methods such as FTIR spectroscopy [54], likely due to hydration and acetalization effects. Severe drying down to 7% relative humidity and high temperatures eventually increased the C = O vibrations [55, 56], which indicated a large extent of hydration of the carbonyl groups in these substrates. Other explanations are strong cross-linking by the formation of hemicaetals with neighboring hydroxyl groups. Such intra-molecular and inter-molecular cross-linking has been postulated [57], but only be proven indirectly by changes in macro-

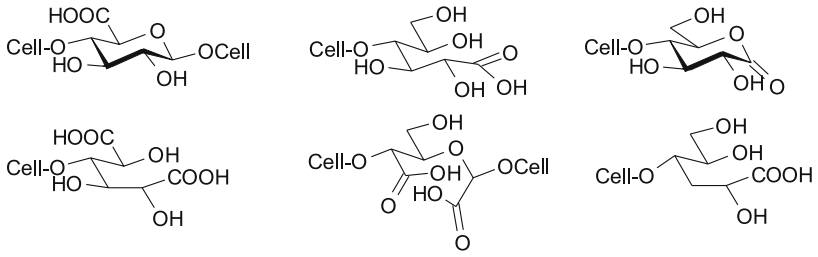


Scheme 6 Examples of the equilibrium between keto and hydrate forms in aqueous solution, including the respective ^{13}C NMR shifts of the carbonyl/hydrate atoms. Glucose data from [44]

scopic properties, e.g., solubilities. The interchange between hydrated carbonyls and those present in the sp^2-hybridized form can be investigated combining the CCOA method (see next chapter) and the resonance Raman technique. While the CCOA method detects all carbonyl groups independent of hydration or hemiacetalization/hemiketalization, resonance Raman records only sp^2-hybridized carbonyl groups. It was demonstrated, that carbonyl functions in cellulose are present to a large extent as hydrates and/or hemiacetals/hemiketals in addition to their free, double-bonded form [58].

1.3 Carboxyl Groups in Cellulose

Only second to carbonyl groups, carboxyls are a very important oxidized function in celluloses (Scheme 7). Whereas hemicelluloses inherently contain a high number of different acid groups, carboxyl groups in cellulose are artifi-

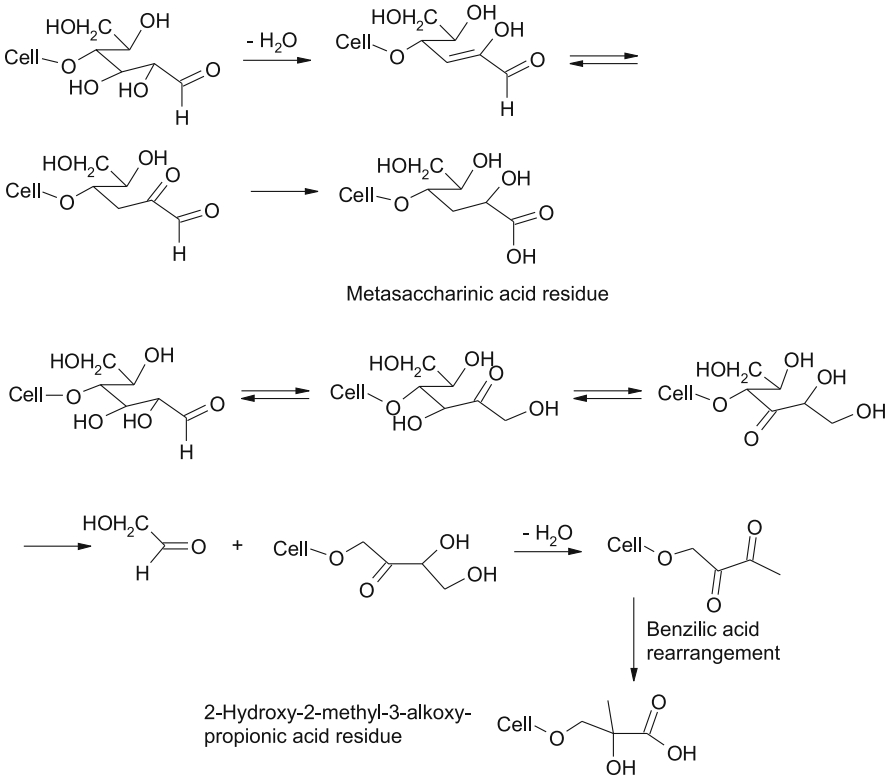


Scheme 7 Examples of different carboxyl structures in cellulose

cially introduced by pulping and bleaching processes. The primary positions for oxidative attack are the reducing end groups, which are oxidized to the corresponding gluconic acid end groups. Oxidation at the primary C6 hydroxyl group leads to glucuronic moieties. The pyran ring structure of an AGU and the integrity of the cellulose may be destroyed by cleavage at the ring-oxygen or by formation of acids at C2 and/or C3 (Scheme 7).

1.3.1 Gluconic Acids

Oxidation of the reducing end group leads to the corresponding gluconic acid. Since the REG is the starting point for the peeling process under alkaline conditions, an oxidation represents a stabilization of the polymer chain. Especially under kraft pulping conditions additives or modifications aimed at improving the process yield often involve the oxidation of the REG (e.g., polysulfide, AHQ) [59].



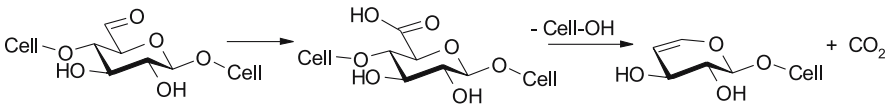
Scheme 8 Formation of acidic residues, stopping the peeling process of cellulose under alkaline conditions

As a result of the peeling/stopping process, metasaccharinic acid end groups formed by the Cannizzaro reaction, or 2-hydroxy-2-methyl-3-alkoxypropanoic acid residues are generated (Scheme 8) [60].

1.3.2 Glucuronic Acids

Depending on the type and the origin of the pulp a major source for glucuronic acids are the hemicelluloses. The glucuronoxylans of hardwoods and the (arabino)glucuronoxylans of softwoods contain α-D-glucuronic acids and/or their 4-*O*-methyl derivatives attached to position C2 of the xylan backbone [61]. Chemical generation of uronic acids in cellulose can be accomplished by TEMPO oxidation [62], or, less selectively, by nitrogen oxide treatments [63, 64].

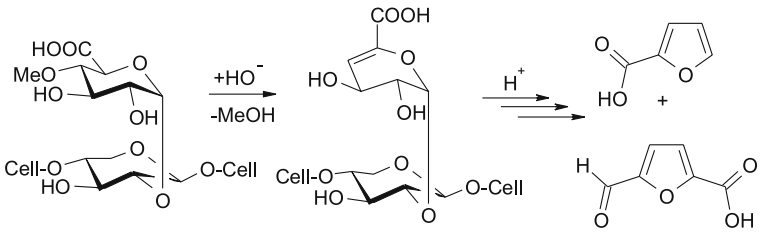
Also under conditions of ozone bleaching, further oxidation of 6-aldehyde cellulose to glucuronic acids proceeds. Decarboxylation of the resulting C6-carboxyl group is made responsible for CO_2 formation and DP loss during this process [4, 14] (Scheme 9).



Scheme 9 Decarboxylation of acids under the conditions of ozone bleaching

1.3.3 Hexenuronic Acids

Hexenuronic acid (i.e., 4-deoxy-L-*threo*-hex-4-enopyranosyl-uronic acid) is formed under alkaline conditions by elimination of methanol from side chain residues in xylans [65] (Scheme 10). The reaction is promoted by both increasing alkali concentration and temperature [66, 67]. After kraft pulping, only

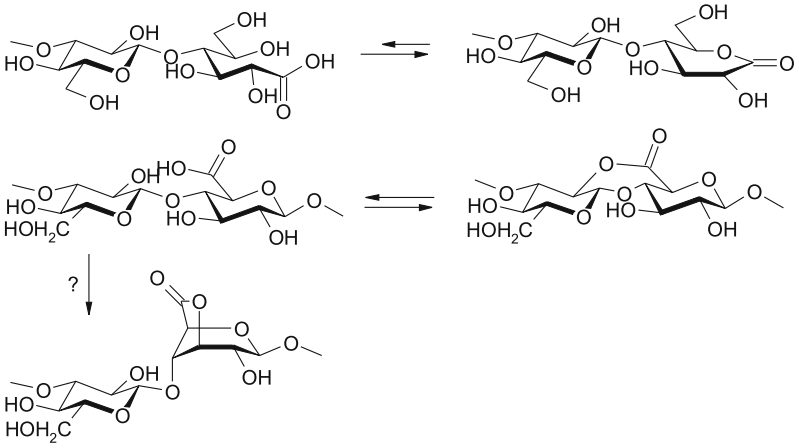


Scheme 10 Formation of HexA residues from xylan under alkaline conditions and degradation to furan products in acidic media

about 12% of the remaining carboxyl groups in accessible xylan are still of the 4-*O*-methylglucurono type [68]. Hexenuronic acids add to the total carboxyl group content and also to the kappa number. HexAs are seen as being partly responsible for diminished brightness stabilities of bleached pulps. Under acidic conditions hexenuronic acids are unstable (Scheme 10), so that a mildly acidic treatment can thus be used to selectively remove HexA from the pulp [69].

1.3.4 Lactone Entities

Within oxidized polysaccharides, carboxylic units may occur at the former reducing end as gluconic groups as well as along the chain in the form of uronic acid residues or at the C-2 and C-3 positions in periodate-cleaved and oxidized products. Under appropriate conditions induced by elevated temperatures, dehydration or low pH, the carboxylic groups are prone to intra-molecular ester formation, which in case of cellulose includes the 5-OH group of the proximal cellobiose moiety of the cellulose chain or the 2-OH group of a glucose unit preceding the glucuronic acid residue within the polysaccharide backbone. Whereas in the former case a six-membered lactone moiety in a distorted half-chair conformation will be generated, an inter-residue lactone in a sterically feasible seven-membered ring will be present in the latter case. This arrangement resembles the positioning of the hydrogen-bond pattern stabilizing the cellulose I structure by hydrogen bonding from OH-2′ to O-6. In the literature [133], the occurrence of 6,3-lactone units has also been discussed. The formation of this intra-residue lactone, however, requires a transition from the $^4C_1$ conformation into a boat conformation which would meet with considerable steric restraints (Scheme 11).

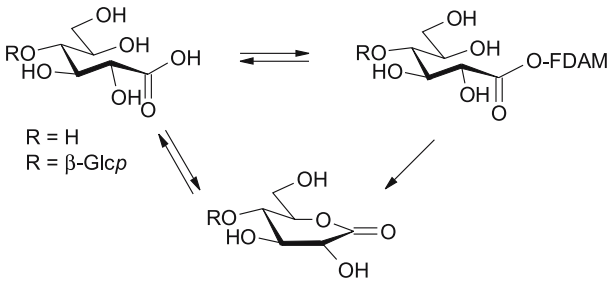


Scheme 11 Formation and hydrolysis of glucono-1,5-lactone and 2′,6- and 3,6-lactone entities

1.3.4.1 D-Glucono-1,5-Lactone Formation and Stability

In contrast to D-glucono-1,4-lactone, the 1,5-lactone exhibits pronounced instability. Depending on the applied conditions, the lactone will be in equilibrium with the corresponding gluconic or cellobionic acid moiety, respectively, which in solution is shifted to the open-chain form. Semiempirical calculations of the hydrolysis of 1,5-gluconolactone indicated preferential cleavage of the C1-oxygen linkage, which was supported by ^{13}C NMR data of a hydrolyzate obtained from gluconolactone in $H_2{}^{18}O$ at pH 7.5 [70]. Thus, isolation of cellobiono-1,5-lactone has formerly only been achieved by removal from the mixture by selective crystallization [71]. The formation of gluconic acid lactones in cellulosic substrates has been implicated in various FAD-assisted enzymatic reactions involved in cellulose degradation. As an example, in addition to the well-studied cellobiose dehydrogenases, a glucooligosaccharide oxidase has been described, which also accepts cellodextrins as substrate [72, 73]. Mechanistic and crystal structure data showed the formation of the glucono-1,5-lactone intermediate in the oxidation step, which subsequently hydrolyzes spontaneously to give the open-chain form. Due to the inherent lability of the 1,5-lactone, a cellobionolactame analogue has therefore been used for cocrystallization experiments with a cellobiose dehydrogenase, and these lactone mimetics are of general interest as potential glycosidase inhibitors [74]. In addition to the crystallographic data, direct ^1H NMR-spectroscopic evidence has been obtained for the enzymatic conversion of cellobiose into cellobionic acid [75].

Cellobionic acid was also found as the major product arising from ozonation of cellobiose and was isolated by HPLC and fully characterized by ^1H and ^{13}C NMR spectroscopy [76]. Ozonation of methyl β-D-glucopyranoside afforded a lactone of arabinonic acid arising via a Ruff-type degradation of gluconic acid followed by subsequent oxidation [16]. Alkaline conditions, such as those which have been applied to open lactone rings, are prone to generate additional carboxylic groups. Kraft pulps, which have undergone

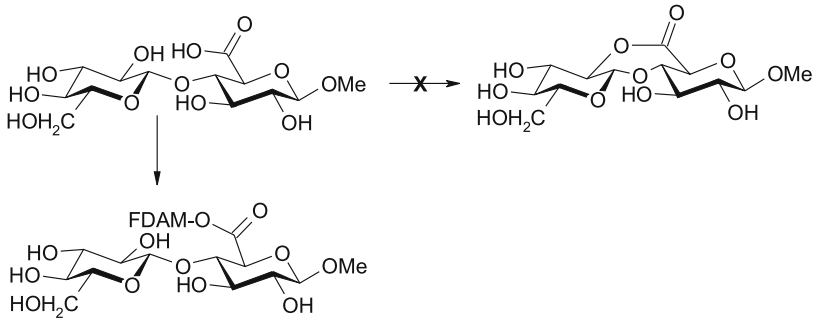


Scheme 12 Formation and hydrolysis of FDAM-labeled aldonic acids

alkaline treatments contain metasaccharinic end units in addition to gluconolactones. These end groups have been reported to form stable lactones, which are fairly resistant towards iodine-induced lactone opening [77]. Attempts to label gluconic acid residues with the fluorescence label FDAM (see forthcoming chapter) reflect the ambiguous reactivity of 1,5-lactones (Scheme 12). In model labeling experiments with xylonic, gluconic and cellobionic acid, formation of the labeled products could be monitored by TLC. Isolation of the derivatives by column chromatography, however, provided trace amounts only due to direct hydrolysis or transesterification reactions, respectively [78].

1.3.4.2 Formation of Intra-Chain Lactones

The formation and hydrolytic stability of intra-chain lactones has been inferred from oxidized cellulose samples (oxycelluloses). Little is known on the structure and occurrence of lactones in bleached pulps. The amount of glucuronic acid in bleached wood pulps is rather small. The potential involvement of lactones and ester bridges in inter-chain cross-linking has been controversially discussed in the literature [79]. Nevertheless carboxylic and lactone groups have been linked to numerous processes and product properties such as hornification, yellowing, aging or tensile strength [79, 80]. Model studies for the labeling reaction and for lactone formation in solution have been performed using synthetic methyl β-D-glucopyranosyl-(1 $\rightarrow$ 4)-β-D-glucopyranosiduronic acid. In situ NMR experiments at pD 1–2 did not indicate the presence of lactones [81]. The situation in a solid matrix, however, might be different where additional parameters such as accessibility, Donnan effects, hydration and the steric environment are operative. Furthermore, in striking contrast to the labeling experiments with aldonic acids, the FDAM-labeled uronic acid derivative could be isolated in preparative yield (Scheme 13).



Scheme 13 Labeling of a disaccharide uronic acid derivative

2
Determination of Oxidized Functionalities

The quantitation of oxidized groups can in principle be performed at three levels of information. The variant mostly utilized so far is the determination of the total amount of a respective functional group as a sum parameter. Examples of different methods are given in the following section. To gain more detailed information in which part of the polymer these functional groups are situated a combination of group-selective labeling and size exclusion chromatography can be used. The third level is the determination of the type of functional group within the AGU. For cellulose no methods are currently available to quantify different types of oxidized groups within the AGU. Such an investigation would require for instance total hydrolysis and analysis of the fragments obtained, and determination despite the extremely low concentration of differently oxidized units remains a future challenge.

2.1
Carbonyl Functionalities (Aldehyde and Keto Groups)

2.1.1
Conventional Methods for Carbonyl Analysis in Celluloses

The quantitation of carbonyl groups in cellulose was so far limited to the measurement of the total carbonyl content by different methods, which are summarized in Table 1.

The so-called copper number is still the method of choice in the pulp and paper industry in process control, but sometimes also in the research lab. The reducing power of cellulose is measured by reaction with an alkaline Cu^{II}-salt under defined conditions, the formed Cu^{I} ions can be titrated after re-oxidation [82]. The underlying reaction mechanisms are still not entirely understood, neither are the types of oxidized structures recorded. However, even though the copper number is only a sum-parameter, the data of which cannot be directly linked to the quantity of a specific oxidized function, it remains a valuable parameter for control in a number of industrially relevant processes.

Cyrot [83] proposed the reaction with hydroxylamine to the corresponding oxime as a means of monitoring, since oximation was more sensitive than reaction with similarly reacting reagents such as hydrazine. The degree of nitrogen fixation can be measured either by a Kjeldahl procedure, or, as more recently applied, by elemental analysis. According to Rehder et al. [84], running the reaction in a zinc acetate buffer eliminated side-reactions with acidic groups and minimized those with lactones. According to Lewin [85] the formation of cyanohydrins by reaction of carbonyl groups with cyanide can also be taken as a measure of oxidized functionalities. Excess cyanide is removed

Table 1 Classical methods for determination of carbonyl groups in cellulose

Method	Reaction	Detection	Comment	Refs.
Copper number	(Unspecific) Reducing power	Titration	Only relative data, mechanism ill-defined	[82]
Hydrazinium-salt	Formation of charged groups on cellulose	Photometric	Only semi-quantitative	[88]
Hydroxyl-amine	Oximation	Elemental analysis, photometric, titration	Sensitivity depends highly on detection mode	[83, 89]
Cyanide	Formation of cyanohydrins	Titration	Toxic reagents, overestimation	[85]
$NaBH_4$	Reduction to the alcohol	Titration	Rather insensitive	[86, 87]
TTC-reaction	Formation of a red dye from reducing ends	Photometric	Only for REG, cellulose degradation, overestimation	[90, 91]

from the pulp and determined by titration with $AgNO_3$. This cyanide method often yields too large values due to adsorption phenomena, and is, moreover, less often used today because of the toxicity of the reagents.

The consumption of sodium borohydride (reduction method) upon reduction of carbonyl groups can also be used for their quantification. Remaining $NaBH_4$ is quantified by the amount of hydrogen formed after reaction with acid [86, 87].

Determination of dialdehydes in periodate cellulose can also be based on consumption of hydroxyl ions by the Cannizzaro reaction [92]. The β-elimination reaction of 2,3-dialdehyde celluloses in combination with DP-determination is proposed as a means to roughly determine the extent of oxidation [93].

An interesting procedure for quantification of C6-aldehyde groups of highly substituted C6-aldehydo derivatives by reduction with $NaBD_4$ and MS analysis of the hydrolyzed material is given by Clode and Horton [51].

2.1.2 Differentiation Between Keto and Aldehyde Groups

An important issue is the differentiation between aldehyde and keto groups in cellulosic materials. 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 overall amount of keto structures determined, or a selective "masking" of either of the two oxidized species by oxidation or reduction [94, 95]. Classical approaches follow the latter mode. However, all of these approaches give only a rough estimate of the aldehyde/keto ratio. An interesting approach was followed by Sihtola et al. [96] who correlated different rates of oximation with different carbonyl types present, but without making assignments of the rates to underlying structures, a similar principle was later applied by Blaha et al. [97]. Different oximation rates have been observed for differently bleached samples and aged pulps. However, the extent of morphological influences in this procedure is quite difficult to assess.

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 is 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, also the calculation approach has considerable drawbacks: the error of the measurement of M_n is rather large and depends highly on the data evaluation of the GPC measurements, and so does the calculated REG content. In addition, pulp samples and also aged papers contain oxidized reducing end groups in the form of acid residues, since the REG is a primary site of oxidative attack. However, if molecular weight data (M_n) are available, the number of REG can be roughly estimated.

The oxidation of reducing end groups of cellulose to the corresponding aldonic acids by chlorous acid was thought to proceed selectively enough to be used as a means of differentiation between keto and aldehyde groups. A second determination of either remaining keto carbonyls or newly formed carboxyl groups would finally yield the keto/aldehyde ratio [98]. However, the oxidation with chlorous acid proved to be not sufficiently selective (unpublished results). Another method was based on the work by Siggia and Maxcy [99] who estimated aldehydes by bisulfite addition and titration of the excess bisulfite with alkali. As this addition proceeds also with keto groups, albeit at a slower rate, the method must be applied with great care only [100].

The reducing power of aldehyde groups can be used to convert TTC (2,3,5 triphenyltetrazolium chloride) into triphenylformazane, a red dye, which can be quantified spectrophotometrically [90]. As the reaction is carried out in aqueous alkaline media, it induces β-elimination and hence results in overestimation of aldehyde groups. The same principle was applied by Strlic and Pihlar [90] in homogeneous solution (DMAc/LiCl) yielding results more reproducible than those according to the heterogeneous procedure in aqueous solution. However, also under these modified conditions, most industrial pulps suffer considerable DP-loss which results in an overestimation of REGs.

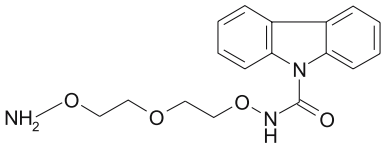
Only pulps from cotton linters and rag paper proved to be stable enough to be safely subjected to this method. A combination of the TTC protocol and the CCOA method to obtain pure keto group profiles is given by Nagel et al. [177].

2.1.3 The CCOA Method for Carbonyl Quantification

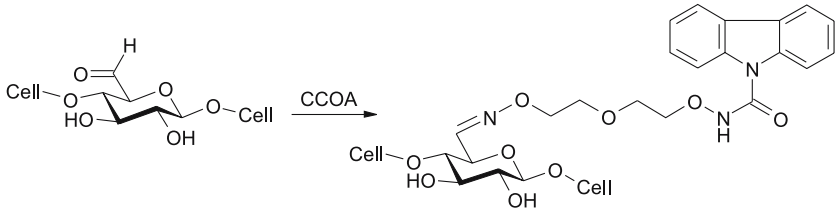
The classical procedures described above yield the carbonyl value as a sum parameter with varying accuracy. An additional drawback is the large sample amount required to obtain sufficient sensitivities. The comparably high reactivity of the carbonyl groups allows the application of carbonyl-selective reagents being UV-active or fluorescent compounds. This already lowers the sample demand significantly (see *Hydrolysis Method* in this chapter). The actual advantage, however, represents the opportunity of combining fluorescence labeling with a separation technique (see *Combination with GPC*). A suitable carbonyl-selective fluorescence label was synthesized: the reagent carbazole-9-carboxylic acid [2-(2-aminooxy-ethoxy)ethoxy]amide (carbazole-carbonyl-oxyamine, CCOA) (Scheme 14).

The CCOA label contains a fluorophore and a flexible spacer. The reactive anchor group is an oxyamine. Oxyamines showed a higher reactivity towards carbonyl groups as compared to hydroxyl amines [101]. Due to the fluorescence characteristics (λ_{ex} = 340 nm in DMAc) no interference with MALLS detection (λ = 370 nm or higher) is encountered. CCOA can be applied both in aqueous buffer systems and in the solvent DMAc/LiCl, as tested with model compounds [102], and gives generally neat and quantitative conversion of carbonyl functions into the corresponding *O*-substituted oximes (Scheme 15).

The label is completely stable in this solvent system at ambient temperature. Calibration, which is required for quantification of the carbonyl content,



Scheme 14 Structure of the fluorescence label CCOA for carbonyl-detection in cellulose



Scheme 15 CCOA-Labeling of cellulose by the example of a C6-aldehyde structure

can be performed with the label CCOA (Scheme 14) which has exactly the same fluorescence properties as the labeled compounds. The integrity of the cellulose was not affected upon labeling, i.e., no degradation occurred.

The advantages of the CCOA method are:

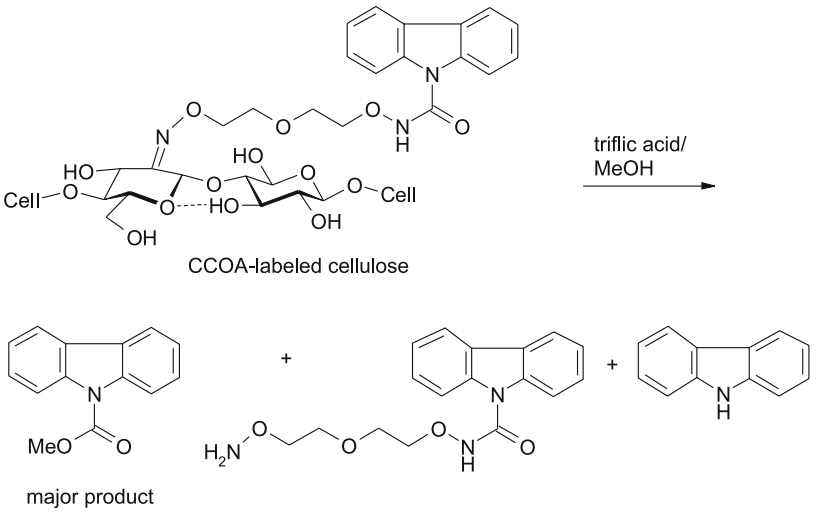
- Relatively low sample amounts required (5–25 mg)
- High selectivity and high sensitivity (LOD: 0.01 μmol/g; LOQ: 0.05 μmol/g)
- Yields all MWD data in addition to carbonyl profiles, combined with GPC
- MWD does not change after labeling (no aggregation phenomena)
- Separate numeric analysis of different MW regions is possible
- Practical on a routine basis

The following points can be considered as drawbacks of the method:

- The label is commercially unavailable.
- There is no differentiation between aldehyde and keto groups.
- There is no differentiation between lignin-carbonyls and cellulose-carbonyls.
- The combination with GPC is limited to pulps and papers soluble in DMAc/LiCl.

2.1.4 The CCOA-Hydrolysis Procedure

The hydrolysis procedure is based on heterogeneous carbonyl-selective fluorescence labeling with CCOA, which is subsequently released with triflic



Scheme 16 Hydrolytic cleavage of CCOA-labeled cellulose by triflic acid in methanol under release of *N*-(methoxycarbonyl)carbazole, CCOA, and carbazole

acid from the labeled pulp in a quantitative manner, and the concentration of CCOA and CCOA-derived products are determined by HPLC with fluorescence detection [103] (Scheme 16). The procedure requires material in the mg range only and can be applied to pulp and paper samples which do not readily dissolve in DMAc/LiCl. Calibration can be performed against DMAc/LiCl-soluble standard pulps. The data obtained by this approach correlate well with data from the traditional CCOA procedure. Limit of detection (3σ) and determination (10σ) are very low with 0.042 μmol/g and 0.14 μmol/g, respectively, as compared to conventional methods. Scheme 16 shows the reaction and the products obtained after hydrolysis.

2.2
Carboxyl Groups

2.2.1
Conventional Methods for Carboxyl Analysis in Cellulose

Two procedures for the determination of carboxyl groups predominate today: titration of the acids under formation of salts with different cations and utilization of the anion exchange capacity of pulps, which is a consequence of the presence of carboxyl groups. The acidic groups are transferred into their salt forms, using either an inorganic cation as in the former case, or an organic cationic dye, such as methylene blue or crystal violet, in the latter. The cation is subsequently quantified either after recovery from the pulp or by depletion analysis of the remaining species in solution. Also decarboxylation and determination of the freed carbon dioxide has been applied to quantify cellulose carboxyls [104, 105]. Investigations of Wilson [106] showed a strong dependence of the ion exchange capacity of carboxyl groups in pulp on the ion strengths. Various modifications of the method have been published [107–109]. Samuelson and Törnell [110] studied the determination of carboxyls in the presence of carbonyl groups. The pH of the alkaline sodium chloride solution to obtain the sodium salt of the carboxyl groups did not exceed pH 8 in order to prevent β-eliminiation reactions of the carbonyl groups. Sihtola [111] used a potentiometric determination after desalting by electrodialysis, displacement by potassium ions, and subsequent titration to pH 7.0. The iodide-iodate method [112] has the advantage of detecting carboxyl groups, lactones and enediol groups. The conductometic titration allows also differentiating between strongly acidic groups (e.g., sulfonic acids) and carboxyls. Table 2 gives an overview on conventional methods to estimate carboxyl groups in cellulosics.

Table 2 Classical methods for determination of carboxyl groups in cellulose

Method	Reaction	Detection	Refs.
(Reversible) Methylene blue	Ion exchange	Photometric	[113–116]
Sodium bicarbonate/NaCl	Neutralization	Titration	[117, 118]
Zinc acetate	Ion exchange	Gravimetric, complexometric	[119]
Crystal violet base	Acid-base reaction in benzene	Photometric	[120]
HCl	Neutralization	Conductometric	[121, 122]

Problems frequently encountered with the above described methods are:

- Inaccuracy due to unspecific binding of the cations
- Large sample demand
- Limited sensitivity
- Lactones have to be "opened" prior to the measurement [84, 89] but exhibit different rates of saponification depending on their type.

For a recent review on a critical evaluation on classical methods see Fardim et al. [123]. In addition to the wet chemical methods, FTIR spectroscopy and ESCA [124, 125] techniques have been applied to quantify carboxyl groups in cellulose and pulp. In case of FTIR, satisfactory results in comparison to wet chemical analysis could only be obtained with carboxyl-rich samples [126–128].

2.2.2 Determination of Lactones

In most cases, uronic acids are liberated from acidic polysaccharides by hydrolysis leading to irreproducible concomitant formation of lactones. Several methods to circumvent this problem have been published describing conversion of the uronic acid into methyl esters followed by reduction with borohydride or borodeuteride reagents and subsequent hydrolysis and GC-MS detection [129]. Other techniques are based on the liberation and quantification of carbon dioxide. Direct determination of uronic acid residues in hydrolyzates has frequently been performed according to colorimetric assays, which are rather insensitive and have thus mostly been replaced by high-performance anion exchange chromatography (HP-AEC) methods [130–132].

Due to the inherent chemical propensity of lactones with respect to hydrolysis and reformation, the determination has mostly relied on the total quantification of carboxyl groups (assuming a complete hydrolysis of lactones under alkaline conditions and negligible regeneration under acidic condi-

tions needed for the deionization of carboxyl groups) followed by measurement of free carboxylic groups using a variety of alkalimetric or complexometric protocols. The direct and unambiguous detection and differentiation of lactone moieties has not been accomplished thus far. An estimation of the lactone content has been gained by a variant of the iodometric titration of carboxyl groups [133, 134]. Hydrolysis of lactones under non-alkaline conditions is achieved by KI – KIO_3 which releases iodine – albeit at a slower rate than free carboxylic groups – which is scavenged in the presence of thiosulfate and quantified by an indirect iodometric titration [135]. The different types of lactone moieties, however, display varying degrees of reactivity towards hydrolysis.

2.2.3 Determination of Hexenuronic Acids

The following gives a brief compilation of procedures to determine hexenuronic acids in cellulosic pulp samples. The common methods are based on hydrolysis of HexA moieties from pulp, either enzymatically or chemically, with subsequent quantification of the hydrolysis products either directly or after chemical conversion into UV active compounds. A comparison of these three methods is given by Tenkanen et al. [136]. For comparison rather than exact determination of HexA, e.g., during bleaching stages, the diffuse reflection UV VIS method can be applied [137]. A photoacoustic FTIR procedure based on chemometric analysis has been described as well [138]. In Table 3, the available methods to analyze HexA moieties in cellulosic material are summarized.

Table 3 Methods for the determination of hexenuronic acids in cellulose

Method	Principle	Detection	Refs.
VTT method	Enzymatic hydrolysis	HPAEC-PAD, CE	[139, 140]
HUT method	Acid hydrolysis	Photometric	[69]
KTH method	Hg-salt, hydrolysis	Photometric, HPLC	[141, 142]
Resonance Raman	Direct detection of double bond	Resonance Raman	[143, 144]
Cadoxen method	Direct detection of double bond	UV	[145]

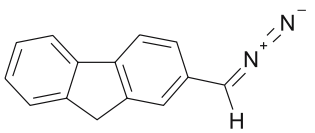
2.2.4 The FDAM Method for Carboxyl Group Determination

An approach different from the classical methods described in the previous chapter is the mild esterification of carboxylic acids as described in

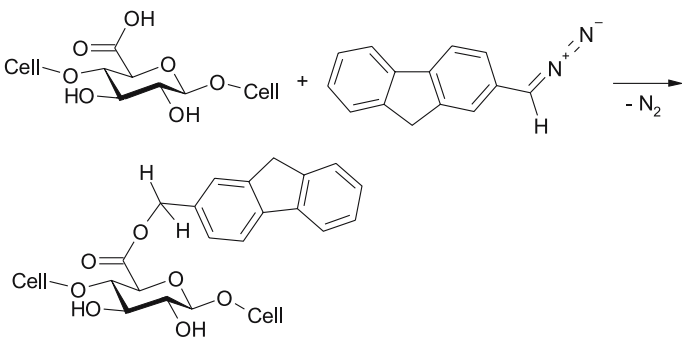
protein chemistry [146, 147] or utilized for fatty acid analytics [148–150]. However, most of these methods are not applicable towards quantification of sugar acids in polysaccharides due to insufficient yields. So far, the only appropriate reaction for sugar acids turned out to be that with diazomethane reagents [151]. Aromatic diazomethane derivatives do not require an activation step, no auxiliaries are necessary, traces of water do not interfere with the conversion, and the reaction proceeds at ambient temperatures to afford stable esters. Hydroxyl groups do not react under these conditions. If a fluorophore is linked to the diazomethane anchor group, the detection limit can be lowered significantly. In addition, a combination with other detection modes becomes possible. 9-Anthryldiazomethane (ADAM) [152–154], 1-pyrenyl-diazomethane (PDAM) [155, 156], and 4-diazomethyl-7-methoxycoumarine (DAM-MC) [157] have been described in the analytics of fatty acids and similar analytes, but could not be used in analytics of cellulosic carboxyls due to the limited stability of the product esters and because of the interference of the fluorescence label with the MALLS detection in GPC analysis.

For carboxyls in cellulose the FDAM label (Scheme 17) was found to be most suitable in combination with GPC-MALLS analysis [158].

The reaction was tested on carbohydrate model compounds and was shown to provide quantitative yields [78, 81] (Scheme 18). Following from the reaction mechanism, the carboxyl groups must be present in free form (COOH) for the labeling to proceed. Carboxylates (salts), lactones or esters



Scheme 17 Structure of the fluorescence label FDAM for carboxyl-detection in cellulose



Scheme 18 FDAM-labeling of cellulose by the example of a C6-carboxylic (glucuronic) acid structure

are not detected. At present, the statement of neat labeling with FDAM and reliable GPC detection must be restricted to uronic acids. Even though preparative experiments showed a conversion also for aldonic acids, the primarily formed fluoren-2-ylmethyl onates appeared to behave like activated esters under normal phase chromatography conditions, promoting the formation of lactones (see Scheme 12 above) with concomitant release of fluoren-2-ylmethanol from the FDAM label.

The reaction conditions for FDAM labeling were optimized for cellulose to fit into the usual activation protocol prior to dissolution in DMAc/LiCl (9%). FDAM can be prepared in the GPC eluant DMAc from 9*H*-fluoren-2-yl-carboxaldehyde via its hydrazone by oxidation with excess manganese dioxide [78].

The advantages of the FDAM method are:

- Relatively low sample amounts required (5–25 mg)
- Yields all MWD data in addition to carboxyl profiles in combination with GPC
- Separate numeric analysis of different MW regions is possible
- Practical on a routine basis
- FDAM label is easily and neatly synthesized prior to derivatization

The following points can be considered as drawbacks of the method:

- There is no differentiation between lignin-carboxyl and cellulose-carboxyls
- Combination with GPC is limited to pulps and papers soluble in DMAc/LiCl
- Degree of labeling of gluconic acid residues at present is unknown

2.3 Combination of Group-Selective Fluorescence Labeling with GPC

A substantial progress in analytics of oxidized groups in celluloses was achieved with the possibility to locate carbonyl and carboxyl groups along the cellulose macromolecule [159, 160]. This technique combined group-selective fluorescence labeling with GPC analysis. Addition of a fluorescence detector reports the amount of fluorescence in every slice of the MWD, and provides directly the concentration of the respective functional group after signal calibration. The standard GPC setup used in such approaches is given in Fig. 3.

From the integrated signals of the RI detector, which is concentration-sensitive, and the fluorescence detector, which is functional group-sensitive, the total amount of carbonyl or carboxyl groups can be calculated.

The MALLS detector allows the absolute determination of the molecular weight, provided that the dn/dc value of the polymer in solution is known. The minute concentration of functional groups (labeled carbonyls or carboxyl groups) does not influence the dn/dc value. From the MALLS and the RI signal the MWD is calculated. Combination of both, the fluorescence signal and the MWD allows the calculation of the functional group profile. Figure 4

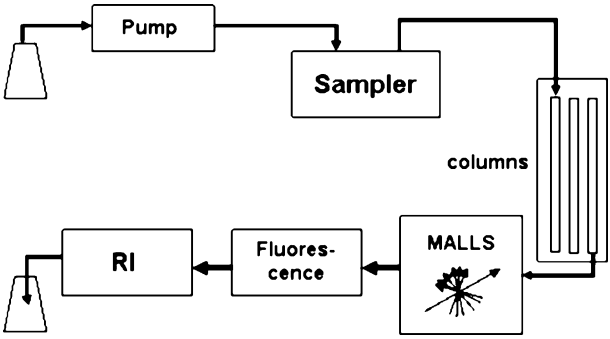


Fig. 3 GPC setup for functional group profiling. RI: refractive index detector; Fluorescence: fluorescence detector; MALLS: multi-angle laser light scattering detector

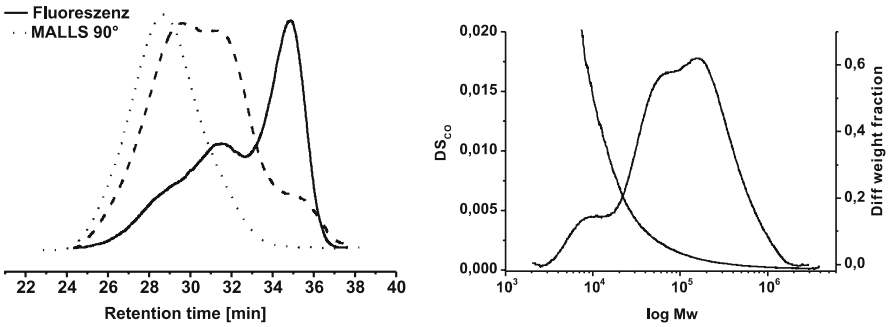


Fig. 4 *Left*: Fluorescence, MALLS (90°) and RI signals from a CCOA-labeled cellulose sample. *Right*: Differential molecular weight distribution and degree of substitution of carbonyl groups (DS_{CO}) as calculated from the detector outputs

shows an example of the signals obtained during GPC and the resulting MWD and carbonyl profile.

This approach requires a sensitive means of tagging the functional group since the absolute amount analyzed by GPC ranges in the μg range. The sensitivity needed is achieved with the above described fluorescence labels. However, in order to combine the fluorescence labeled material with GPC in combination with MALLS detection, the label must not emit light close to the laser wavelength of the MALLS device, which puts some restrictions to the chromophore of the marker. Classical carbonyl labels such as dansyl hydrazine [161], dansyl oxyamine [101], phenylhydrazine [162] do react with cellulose carbonyl groups as well [163] but are not suitable in combination with GPC-MALLS. The CCOA and FDAM labels described above have been designed to work in combination with GPC detection.

In cellulose chemistry, the term "degree of substitution" (DS) usually denotes the number of substituted OH groups per anhydroglucose unit; with

this meaning it is most frequently used for cellulose ethers or ethers. The DS thus reflects the completeness of a chemical modification at the hydroxyl groups of the polysaccharide. However, the term DS was also used to describe the average content of CO or COOH groups per anhydroglucose unit, hence the terms DS_{CO} and DS_{COOH} have been used by analogy to illustrate the amount of functional groups in relation to the MWD.

The outputs of the respective detector signals are used as described above to calculate the DS profiles, i.e., the concentration of the respective functional group in relation to the molecular weight distribution. Figure 5 presents examples of DS_{CO} and DS_{COOH} profiles, obtained from a sulfite dissolving pulp. The DS_{CO} generally increases with decreasing molecular weight due to the increasing number of reducing end groups. The DS_{COOH} profile differs significantly from the DS_{CO} profile. In the example shown, the number and distribution of carboxyl groups is mainly governed by glucuronic acid moieties in the hemicelluloses. The signal passes through a maximum and decreases towards both small and large MW fragments.

ΔDS plots, which simply give the difference between two DS curves, facilitate the comparison of two samples with regard to their carbonyl or carboxyl contents relative to the molecular weight. ΔDS plots are very suitable graphic representations to report even slight differences in carbonyl or carboxyl contents in order to better visualize changes occurring during specific treatments starting from the same material. They allow, for instance, to analyze in a very straightforward way, how a chemical treatment increases or decreases functional groups in certain molecular weight ranges. Examples of ΔDS plots are given in the following application chapter.

Carbonyl or carboxyl group profiles can also be evaluated numerically. This way, the amount of functionalities in preselected regions of the MWD are compared, provided that the samples have similar molecular weights. Certain characteristics of the cellulose state of oxidation or the distribution of

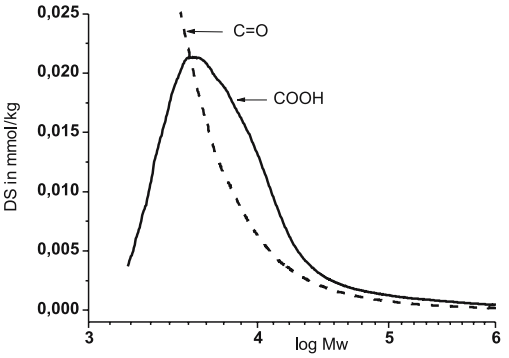


Fig. 5 Degree of substitution plots for the molecular weight dependent distribution of carbonyl groups (DS_{CO}) and carboxyl groups (DS_{COOH}) for an example pulp

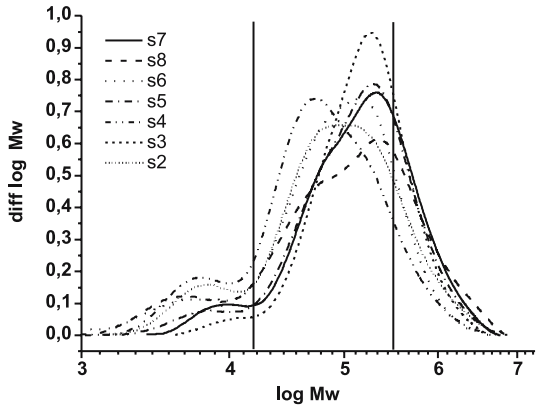


Fig. 6 MWD of different dissolving pulps (s2–s8), the *vertical lines* limit DP ranges of 100 and 2000, corresponding to the carbonyl contents in Fig. 7 below

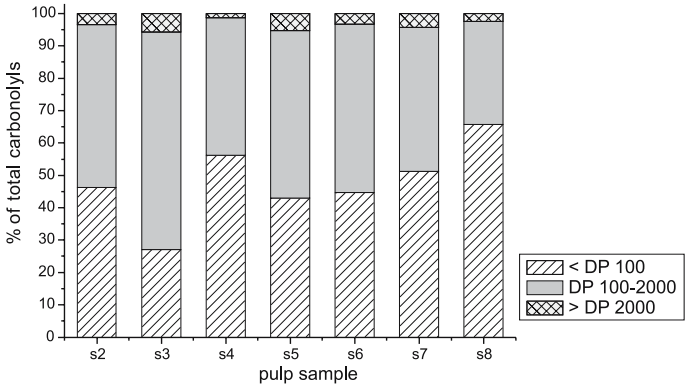


Fig. 7 Distribution of carbonyl groups over three MW regions (DP < 100, 100 < DP < 2000, and DP > 2000), corresponding to the MWD of the pulps in Fig. 6 above

hemicelluloses can easier be visualized. Figure 6 gives the MWD of different dissolving pulps, vertical lines indicating the DP 100 and DP 2000 limits. The corresponding percentages of carbonyls in these regions are displayed in Fig. 7. The data obtained may be further correlated with other pulp parameters.

3 Applications of the CCOA and FDAM Methods: Monitoring Oxidative Processes

Through combination of MWD analysis and functional group analysis it also became possible to examine how functional groups in certain regions of the

molecular weight behave during specific treatments [164]. Often the overall trend of functional groups outweighs effects occurring in specific areas (low MW or high MW parts). These effects can be examined in detail using functional group profiling. If the pulp is degraded during the treatment the profiles report both hydrolysis, by generation of new REG and decrease of M_w, and oxidation. If the MWD does not change significantly, only oxidative changes are considered. The CCOA and FDAM method are thus very powerful tools to follow cellulosic material through different processing steps with regard to oxidative changes occurring thereupon. In the following application chapter, several examples are given: different bleaching treatments, processing of Lyocell dopes, alkalization of pulps, irradiation treatments of cellulose and cellulose aging. In all of these cases, CCOA- and FDAM-monitoring were used to clarify which oxidative changes occur in which molecular weight region. The additional dependence on reaction conditions and reaction time provided mechanistic insights much more detailed than possible by any means available so far.

3.1 Bleaching Treatments

Bleaching can be considered as the major process introducing functional groups, especially carbonyls, into cellulosics. Yellowing and limited brightness stability caused problems in the early days of hypochlorite bleaching, and became an issue again with the introduction of ozone in TCF bleaching sequences. In the following, the effect of different bleaching types and agents on the carbonyl group profiles and the MWD of the bleached celluloses will be summarized.

In Fig. 8 the carbonyl DS and ΔDS plots for ozone-bleached beech sulfite pulps are shown. With increasing bleaching intensity, the ΔDS_{CO} was increased for medium and high-molecular weight regions, but dropped below the value of the starting material for the low MW range. The boundary region between DS increase and DS decrease was around M_w 10 000–20 000 g/mol. Thus, the ozone treatment comprises an interplay of carbonyl-generating and carbonyl-consuming processes according to the MW regions.

Reducing end groups represent the major part of the total amount of carbonyl groups in cellulose chains with a low DP. Upon ozone treatment, these reducing end groups are oxidized to lactones and carboxylic acids, so that an ozone bleaching stage lowered the carbonyl DS in the lower molecular weight regions. In cellulose chains with a high molecular weight, however, the contribution of reducing end groups to the total amount of carbonyls is much smaller, so that the oxidation of reducing end groups is overcompensated by the introduction of new carbonyl functions. The generation of carbonyls predominantly in higher-molecular weight material might explain the well-known observation that ozone-bleached pulps suffer a severe DP loss

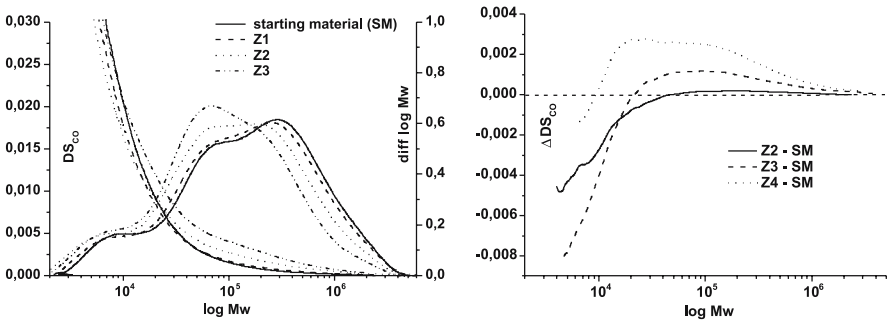


Fig. 8 Ozone bleaching (Z) of a beech sulfite pulp, increasing numbers reflect increasing intensity of the ozone treatment. *Left*: differential MWD und carbonyl DS. *Right*: ΔDS_{CO} plots showing the differences in carbonyl content between the bleached sample and the starting material (SM). Reprinted with permission from Biomacromolecules (2002) 3:969. Copyright (2002) American Chemical Society

in a subsequent P stage: when the carbonyls introduced upon ozone treatment are reduced by $NaBH_4$, the pulp is rendered largely insensitive towards the subsequent peroxide treatment [165].

With the pulp used, a very low ozone charge did not significantly increase the carbonyl content, since preferably the residual lignin present in the material was attacked by the oxidant [16]. The progressing degradation of the polysaccharide material upon further oxidation, as reflected by the decreasing M_W, is clearly visible in Fig. 8.

The effect of a peroxide bleaching stage is demonstrated in Fig. 9, which displays again differential MWD, DS_{CO} and ΔDS_{CO} graphs. A standard beech sulfite pulp was subjected to an ozone treatment followed by a peroxide bleaching stage. Again, it was obvious that the initial ozone treatment increased the carbonyl content at molecular weights above 20 000 g/mol, and decreased it below this value. Also the simultaneous DP loss was evident. The subsequent peroxide stage has a quite beneficial effect with regard to the C = O content: in all molecular weight ranges the carbonyl content was decreased as compared to the ozone-bleached material. The DS profile drops even below the level of the unbleached starting material at molecular weights below 10^5, and ranged only slightly above the curve for the initial pulp above 10^5 g/mol.

As a last example for the applicability of the CCOA method in bleaching chemistry, the effect of an intensive hypochlorite treatment of pulp (H stage) at pH 7.0, aimed at producing highly oxidized pulps, is presented (Fig. 10). The DP loss was progressing with increasing bleaching intensity. The carbonyl DS generally ranged above the values for the genuine pulp, but increased only slightly with enhanced hypochlorite charge in higher-molecular weight regions. Carbonyl functions were mainly introduced into

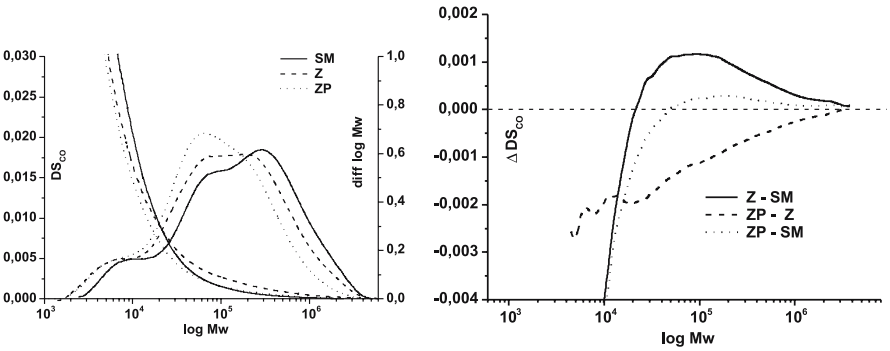


Fig. 9 Ozone bleaching (Z) of a beech sulfite pulp, followed by a peroxide stage (P). SM: starting material, Z: ozone treatment, ZP: ozone – peroxide treatment. *Left*: differential MWD and carbonyl DS. *Right*: ΔDS_{CO} plots showing the differences in carbonyl content between the bleached sample and the respective starting material. Reprinted with permission from Biomacromolecules (2002) 3:969. Copyright (2002) American Chemical Society

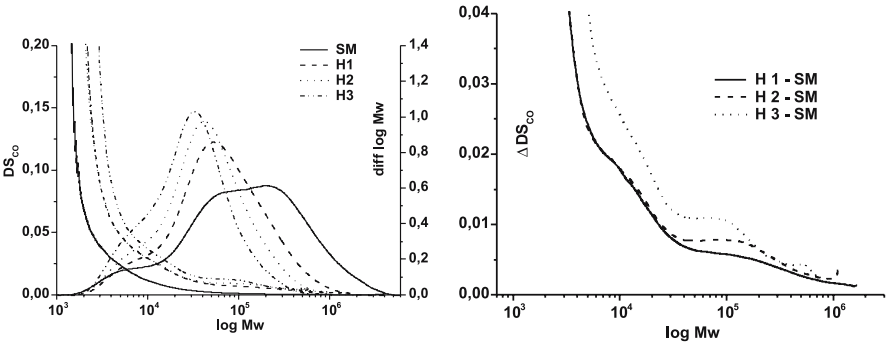


Fig. 10 Hypochlorite treatment (H) of a beech sulfite pulp, increasing numbers reflect increasing intensity. SM: starting material. Reprinted with permission from Biomacromolecules (2002) 3:969. Copyright (2002) American Chemical Society

shorter chains below a MW of about 30 000. This is a major difference to the above-discussed ozone and peroxide treatments of pulps.

In summary, ozone-bleaching increased the DS_{CO} for medium and high-molecular weight regions, but decreased the DS_{CO} for the low-molecular weight range. A peroxide treatment after ozonation decreased the carbonyl content throughout all MW regions. Hypochlorite treatments introduced carbonyl functions mainly into low-molecular weight regions, in contrast to ozone and peroxide treatments. In all three bleaching types a significant decrease in M_w was evident.

3.2 Cellulose in Lyocell Dopes

The cellulose solvent NMMO (*N*-methylmorpholine-*N*-oxide) monohydrate is a white crystalline solid at room temperature with a melting point of 84 °C. Dissolution of cellulose and further processing of the spinning dope is thus carried out at temperatures of about 100 °C. As NMMO is a relatively strong oxidant, which is also frequently used as oxidizing agent in organic synthesis [166], it exerts pronounced oxidative stress on the dissolved pulp material, which is additionally intensified by the elevated process temperatures. Oxidizing solvent and pulp interact sufficiently long to cause possible severe effects, e.g., decreased fiber properties, discoloration of the resulting fibers, thermal instabilities of the dope and even explosions and uncontrolled degradation reactions, sometimes called "exothermic events" [167]. The oxidative action of the solvent NMMO on the solute cellulose was thus a topic of highest importance.

By means of the CCOA method the carbonyl profiles of pulps upon Lyocell processing were studied. It was shown that the overall carbonyl content of the pulp decreased continuously upon dissolution in NMMO at 120 °C. This overall decrease was relatively small, but significant. A detailed evaluation, as given in Fig. 11, revealed that this overall decrease was a superposition of counteracting processes with regard to regions of different molecular weight. Up to a DP of 50, already existing carbonyl groups were consumed, which was mainly attributed to changes of the hemicellulose parts [168]. The same decrease in carbonyls, although less pronounced, was found for a molecular weight region up to DP 200. In contrast to these MW regions, the content of carbonyl groups increased moderately in high-molecular weight material above DP 200.

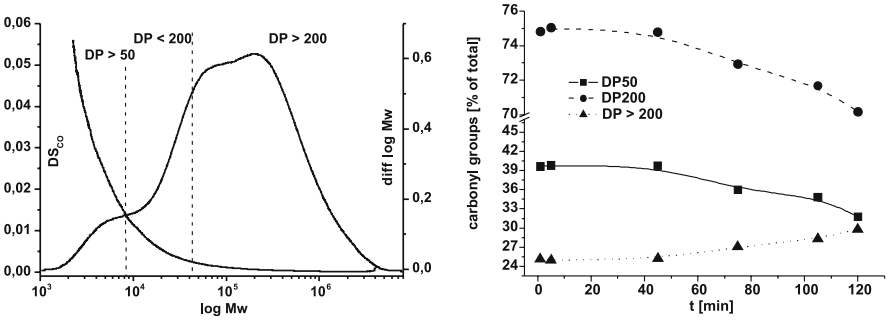


Fig. 11 Cellulose (beech sulfite pulp) dissolved in NMMO (Lyocell dope). *Left*: DS_{CO} and differential MWD of the starting pulp, molecular weights of DP = 50 and DP = 200 are indicated by *vertical dashed lines*. *Right*: Time course of the overall carbonyl content for three selected molecular weight ranges. Reprinted with permission from Biomacromolecules (2002) 4:743. Copyright (2002) American Chemical Society

In the short chain material with a high relative content of reducing end groups (e.g., one per 50 or 100 anhydroglucose units) a major reaction pathway was the oxidation of these structures to carboxylic (gluconic) acid residues, paralleling the behavior of model compounds. At the same time, it was likely that a minor amount of new carbonyl groups was generated along the chain in unselective oxidation at C-2, C-3 and C-6, which was outweighed by the oxidation of reducing ends, so that the observed overall decrease in carbonyl groups resulted. In high-molecular weight parts, in contrast, the relative amount of reducing ends was rather low (e.g., one per 500 or 1000 anhydroglucose units), so that the generation of new carbonyl groups became dominant. The introduction of carbonyl groups, especially into longer chains, was an important result as carbonyl groups have been demonstrated to be directly correlated with chromophore formation in the Lyocell system [169, 170].

In summary, processing of a beech sulfite pulp sample in NMMO at elevated process temperatures caused a net decrease of the carbonyl content. The conversion of reducing ends to carboxylic acids, being the dominant process in lower-molecular weight material, was counteracted by an unselective introduction of keto groups along the chain, which was prevailing in the high-molecular weight region. The latter process will also play a role in subsequent discoloration reactions.

3.3
Alkalization of Cellulose and Aging of Alkali Cellulose

Alkalization ("steeping") is a crucial process step in the production of cellulose derivatives. It is employed prior to certain derivatization reactions, such as xanthation in the viscose process or etherification in the production of carboxymethyl cellulose [171]. Especially in the viscose process the alkalization is not only used for activating the hydroxyl groups, but also to free the pulp from impurities, such as hemicelluloses. The steeping step involves treatment of bleached pulp with strong alkali hydroxides, mostly 18% NaOH, to convert cellulose into sodium cellulose I. Excess sodium hydroxide is pressed off and the press cake, consisting of approx. 34–35% cellulose and 15–16% NaOH, is left in the presence of air for several hours, which is referred to as aging or "ripening". In this process stage the appropriate pulp viscosity, i.e., the cellulose DP, is adjusted to the needs of further processing to viscose. The changes in the molecular weight distribution are brought about by oxidative processes [96, 173–175] which involve both introduction and conversion of oxidized functions, such as keto and aldehyde groups, and chain cleavage by subsequent alkali-induced reactions, such as β-alkoxy-eliminations. The CCOA method was employed to examine these oxidative changes: for the first time the introduction and conversion of oxidized functionalities were monitored in dependence of both time course and molecular weight distribution.

In Fig. 12, the molecular weight distribution and the carbonyl-DS curves of the genuine pulp sample, the pulp after steeping and removal of excess alkali ("pressing"), and the samples after different times of aging are shown. Both the decrease in molecular weight and the intended narrowing of the distribution over time were evident. The overall carbonyl content decreased significantly during the alkali treatment, approx. by about 50%. In contrast, the carbonyl-ΔDS plot in Fig. 13, depicting the difference curve between starting pulp and alkali pulp after steeping, indicated no significant change in carbonyls in the remaining pulp, except for the lowest molecular weight part. The overall decrease was consequently due to a removal of low-molecular weight hemicelluloses and celluloses, being no longer contained in the pulp sample so that the carbonyl functions were thus no longer detectable.

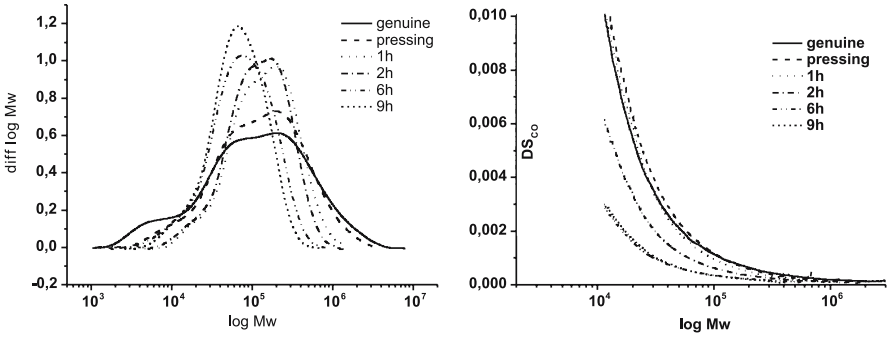


Fig. 12 Alkalization of cellulose and aging of alkali cellulose (beech sulfite pulp). DS_{CO} (*right*) and differential MWD (*left*) after steeping and different aging times. Reprinted with permission from Biomacromolecules (2002) 4:743. Copyright (2002) American Chemical Society

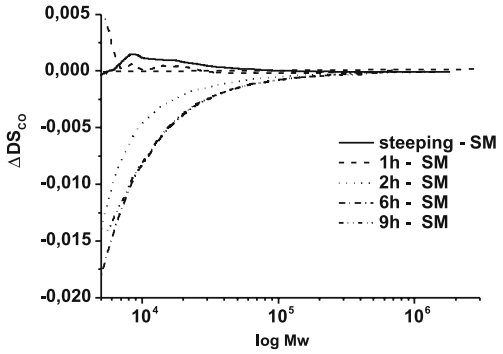


Fig. 13 Alkalization of cellulose and aging of alkali cellulose (beech sulfite pulp). ΔDS_{CO} plots for pulps after steeping and different aging times, SM: starting material. Reprinted with permission from Biomacromolecules (2002) 4:743. Copyright (2002) American Chemical Society

With increasing aging times, the carbonyl content in the pulp decreased further, as shown by the carbonyl-ΔDS plots after 2, 6 and 9 hours (Fig. 13). The main reason for this loss in CO groups was a progressing oxidation of reducing end groups to carboxyl functions, and a removal of keto groups, e.g., by β-elimination and rearrangements. After an aging time of about 4 hours the decline in carbonyls slowed down, and eventually leveled off at a low content of 4 μmol/g (Fig. 14, left). A detailed evaluation revealed once more pronounced differences in the reactivity of different molecular weight regions with regard to the carbonyl groups. In very high-molecular material with a DP > 2000, the amount of carbonyls stayed nearly unchanged (Fig. 14, right) as only very few carbonyl groups (reducing end groups) were contained. The relatively large amount of carbonyl groups and reducing end groups in lowest molecular and low-molecular weight ranges of DP < 50 and DP < 200 caused a continuous decrease during the aging procedure due to the oxidative consumption processes discussed above. Interestingly, the number of carbonyls in the mid-range of 200 < DP < 2000 was found to increase. Thus, only in this region the oxidative introduction of keto groups overcompensated the oxidative and alkali-promoted consumption of carbonyls, which dominated in the other MW ranges.

In summary, the application of the CCOA method disclosed two different processes in the alkalization and aging of cellulose, the initial removal of low-molecular weight material with a high content of carbonyls by dissolution leaving behind a carbonyl-depleted purified pulp, and the subsequent, slower process of oxidatively converting remaining carbonyl groups to carboxyl groups in the pulp. This confirms the traditional concept of alkalization [172, 173] as constructed from the results of conventional analytical methods. In the lower-molecular weight regions, large amounts of carbonyls

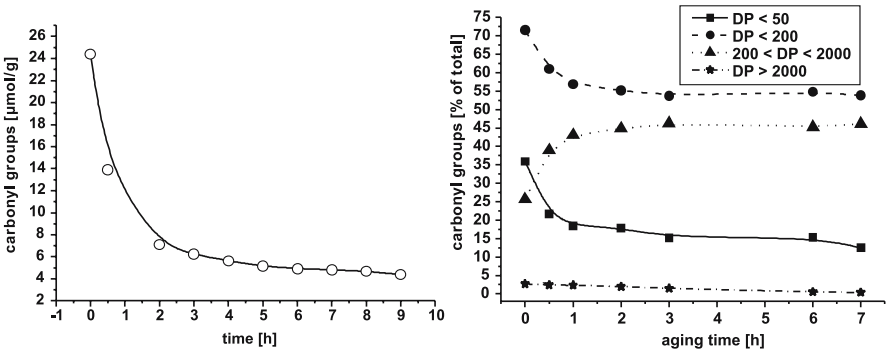


Fig. 14 Alkalization of cellulose and aging of alkali cellulose (beech sulfite pulp). Time course of the total carbonyl content (*left*) and the carbonyl content in different molecular weight ranges (*right*). Reprinted with permission from Biomacromolecules (2002) 4:743. Copyright (2002) American Chemical Society

were oxidatively removed, while in the mid-range generation of new keto groups outweighed this consumption.

3.4 Irradiation of Cellulose

Carbonyl group profiles of samples having undergone high-energy irradiation exhibited significant differences from chemically oxidized samples. Figure 15 shows ΔDS_{CO} plots of a UV-irradiation treatment with differing irradiation times. The cellulose sample used was a bleached prehydrolysis kraft pulp with the hemicellulose part being significantly lowered by alkaline extraction. This cellulose, free of lignin and most hemicelluloses, proved to be very stable under the conditions of UV radiation of up to 10 d. No significant reduction in the molecular weight was observed, although a largely uniform increase in carbonyls for the mid- and high-molecular weight areas was found—in addition to a stronger increase in the low-molecular weight range (Fig. 15, left).

How UV irradiation eventually changed the integrity of the cellulose was highly dependent on the origin of the cellulose (pulping) as well as structure and amount of the accompanying polymers (lignin and hemicellulose). While lignin acted as an antioxidant preserving cellulose in TMP pulp upon irradiation [174, 175] and unbleached pulp upon accelerated aging [176], residual lignin in prehydrolysis kraft pulp was shown to have the opposite effect. Radiation with UV light decreased the molecular weight significantly. A similar amount of residual lignin in a beech sulfite pulp, however, did not show such an effect on the DP of cellulose.

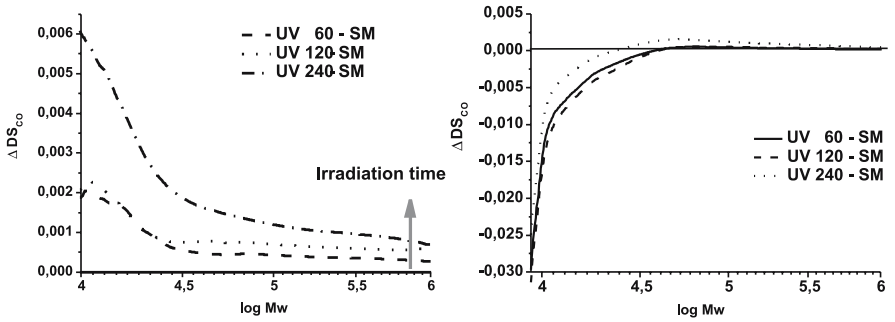


Fig. 15 UV radiation of cellulose for different times (h). ΔDS_{CO} plots of a pulp without hemicellulose (*left*) and with hemicellulose (*right*). The ΔDS_{CO} plots show the MW-dependent differences in the carbonyl content between the respective irradiated pulp samples and the non-irradiated starting material (SM). Reprinted with permission from Holzforschung (2004) 58:597. Copyright (2002) Walter de Gruyter

Also the presence of hemicellulose influenced the oxidation of the cellulose by UV light. After removal of the hemicelluloses, the UV irradiation caused significant introduction of carbonyls into the low-molecular weight part. A strong decrease in carbonyls of the low-molecular weight cellulose was found when hemicellulose was present, see the ΔDS_{CO} plot in Fig. 15 (right). Thus, the hemicelluloses responded differently, somehow acting as a sacrificial substrate which is further oxidized to carboxyls, saving the cellulose from being oxidized itself.

β-Irradiation, usually called "electron beaming" or "e-beaming", was found to induce severe changes due to the much higher energy input as compared to UV. The cellulose was significantly degraded, and this loss in DP was accompanied by the introduction of carbonyl groups over the whole molecular weight range (Fig. 16). In comparison to UV radiation, the amount of carbonyls introduced by e-beaming was about threefold.

In summary, from the studies on different types of oxidative modifications of celluloses it can be generalized that chemical means affect the low-molecular weight areas primarily. Higher oxidant dosages or more severe oxidation conditions increased the effect also on the high-molecular weight parts. By contrast, irradiation by UV light or by electron beams caused a constant increase in carbonyl groups also in the mid- and high-molecular weight areas. It was concluded that chemical oxidations, carried out under heterogeneous conditions, mostly influenced the readily accessible less ordered regions in cellulose, while radiation treatments were also able to penetrate into well ordered "crystalline" regions, causing a more uniform oxidation of the material over all MW areas.

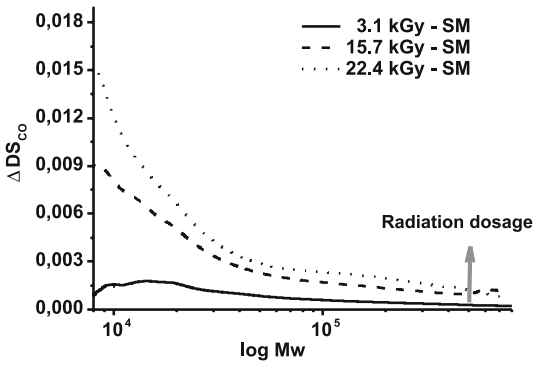


Fig. 16 ΔDS plots for β-irradiation (electron beaming) of cellulose with different dosages. The ΔDS_{CO} plots show the MW-dependent differences in the carbonyl content between the respective irradiated pulp samples and the non-irradiated starting material (SM). Reprinted with permission from Biomacromolecules (2002) 4:743. Copyright (2002) American Chemical Society

3.5 Aging of Cellulose in Paper

Two major processes are involved in aging of paper, hydrolysis induced by the presence of water and acids, and oxidative processes triggered by different factors such as metal ions, air contaminants, or simply the presence of air over long periods of time. Both pathways cause an increase in carbonyl and carboxyl groups. However, aging is also influenced by endogenic factors of the paper itself. Origin of the cellulosic material, pulping and papermaking procedures and especially papermaking additives, such as sizing agents, play a crucial role in this respect. Storage, mainly under ill-defined conditions, sometimes over centuries in the case of historical papers and documents, is an additional unknown variable. A straightforward assessment of the mechanisms of paper aging is thus rather problematic. The following section will illustrate how CCOA and FDAM method can assist in conservation and aging studies.

The molecular weight distribution of rag papers from four centuries is shown in Fig. 17. The highest M_w in this example was observed for the oldest paper. Endurance and stability of rag paper is highly influenced by the provenience of the material, the papermaking procedure and the storage conditions. Thus, no conclusion as to the age can be drawn from the molecular weight distributions measured.

A common feature observed at naturally aged rag paper samples is the relatively high amount of carbonyl groups at the low- and mid-range of MW, approx. below 10^5 g/mol or DP 6000. Roughly 50% of all carbonyls fall below DP 300. From the amount of reducing ends as estimated from M_n it was

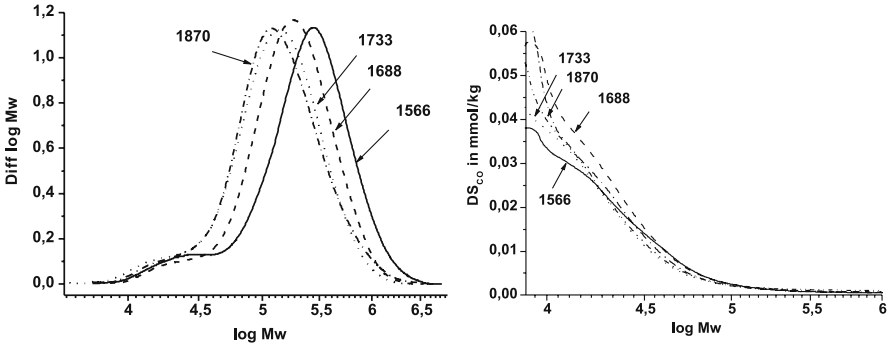


Fig. 17 Molecular weight distribution (*left*) and DS_{CO} plots (*right*) of rag papers from different centuries. Data given in the graphs correspond to the approximate year of production. The total amounts of carbonyl groups and the REG estimated from M_n (in parenthesis) were as follows: 1566 – 17.7(7.5) μmol/g; 1688 – 21.8(8.1) μmol/g; 1733 – 24.2(11.8) μmol/g; 1870 – 25.9(10.7) μmol/g

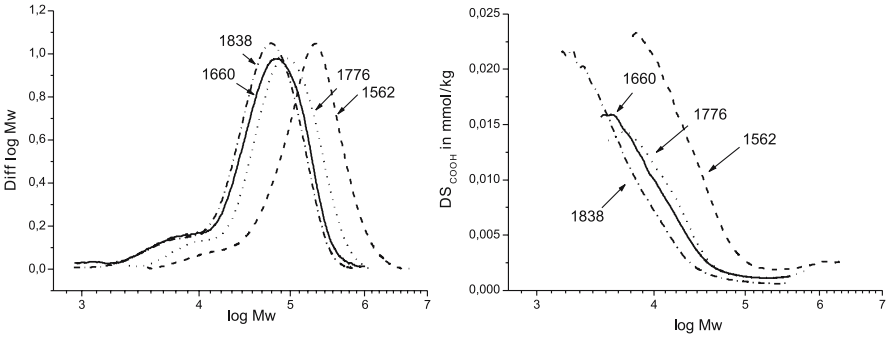


Fig. 18 Molecular weight distribution (*left*) and DS_{COOH} (*right*) of rag papers from different centuries. Data given in the graphs correspond to the approximate year of production. The total amounts of carboxyl groups were as follows: 1562 – 23.6 μmol/g; 1660 – 20.5 μmol/g; 1776 – 17.3 μmol/g; 1838 – 15.44 μmol/g

calculated that the amount of keto functions, which are related to oxidative processes, is about 50% of the total carbonyls, while the remaining half is attributed to reducing ends that are naturally present or a result of hydrolysis. Rag paper samples having experienced accelerated aging exhibited carbonyl profiles comparable to naturally aged material.

The MWD and carboxyl group distributions for different rag papers are given in Fig. 18. The amount of carboxyl groups also increased in the low MW region. Hence, it was concluded that during natural aging oxidation preferentially occurred in the low MW region, or that the progressing degradation of rag paper produced higher amounts of acidic low-molecular weight material with consequently increased amounts of carboxyl groups in this region.

3.6 Visualization of Oxidized Groups on Paper Surfaces

Analysis of historic paper materials and chemical processes occurring at specific locations of paper, such as ink or pigment lines, often calls for a visualization method. Since CCOA and FDAM emit fluorescence in the UV region, these labels cannot be used for direct imaging. Hence, other chromophores were applied, but the labeling principle and the underlying chemistry was maintained [163]. A representative example is given in Fig. 19, showing a model paper with a grid of copper pigments subjected to accelerated aging. Next to the pigment lines hydrophobic areas developed which became visible upon wetting with water (Fig. 19, left). These hydrophobic areas coincided with locations of higher oxidative damage in terms of a higher concentration of carbonyl groups. This was caused by migration of copper ions into the surrounding paper parts as shown by laser ablation MS techniques. The oxidative injure was visualized using a fluorescence label

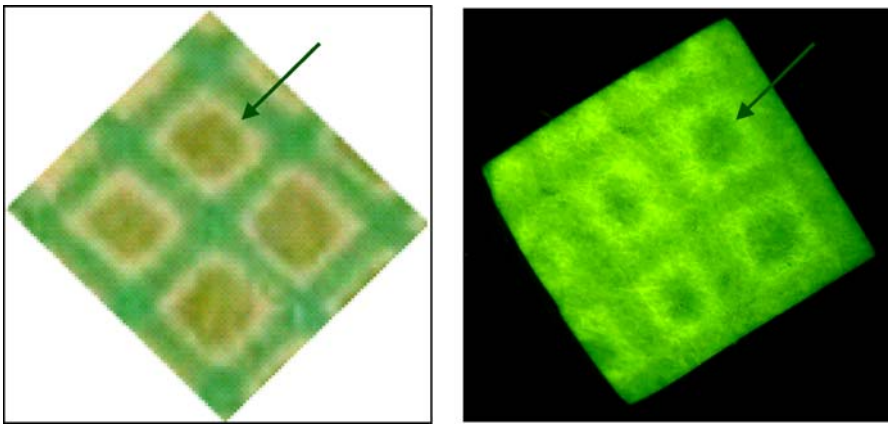


Fig. 19 Simulated copper corrosion in paper. Wetting (*left*) and fluorescence labeling (*right*) to visualize areas of pronounced oxidative damage

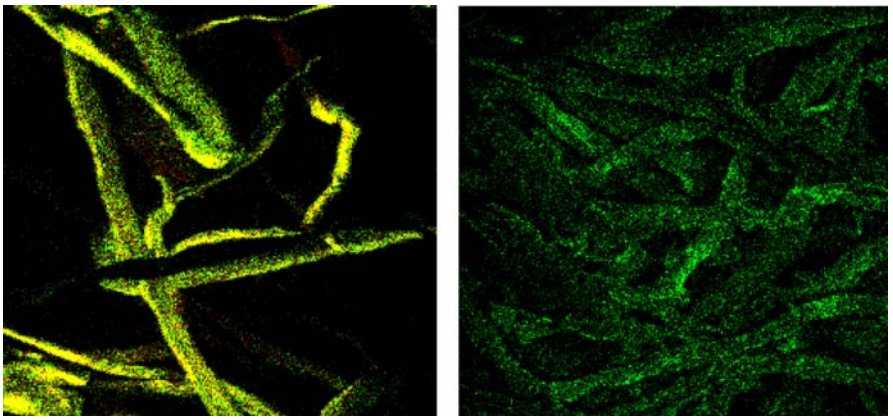


Fig. 20 Group selective labeling agents for chemical microscopy by TOF-SIMS. Analysis of a CCOA-labeled (*right*) and an FDAM-labeled beech sulfite pulp

emitting in the visible region, showing a stronger fluorescence emission for the hydrophobic, stronger oxidized areas (Fig. 19, right). Hence, also relatively minor spatial differences in oxidation states of paper surfaces can be made visible.

CCOA and FDAM are also suitable labels for surface analysis by TOF-SIMS. Both labels exhibit a distinct mass spectrum which can be used to visualize the surface distribution of carbonyl or carboxyl groups across single fibers by chemical microscopy (Fig. 20) (courtesy of Fardim P, Abo Academy Turku, Finland).

3.7 Outlook

The application of fluorescence labels in combination with GPC can be considered a step forward in the analysis of oxidized functionalities in cellulosics. However, a large number of questions still remain to be addressed in the future. If oxidized functionalities are considered as "substituents" along the polymer chain of cellulose, then a thorough analysis of the substituent distribution within the cellulose chains and per anhydroglucose unit should provide many new insights. The differentiation of aldehyde and keto functions will be a next step. Also the exact position of carbonyls (keto or aldehyde) within the AGU needs to be resolved, and differences in their reactivity determined. Furthermore, it is an open question whether oxidation occurs statistically within cellulose chains or forms clusters of highly oxidized areas.

With selective labels available, visualization of oxidative damage in all spatial dimensions of paper materials will become a major issue, especially with regard to conservation issues and restoration of historical paper documents. For the determination of carboxyl groups improvements in the reliable quantification of lactones can be considered a future challenge.

The analysis of oxidized groups in celluloses will remain a hot topic in cellulose chemistry and analytics, as we are only beginning to understand the enormous importance of these groups in governing chemical behavior and macroscopic properties of cellulosic material.

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