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The FDAM Method: Determination of Carboxyl Profiles in Cellulosic Materials by Combining Group-Selective Fluorescence Labeling with GPC

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A novel method for accurate determination of the carboxyl content in cellulosic materials by fluorescence labeling with 9*H*-fluoren-2-yl-diazomethane (FDAM) has been developed. The procedure can readily be implemented into a GPC system with RI and MALLS detectors, requiring additional fluorescence detection. The labeling conditions were optimized by means of sugar acid model compounds and were transferred to the cellulose case. Kinetics of the labeling and the influence of reaction parameters were comprehensively studied. For the first time, carboxyl profiles of cellulosics, i.e., the carboxyl content relative to the molecular weight distribution, were obtained.

Introduction

Carboxyl groups in cellulosics are not naturally present. They are moreover introduced by different production processes, such as pulping, bleaching, and processing of cellulosic materials and fibers, or they are the result of natural aging, irradiation impact, or oxidative stress. They also can originate from hemicelluloses still contained within the cellulosic material.

Oxidized positions along the carbohydrate chain constitute spots of a distorted microenvironment, which results in pronounced chemical instability and altered properties. Carboxyl groups—or oxidized functionalities in cellulosics in general—are the cause of many macroscopic phenomena, even though they are present in very low concentrations only (μmol/g range). They are held responsible for yellowing and aging processes, strength loss, and decreased performance of cellulosic materials, being involved also in development of paper surface charge and chemical retention during paper making. Metal binding onto pulps is facilitated by carboxyl groups. Spinning problems of cellulosic fibers (viscose, acetate) as well as chromophore formation in the Lyocell process are linked to some extent to increased amounts of carboxyl groups. Decreased thermal stability of papers is another facet of the presence of such acidic groups. Furthermore, when it comes to paper permanence, carboxyls are involved in losses of strength and brightness, besides carbonyls. The COOH levels increase upon paper aging and through corrosion by ancient inks or color pigments as the major triggering factors in historic paper materials. Hemicelluloses are a major source of carboxyl groups—present as uronic acid moieties in cellulosic pulps. This short compilation may show how large the range of macroscopically relevant changes of properties is that can be affected by small changes in the carboxyl content of cellulosics.

Generally, conventional methods for carboxyl quantification in carbohydrate oligomers and polymers provide only an estimate of the overall content, which is a sum parameter. All these methods rely on the conversion of free acids into salts, and are thus basically mere variations of an acid−base titration. In other words, these traditional ways of carboxyl determination in cellulosics depend on the cation exchange capacities of the material. The various approaches differ mainly in sample preparation, in the cation used, and in the direct or indirect measurement of the exchanged cations. Direct alkalimetric titration of the fully protonated carboxyl group with NaOH was used, as was ion exchange with chromophoric cations. The most common technique, the direct[1] or reversible[2] methylene blue method, is based on the quantification of the colored organic base, measuring either its absorption to the polymer or its depletion in the titer, respectively. Major problems arise with accessibility, nonspecific adsorption, and reproducibility. The situation is additionally complicated by the extremely low average contents of cellulosic carboxyls in the μmol/g range, but still, this method has been widely used due to the lack of alternatives. Philipp et al. have summarized titrimetric carboxyl determination methods.[1] In addition to those, decarboxylation and quantification of the formed CO_2 has been reported for carboxyl quantification.[3]

Presently, the reversible methylene blue method,[4] the sodium bicarbonate procedure according to Wilson,[5] and the zinc acetate method[6] are widely applied in pulp and paper laboratories. They require large amounts of material, and naturally yield no information on the molecular weight related distribution of the carboxyls. By far the major drawback is the low reproducibility: All these methods generate values of severely deficient comparability, in both interprocedural and interlaboratory comparisons. This was again demonstrated in a recent round-robin test.[7]

With this situation in mind, finding a novel and better defined approach to determine carboxyl groups in cellulose seemed valuable. To gain more information on the localization of such groups in relation to the molecular weight, we selected a combination with gel permeation chromatography (GPC)—as realized for carbonyl functionalities by the CCOA method recently developed in our laboratory.[8−10] Fluorescence spec-

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troscopy was to be used as the reporting method, as it is highly sensitive and reports minute amounts of oxidized structures. To circumvent problems of nonspecific adsorption and ill-defined reactions, we aimed at a covalent derivatization ("labeling") of carboxyls as esters. The required compatibility with subsequent GPC analysis imposed a number of further limitations: The reaction must proceed either in aqueous solution or in the somewhat "exotic" solvent *N*,*N*-dimethylacetamide (DMAc), and the label must be stable under GPC conditions using DMAc/LiCl (9−0.9%) as the GPC solvent and eluant. Most importantly, any conditions impairing the integrity of the polysaccharide chains were to be avoided, calling for labels of high reactivity and mild derivatization conditions at the same time. In addition, the attached label should emit fluorescence light not interfering with the multiangle laser light scattering (MALLS) detection wavelength (e.g., at 488 nm or higher), it should have a high fluorescence yield to guarantee that even the small amounts of carboxyls present in cellulosics can be reported, and it must be stable under derivatization and GPC conditions.

The following individual tasks were necessary, which will be described in detail in the present part of our studies:

(1) Selection of an appropriate fluorophore and anchor group;

(2) Optimization of the derivatization reaction by means of oxidized carbohydrate model compounds, which includes comprehensive kinetic studies in terms of reaction temperature, reagent concentrations, reagent stability, product stability, catalysts to be used, solvent composition, and side reactions;

(3) Transfer of the elaborated methodology from model compounds to genuine cellulosic material and fine-tuning of the reaction conditions and kinetic studies;

(4) Incorporation as off-line derivatization into a GPC system for cellulose analytics;

(5) Calibration of the method and optimization of the GPC conditions.

Materials and Methods

Chemicals. Chemicals were obtained from commercial sources and were of the highest purity available. DMAc was obtained from Promochem Chemicals, Germany. Pulps from different origins and sources were used (e.g., beech, Mg sulfite pulp, TCF bleached 92% ISO brightnsess; eucalyptus prehydrolysis kraft pulp, TCF bleached, 91.7% ISO brightness). Activation of pulp samples was carried out as published recently.[8] (Methyl β-D-glucopyranosid)uronic acid and (methyl 4-*O*-methyl β-D-glucopyranosid)uronic acid were synthesized according to a recently published procedure.[11]

General Analytics. Column chromatography was carried out on silica gel 60 (0.04−0.063 mm). 2D fluorescence spectra were obtained on a Hitachi F-4500 and Agilent FLD G1321A. GPC measurements used the following components: online degasser Dionex DG-2410, pump Kontron 420; puls damper, autosampler HP series 1100, column oven Gynkotek STH 585; fluorescence detector Agilent FLD G1321A; MALLS detector Wyatt Dawn DSP with argon ion laser ($\lambda_0 = 488$ nm), RI detector Shodex RI-71. Data evaluation was performed by the standard Chromeleon and Astra software and GRAMS/32AI.

GPC System. For GPC measurements, the system as described earlier[8] was used, with DMAc/LiCl (0.9%, m/V), filtered through 0.02 μm filter, as the eluant. The sample was injected automatically, chromatographed on four serial GPC columns, and monitored by fluorescence, MALLS, and refractive index (RI) detection. Molecular weight distribution and related polymer-relevant parameters were calculated by software programs, based on a refractive index increment of 0.136 mL/g for cellulose in DMAc/LiCl (0.9% m/V).

The following parameters were used in the GPC measurements: flow, 1.00 mL/min; columns, four PL gel mixed A LS, 20 μm, 7.5 × 300 mm; detectors, MALLS-fluorescence-RI; fluorescence detection: excitation, 252 nm; emission, 323 nm; injection volume, 100 μL; run time, 45 min.

General Procedure for the Determination of Carboxyls in Pulp by Heterogeneous Fluorescence Labeling. For pulp preconditioning, pulp corresponding to 20 mg of dry pulp was suspended in 0.1 M HCl and agitated for 20 s in a mixer. The pulp was washed with 0.1 M HCl, ethanol 96%, and DMAc, and filtered and transferred into a 4 mL vial. For derivatization, the pulp was suspended in 3 mL of DMAc, and 1 mL of FDAM solution (approximately 0.125 mol/L in DMAc) was added. The suspension was agitated in a shaking bath at 40 °C for 7 d. The pulp was filtered off, washed with DMAc, and transferred into a dry vial. For dissolution of the cellulose, 1.6 mL of DMAc/LiCl 9% (m/v) was added. After complete dissolution with no visible residues, the sample was diluted and filtered through 0.45 μm filters.

Results and Discussion

Selection of the Carboxyl Label. The major difficulties on the way to a reliable labeling protocol for carboxyls in cellulosics were low carbonyl reactivity of the carboxyl group and the mild conditions required to make sure that the polysaccharide chain remained integer. Generally, there were two basic approaches to mild esterification: activation of either the acids or the reagents. The first variant, widely used in protein[12,13] and fatty acid analytics,[14−16] did not provide satisfactory results with low molecular weight carbohydrate compounds and celluloses, although several reagents (based on carbodiimide and triazine coupling agents) were tested under a wide variation of reaction conditions. A prominent example of the second variant is 4-bromomethyl-7-methoxycoumarin, that is a frequently applied reagent for the derivatization of fatty acids, α-hydroxy acids, or heterocyclic carboxylic acids.[14,16] However, this type of reagent failed to react neatly with the corresponding sugar acids. We thus turned our interest toward aromatic diazomethane reagents, which are more stable than their aliphatic counterparts and carry a fluorescence-active moiety in addition. Aromatic diazomethane derivatives do not require an activation step, traces of water do not significantly interfere with the conversion, and the reaction proceeds under mild conditions (room temperature, no auxiliaries necessary) to afford stable esters.

The first promising derivatization example was the coupling of (methyl β-D-glucopyranosid)uronic acid and similar carboxyl carbohydrate model compounds with the commercially available UV-label 1-(4,5-dimethoxy-2-nitrophenyl)-diazoethane, which gave nonoptimized yields above 50%. However, the marker had too low an extinction coefficient to operate in combination with a GPC UV detection, so that no carboxyl group profiles of cellulose could be recorded. Furthermore, its fluorescence properties were inferior, so that fluorescence detection was similarly ruled out. The diazo labels described in the literature were inappropriate for our purposes, as their fluorescence characteristics did not fit our spectral requirements (no interference with the MALLS wavelength), some of them suffering in addition from limited stability. Consequently, we set out to synthesize several diazo-based fluorescence labels, to find a reagent which would comply with the special demands of our approach.

Finally, 9*H*-fluoren-2-yl-diazomethane (FDAM, **3**) proved to be the reagent of choice that met all our requirements. FDAM was readily prepared in the GPC eluant DMAc from 9*H*-fluoren-2-yl-carboxaldehyde (**1**) via its hydrazone **2**[17] by oxidation with excess manganese dioxide. The oxidant is completely removed by filtration so that the derivatization solution contains only the diazo reagent (**3**) and the corresponding inert azine. The

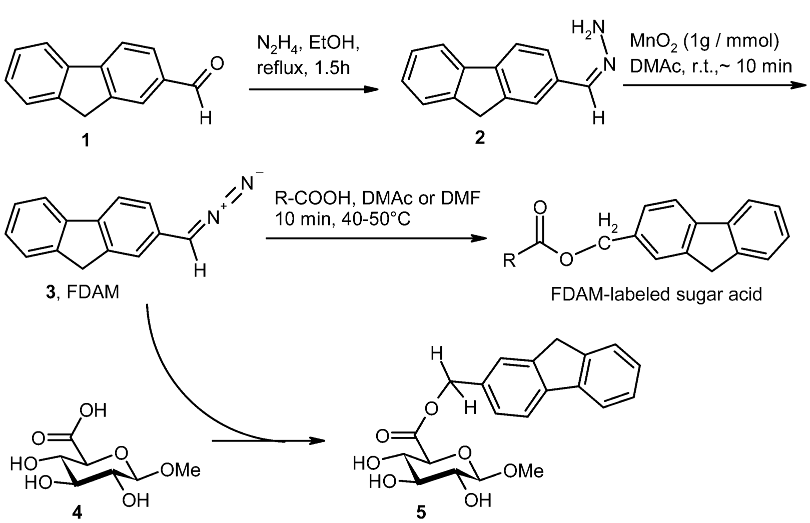

Figure 1. Synthesis of the carboxyl label FDAM and its reaction with sugar acids in general (RCOOH) and model compound **4**.

stability of the reagent was convincingly high, excess reagent being readily detectable by its red color even after days. With an excitation maximum of 252 nm, the label emits fluorescence with a maximum of 323 nm[18] in DMAc/LiCl (0.9% m/V), far off the range of MALLS detection. The fluorescence properties of the label were independent of the carboxyl compound labeled. The neutral derivatization conditions were fully compatible with the integrity of both cellulose and carbohydrate model compounds. Details on the derivatization of the latter and complete analytical data of FDAM-labeled model compounds have been reported elsewhere.[11] Synthesis of the label, the general FDAM-labeling reaction with sugar acids, and the conversion of the model compound (methyl *β*-D-glucopyranosid)uronic acid (**4**) into (9*H*-fluoren-2-yl-)methyl (methyl-*β*-D-glucopyranosid)-uronate (**5**), are shown in Figure 1.

Optimization of the Labeling Procedure. *Reaction Conditions.* Our general approach consisted of a selective fluorescence labeling of carboxyls, i.e., prior to dissolution of the cellulosic materials or model compounds in DMAc/LiCl and subsequent GPC analysis with multiple detection. The first part of the optimization was concerned with finding optimum conditions for the reaction of the label with carboxyl-containing low molecular weight carbohydrate model compounds. Temperature, reagent concentration, auxiliary type and concentration, sample−reagent ratio, reaction time, and solvent were varied as parameters. Synthesis of the carboxyl model compounds and their reaction with FDAM has already been described.[11] The second part consisted of the transfer of the conditions optimized with model compounds to the “polymer case”, i.e., the application of the labeling procedure to different cellulosics, followed by a fine-tuning of the procedure.[19] In addition, some peculiarities of the reaction with cellulose had to be considered, which go beyond reactions with low molecular weight models as described in the following.

At a reaction temperature of 4 °C, the labeling reaction proceeded rather slowly, being incomplete even after 6 days, as seen in Figure 2. At 30 °C and even better at 50 °C, the reaction proceeded smoothly. At 70 °C, the labeling was very fast, but at extended reaction temperatures, the number of labeled groups decreased slightly, which indicated a temperature sensitivity of the fluorenyl methyl esters produced. As a result, standard labeling is performed at 40 °C reaction temperature throughout.

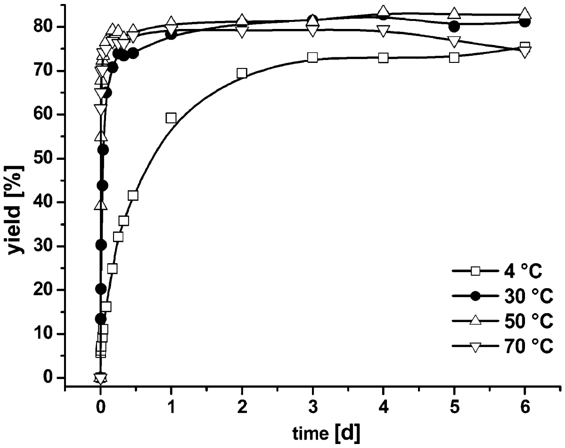

Figure 2. Effect of the reaction temperature on the formation of the FDAM coupling product **5** from uronic acid **4**.

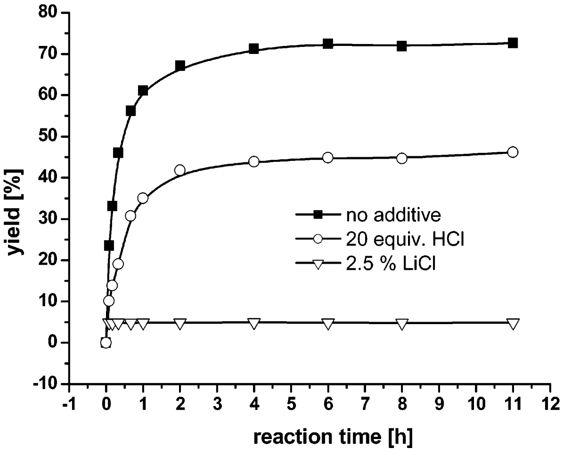

Figure 3. Effect of additives on the formation of the FDAM coupling product **5** from uronic acid **4**.

For completeness, the effect of several additives on reaction rate and yield were tested; some examples are shown in Figure 3. Both acidic and basic auxiliaries impeded the reaction. In the case of ethereal HCl, 20 equiv relative to the carboxyl content for the reaction with cellulose model compounds were used. Because of the large excess of FDAM, the negative outcome cannot be due to a consumption of the labeling agent only. It can be speculated that the presence of both label and ethereal HCl induces side reactions of some carboxyls in the cellulose, which makes them inaccessible for labeling, for instance, lactone formation.

Interestingly, the addition of LiCl into the derivatization solvent had the most detrimental effect. Only a small fraction of carboxyls was labeled, but soon, the reaction stalled at a very low yield level. Evidently, the carboxyls are prevented from reacting with the fluorescence label by the salt addition. It should be noted, however, that this process cannot consist of a simple salt formation, as the equilibrium in eq 1 is always shifted far to the right due to the much higher acid strength (pK_A) of HCl as compared to organic carboxyls in cellulose. It is more likely, instead, that the LiCl anchors the carboxyl protons in tight hydrogen bonding so that they are rendered unavailable or much less reactive for the labeling reaction.

$$\text{Cellulose-COOLi} + \text{HCl} \rightarrow \text{Cellulose-COOH} + \text{LiCl} \quad (1)$$

Variation of FDAM excess was performed between 8-fold and 741-fold equivalent amounts relative to the estimated carboxyl contents in the pulps (estimation by the methylene blue method). As was confirmed by the model studies, a 50-fold excess proved to be completely sufficient for all pulps used. Higher concentrations of FDAM gave no measurable gain in yield and reaction rate. According to the standard labeling protocol, an FDAM concentration was used that corresponded to a 50-fold molar excess relative to a carboxyl group content of 100 μmol/g pulp. This covers, by far, most pulps of practical relevance; for special cases, such as oxidized materials or pulps carrying carboxyl-containing reagents, the ratio must be adjusted accordingly.

An important practical question regarding the labeling protocol was whether it would be best to conduct the reaction under homogeneous or heterogeneous conditions. In initial runs, the labeling with FDAM was carried out homogeneously, i.e., after dissolving the pulp in DMAc/LiCl (9%, m/v) and diluting to 6.4%, 2.5%, and 1.6% LiCl (m/v) prior to derivatization. According to the homogeneous procedure, a slight, but noticeable, degradation of the polymer was observed, which was a definitive drawback as compared to the heterogeneous alternative. Taking into account that the homogeneous approach is by far more laborious—as the pulp has to be precipitated to remove excess label and redissolved for GPC analysis—it was easy to select the heterogeneous procedure as the protocol of choice. Interestingly, also in the case of the CCOA labeling of carbonyls in pulps, the heterogeneous variant proved to be superior to the homogeneous alternative. As result of the optimization, the standard FDAM labeling procedure was elaborated as described in the Experimental Section.

Pulp Preconditioning. As described so far, the variations in the reaction conditions were aimed at converting as many of the *free* pulp carboxyls into the labeled esters. However, there should also be a minor fraction of *bound* carboxyls—present as salts, lactones, or other forms inert toward the labeling agent. Thus, especially when working with real-world pulp samples having undergone a number of processing steps, the labeling could be hampered by various processes, such as salt formation by metal ions (which corresponds to a "deprotonation" of the acid) or formation of lactones and other esters.

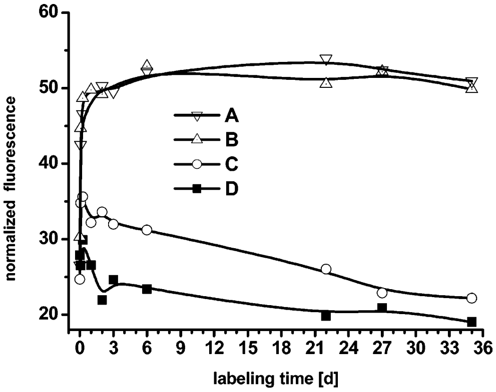

Figure 4. Effect of pulp treatments on the FDAM-labeling reaction of cellulosic pulps. A, DMAc-activated pulp, labeled in pure DMAc; B, air-dried pulp, labeled in pure DMAc; C, air-dried pulp, labeled in aqueous $ZnCl_2$; D, air-dried pulp, labeled in zinc acetate buffer, pH 4.

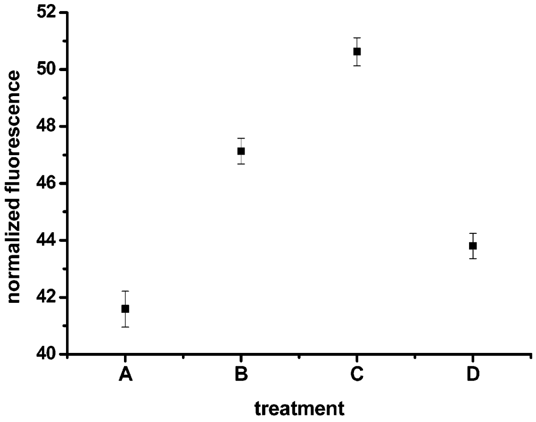

Figure 5. Effect of solvents and solvent exchange on the labeling with FDAM in DMAc, measured as overall fluorescence yield. Treatment A: mixing in water, solvent exchange via ethanol to DMAc. Treatment B: mixing in 0.1 M HCl, washing with water, solvent exchange via ethanol to DMAc. Treatment C: mixing in 0.1 M HCl, solvent exchange with ethanol to DMAc. Treatment D: mixing in DMAc.

The results obtained for the preconditioning of the pulp agreed with the outcome of adding LiCl to the derivatization mixture. A treatment with zinc chloride or zinc acetate, used, for instance, to open pulp lactones to prevent their reaction with hydroxylamine according to the traditional carbonyl determination method,[20] had a pronouncedly negative effect (Figure 4). The activation of the pulp by suspending in DMAc and subsequent freeze-drying, as sometimes used in the CCOA labeling protocol, gave no significant improvement.

Much screening work was invested into finding the optimum procedure for pulp preconditioning, giving the highest amounts of labeled carboxyls in the subsequent GPC run. The most promising protocol was a short-time suspension (approximately 20 s) by mixing the pulp in 0.1 M aqueous HCl followed by thorough washing with ethanol and DMAc prior to labeling. Generally, suspending the pulps in a polar solvent enhanced yield and reactivity by improving accessibility. Likewise, treatment with a strong (according to the pK_S) inorganic acid boosted the yield by converting salts into the free acid form, thus performing a metal ion-to-proton ion exchange (Figure 5). Even though the overall contact time between pulp and acid was rather short (less than 1 min), this step was crucial. It should be noted that an aqueous washing after suspending the pulp in the acid lowered the yield again. Immediate washing with ethanol and DMAc, i.e., solvent exchange from aqueous to protic to polar aprotic medium, yielded the best results.

Naturally, the integrity of the cellulose in the pulp sample after preconditioning and labeling was a crucial issue, especially as an acidic preconditioning step was involved, although being rather short. Thorough GPC screening demonstrated that the molecular weight distribution was unchanged by suspending the pulp in water and in 0.1 M HCl, even for much longer times

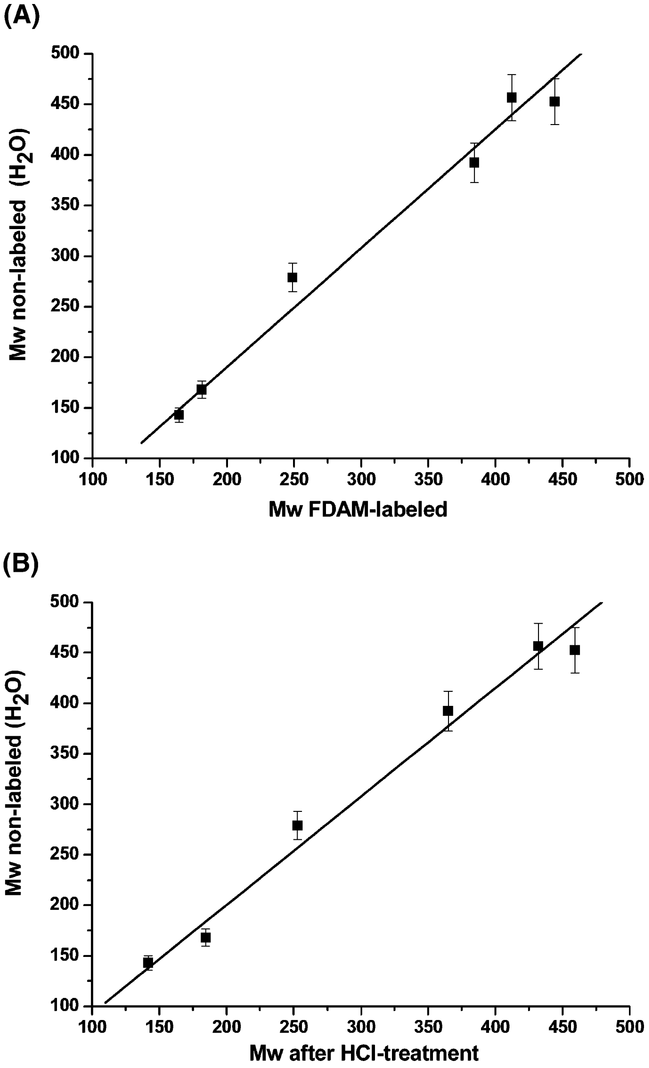

Figure 6. Effect of FDAM labeling and HCl pretreatment on the M_w of the pulp. (A) Correlation between FDAM labeled and nonlabeled pulp; slope 1.17. (B) Correlation between nonlabeled pulp treated with water and treated with 0.1 M HCl; slope 1.07.

than used in the standard mixing procedure (20 s at room temperature). More importantly, the molecular weight distribution remained unchanged throughout the whole labeling procedure, which is a prerequisite if the genuine pulp data and carboxyl profiles shall be monitored. In summary, it is safe to state that neither the pulp preconditioning protocol nor the actual labeling procedure (7 d at 40 °C in DMAc) caused a degradation of the cellulosic materials, which was larger than the analytical error of the M_w measurement (Figure 6).

Calibration of the Analytical System. For determination of the absolute content of carboxyl groups in cellulosic materials, the analytical system must be calibrated. This requires a calibration standard with the same excitation and emission characteristics, and also the same fluorescence yield, as labeled pulp under GPC conditions. Since these requirements were fulfilled for the labeled sugar acid **5** (cf. Figure 1), this model compound was chosen as a calibration standard. To level out differences during labeling and workup, and in order to avoid repeating the rather laborious calibration procedure every GPC batch, well-characterized standard pulps were thoroughly calibrated with the model compound and were then further used as internal calibration standards in each GPC batch.

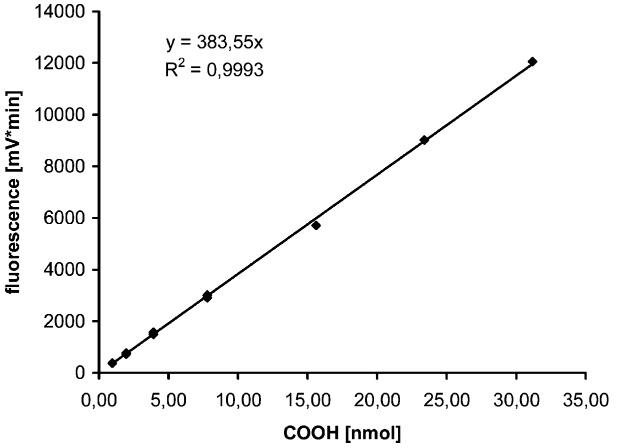

Figure 7. Calibration of the analytical system with model compound **5**.

Upon injecting differently concentrated solutions of compound **5** into the GPC system, the fluorescence was detected in the salt peak, which was rather narrow (about 3 min, in contrast to approximately 10−15 min elution time for labeled pulp). Thus, the peak height exceeded in some cases the dynamic range of the detector. To circumvent this problem, we used repetitive injections of smaller portions: Instead of injecting the calibration standard at once, the injection volume was quartered and injected four times in 1 min intervals. The peaks obtained according to this injection mode were fully comparable to peaks from labeled pulp with regard to both peak width and height. For calibration, the run time was prolonged due to the slow elution of the low molecular weight compounds. From the calibration curve, which showed satisfying linearity over the whole calibration range (Figure 7), the amount of carboxyls in the labeled pulp—most commonly given in the unit as μmol/g of cellulose—was directly determined. The injected mass of cellulose was calculated from the integral of the pulp peak in the refractive index (RI) detector signal, with the RI constants having been thoroughly determined beforehand. Validation of the complete analytical procedure will be presented in part 2 of this study.

FDAM Labeling: Characteristics and Example Applications. *Stability of Label and Labeled Structures.* FDAM solution is freshly synthesized up to a day before the labeling is carried out. The solution can be kept at 4 °C for at least one week without significant reagent loss. During labeling of the pulp, the presence of active FDAM can be easily recognized by the red color of the solution, which remained visible even after 14 days. Loss and replacement of FDAM during the labeling time (7 d) was no issue, as addition of fresh reagent solution during the labeling did not change the outcome in any way.

To make sure that all labeled carboxyls are actually reported by fluorescence detection, the stability of labeled carboxyls—both in models and in pulp—was tested. The corresponding fluoren-2-yl methyl cellulose esters were subjected to prolonged treatments with DMAc/water mixtures (0%, 0.1%, 1%, 9%, 33%, 50% water (v/v)), and DMAc/LiCl (3.6%, injection concentration) for up to four weeks at room temperature (rt) (Figure 8). All these treatments did not impair the integrity of the labeled moieties, and there was no reduction of the fluorescence yield per mass of cellulose. Even a treatment in MeOH/HCl (0.1 M) for 5 h at rt did not result in any de- or transesterification, and the change in the measured fluorescence per mass remained within the error of analysis.

Which Carboxyl Groups Are Labeled. Carboxyl groups in polysaccharides are mainly present as uronic acid groups at C-6 of pyranose units (in cellulose or hemicelluloses). Relatively few onic acids at C-1, such as oxidized “reducing ends” of

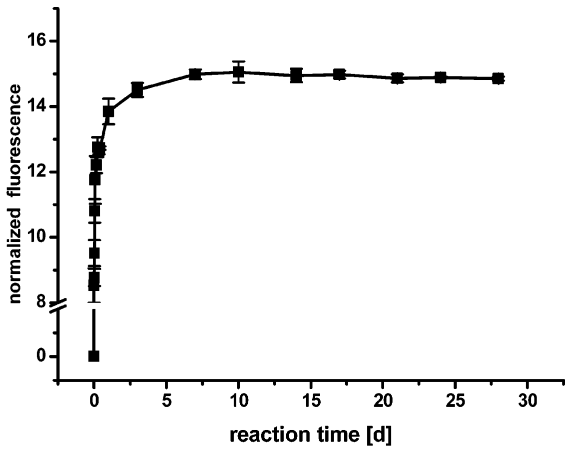

Figure 8. Long-term stability of FDAM labeled carboxyls in pulp (for storage conditions, see main text).

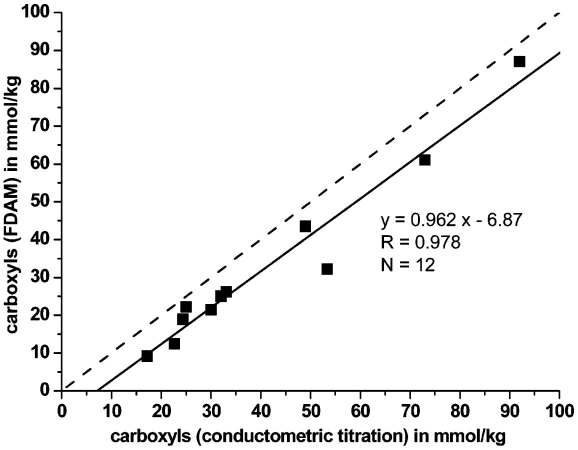

Figure 9. Quantification of carboxyls in pulp: Agreement of the FDAM method with conventional conductometric determination. The dashed line represents the function $y = x$ corresponding to "100% agreement".

cellulose chains, carboxyls at C-2/C-3 after C−C bond cleavage, or metasaccharinic acid moieties (after rearrangement reactions) are contained as well. Many paper pulps manufactured according to alkaline kraft processes contain hexenuronic acids originating from glucuronic acid moieties in xylan. At present, it is not possible to distinguish between different types of carboxyls by FDAM labeling, since the resulting fluorenyl methyl carboxylates exhibit the same fluorescence properties independent of the respective acid structure. Currently, model experiments are underway to determine whether different carboxyls can be distinguished by their reaction rates and labeling behavior. Preliminary results indicated that uronic acids were always neatly and permanently labeled, whereas in the case of onic acids, the primarily formed fluoren-2-yl methyl onates appeared to behave like activated esters, promoting the formation of onolactones with concomitant release of fluoren-2-yl methanol from the label. A comprehensive account of this matter by means of model compound studies will be published soon.

The relation of the FDAM method with respect to conventional approaches toward carboxyl quantitation was comprehensively studied over the full range of commonly observed carboxyl contents in pulps (Figure 9). There was a satisfying agreement that the values of the FDAM method lie on average 10% below the conventionally determined data. It should be noted that this is not at all a conclusive indication that the FDAM method suppressed a minor part of the carboxyls present, as traditional methods suffer from a number of limitations as disussed before. Apart from the ability to report the overall carboxyl content, which the FDAM method and older approaches have in common, it should be kept in mind that the reliability of the FDAM approach and especially the possibility to obtain carboxyl *profiles* are two completely novel qualities which conventional methods are fully unable to provide.

FDAM Labeling of Pulps: Carboxyl Profiles. If only the overall carboxyl content of a cellulose sample is considered, information is lost, since the sum parameter levels possible differences between fractions of different molecular weights. Only carboxyl group profiles, i.e., the COOH content relative to the molecular weight distribution, convey the full amount of information. With the FDAM labeling having been elaborated into a general method, it was possible for the first time to record such profiles, and thus to determine the results of chemical treatments of cellulosics not only in terms of the overall carboxyl changes but also with regard to the carboxyl changes relative to the molecular weight distribution. As the FDAM labeling is actually an off-line derivatization procedure, the subsequent GPC analysis yielded the carboxyl profiles of the respective samples and reported possible changes in these profiles caused by chemical treatments.

In the following, the term "carboxyl DS" shall be used by analogy to the recently introduced "carbonyl DS" as determined according to the CCOA method. The DS_{COOH} describes the average content of COOH groups per anhydroglucose unit.[21] The main prerequisite to obtaining a carboxyl DS relative to the chain length (= the molecular weight) is the simultaneous recording of a mass proportional signal and a substituent proportional signal, which are provided by the RI detector and the fluorescence detector, respectively, in the present method. Both detector outputs were used to calculate the carboxyl DS profiles. ΔDS_{COOH} plots, which present the difference between two DS_{COOH} curves, are very suitable graphic representations to report even slight changes in carboxyl contents, rendering the comparison of two samples with regard to their carboxyl profiles very easy (plots not shown here). In Figure 10, the molecular weight distributions and the FDAM carboxyl profiles of two pulps are given, showing the characteristic differences in modality and polydispersity. In all cases, the carboxyl groups are concentrated in the low molecular weight region.

To visualize oxidative changes in one pulp or differences in the carboxyl content between different pulps even better, a numeric evaluation of the data is usually carried out, displaying the result in pie diagrams (Figure 11). For that purpose, the amount of carbonyls or carboxyls in specific regions of the molecular weight distribution is calculated from the DS curves. The borderlines were set arbitrarily. However, the chosen values have been proven to be most useful for evaluation of carbonyl contents according to the CCOA method, so that an analogous approach for the FDAM procedure was desirable. Figure 11 displays the resulting pie diagrams for the carboxyl contents of two pulp samples. It was evident that the M_w-related carboxyl content will be a new and valuable parameter for the characterization of celluloses and pulps.

Conclusions

An analytical approach to accurate determination of carboxyl groups in cellulosics has been developed, employing COOH-selective fluorescence labeling to convert carboxyl structures into fluorescent fluoren-2-yl methyl esters, detectable by instrumental analysis. The labeling with the diazomethane derivative FDAM was carried out as off-line derivatization, in

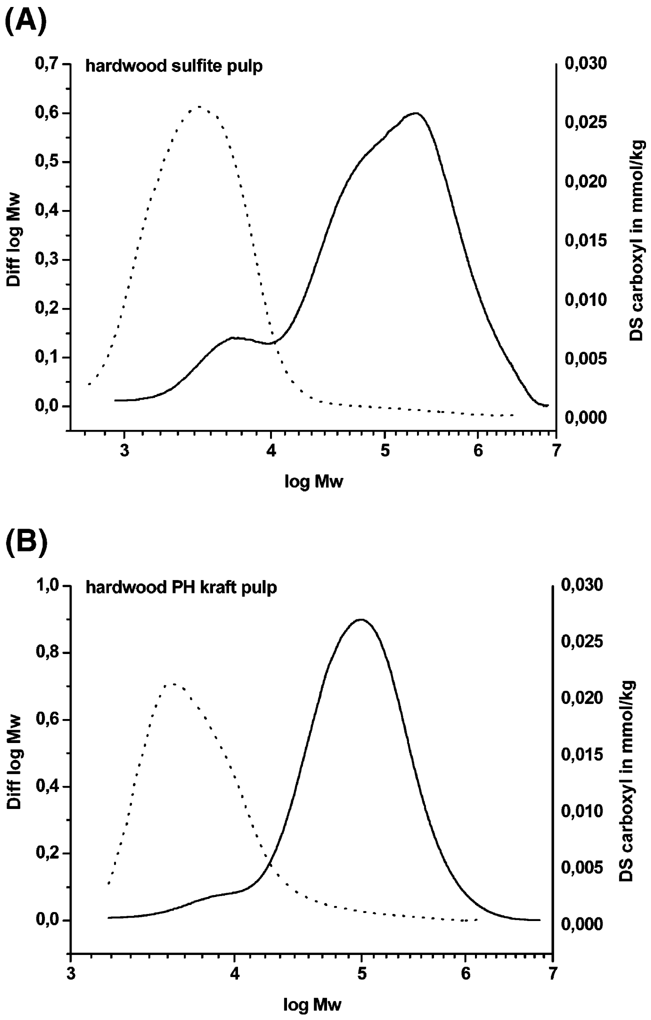

Figure 10. Molecular weight distributions (solid lines) and carboxyl profiles (dotted lines): (A) hardwood sulfite pulp (18.9 μmol/g carboxyls); (B) prehydrolysis hardwood kraft pulp (9.2 μmol/g carboxyls).

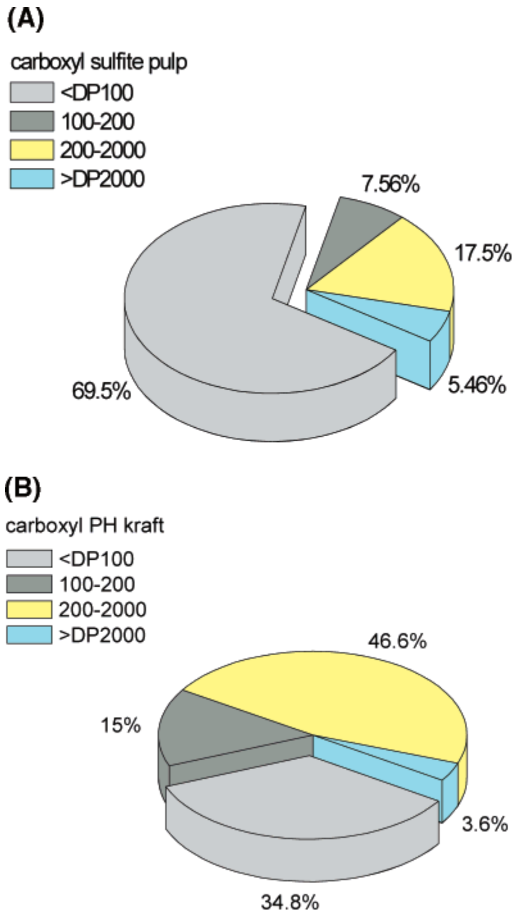

Figure 11. Carboxyl distribution within four of the molecular weight regions in two pulp samples: (A) Mg sulfite pulp; (B) prehydrolysis kraft pulp.

combination with a GPC measurement employing RI, MALLS, and fluorescence detection. The fluorescence label FDAM did not interfere with the MALLS detection and was sufficiently stable. The COOH labeling neither changed the solution state of the pulp nor caused cellulose degradation. A standard labeling procedure has been systematically elaborated, based on experiments with cellulose model compounds and optimization toward the most favorable reaction parameters.

Already, the overall carboxyl content is a valuable parameter to monitor the effect of oxidative chemical treatments. However, for the first time, carboxyl profiles of cellulosic pulps have been recorded, which allowed a much more precise evaluation of oxidative damages or distribution of hemicelluloses. Evaluation of carboxyl profiles was conveniently done by means of "carboxyl DS" plots.

Outlook. A comprehensive validation of the analytical procedure and determination of the analytical standard parameters is required to demonstrate that the approach is more reliable than any competitive method hitherto available. The value of the method will be demonstrated by means of several applications in the field of cellulose chemistry, validation, and applications being reported in part 2 of this work.

Especially in combination with the CCOA method, the full power of the FDAM approach will be evident by imaging the interplay between carbonyls and carboxyls, such as the consumption of the former by further oxidation, causing an increase of the latter. As carboxyl groups play a key role in many cellulose reactions and in determining the cellulose properties, the FDAM method can realistically be expected to find ample applicability in the various fields of cellulose chemistry.

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(21) Strictly speaking, introduction of carboxyl groups by oxidative modifications of cellulose is no "substitution", so that usage of the expression "degree of substitution" would be inappropriate. Nevertheless, the term shall be maintained for convenience.

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