Anal. Chem. **2002,** *74,* 2314−2320

Application of Size Exclusion Electrochromatography to the Microanalytical Determination of the Molecular Mass Distribution of Celluloses from Objects of Cultural and Historical Value

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The molecular mass distributions (MMD) of celluloses from paper and other sources were determined by size exclusion electrochromatography (SEEC). Prior to the separation the celluloses were chemically modified with phenyl isocyanate into their tricarbanilate derivatives (CTC). Sensitive UV detection of the CTC could be performed at a wavelength of 210 nm. The solvent used for separation was acetone. With this solvent, a high electroosmotic flow could be generated in columns packed with bare silica particles. With a column packed with particles with a nominal pore size of 30 nm, use of a mobile phase with a salt concentration of 0.1 mM was found to be optimal with respect to mass selectivity and efficiency. The workable mass range under these conditions was from 2 kDa to at least 500 kDa for (native) celluloses. The SEEC method was compared to classical pressure-driven size exclusion chromatography (PD-SEC). It is shown that the two methods give comparable results for the MMD of celluloses, while SEEC has important advantages in terms of speed and sample consumption. With SEEC, the analysis time was less than 20 min. The method was applied for the study of cellulose degradation during (artificial) aging of paper samples. A clear reduction of the average molecular mass of cellulose during aging was observed. With SEEC, the required sample amount is strongly reduced compared to classical PD-SEC. With a single paper fiber (after derivatization), multiple analyses could be carried out. It is argued that this is not only important for the analysis of unique objects but that it also allows the detection of MMD heterogeneities on a microscale. The strong reduction in sample size may be relevant when local heterogeneities in other types of polymer samples are studied.

Miniaturization of separation techniques is an important strategy to enhance sensitivity, efficiency, and speed of separation and to reduce the required sample amounts.[1−3] Surprisingly, the trend toward miniaturization seems to be largely neglected in polymer analysis. Typical columns for conventional pressure-driven size exclusion chromatography (PD-SEC) have inner diameters of 7.5 mm and total lengths of up to 1 m, which makes polymer characterization by means of SEC relatively time consuming and expensive. Moreover, the sample amounts required for SEC can be problematic in fields where only a very limited amount of sample is available for analysis, e.g., in art conservation studies or forensic analysis.

In capillary size exclusion electrochromatography (SEEC), the sample size constraints are strongly relaxed. In SEEC, the mobile phase is driven by means of electroosmosis, which develops upon the application of a high electric field across the capillary column.[4−12] SEEC has been applied for polymer separations on monolithic columns[6] and on columns packed with silica particles[7−11] or with the polymeric gel-type particles as are often used in conventional SEC.[12] With column dimensions typical for SEEC (inner diameters of 50−100 μm and lengths of 0.25−0.5 m), the sample and mobile-phase consumption can be reduced by a factor of $\sim 10^4$ compared to conventional pressure-driven SEC. Also the efficiency of separation can be significantly improved. The peculiarities of electroosmotic flow (EOF) allow for this high efficiency. An homogeneous flow velocity distribution over the column cross section[4] and the possibility to generate and control

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10.1021/ac0111309 CCC: $22.00
Published on Web 04/17/2002

an intraparticle flow[7−11] make SEEC 2−3-fold more efficient than PD-SEC on columns packed with similar stationary phases. Moreover, the use of smaller particles to generate higher separation efficiencies is more easily realized in SEEC than in pressure-driven chromatography.[4] On the other hand, the mass selectivity in SEEC may be lower than in PD-SEC, so that a careful optimization of the separation conditions may be required.[8]

So far, SEEC has only been tested and applied for the characterization of synthetic polymers. In the present work, its applicability has been studied for the characterization of a natural polymer, cellulose. This polymer is a linear, syndiotactic homopolysaccharide consisting of D-anhydroglucopyranose units connected by β-1,4-glycosidic bonds. It is the major structural component of the wall tissue of most plant cells, and it is the single most abundant polymer in the biosphere.

Cellulose has been processed for many centuries in the fabrication of items of widely diverse nature such as paper, clothing, painting supports (canvasses), etc. Many of these objects may now have a high cultural and historical value and therefore their conservation is important. A major problem with cellulose-based objects is that cellulose may degrade in time under the influence of chemical, physical, and biological agents.[13,14] Eventually this may cause a loss in mechanical or optical properties of those objects. This makes the molecular characterization of cellulose highly relevant in order to obtain an accurate diagnosis of the condition of cellulose-based artifacts. Such diagnosis allows the identification of degraded/unstable objects or collections, as well as the assessment of the effects of conservation measures.

The major chemical mechanism of cellulose degradation in objects of cultural and historical value is the (acid-catalyzed) hydrolytic scission of the β-1,4-glycosidic bonds. Fragments of lower mass are formed, resulting in a change of the molecular mass distribution (MMD) of the polymer. The depolymerization of cellulose will negatively affect the mechanical properties of cellulosic materials (e.g., paper), leading to a loss of strength and flexibility, and to an increased brittleness. The MMD is therefore an important analytical criterion to quantitatively assess the degradation of cellulose-based materials.

Conventional PD-SEC has been used to determine the MMD of celluloses from various origins and to track changes in the MMD after chemical and other treatments that may affect the mass distribution.[15−18] Cellulose is not soluble in the solvents commonly used in PD-SEC. *N*,*N*-Dimethylacetamide (DMAC) containing LiCl has been used for the dissolution and PD-SEC separation of cellulose from various sources.[19,20] However, the sample preparation procedure calls for several solvent exchange and filtering steps and is, therefore, laborious and difficult to miniaturize.

Cellulose is often chemically modified prior to analysis in order to enhance its solubility and detection properties. Several derivatization procedures have been suggested for cellulose, but usually the phenyl isocyanate (PIC) derivative (tricarbanilated cellulose, CTC) is used.[17,18,21,22] It has been shown that chemical modification by PIC does not affect the MMD of the cellulose, while other modification procedures may require harsh reaction conditions that may inflict undesired additional degradation.[23] Moreover, the reaction protocol for derivatization by PIC may be easily modified to be applicable for the MMD characterization of small samples. The sample amounts required for determination of the MMD by means of classical PD-SEC after derivatization with PIC are in the order of 5−50 mg. For typical writing, printing, and drawing paper, this equates to 0.5−10 cm^2, which is clearly not acceptable when unique objects such as drawings and other valuable documents are being sampled.

The main objective of this study is to reduce the amount of sample needed for quantitative characterization of the MMD of celluloses, to an extent that is acceptable for the investigation of cellulose-based objects of cultural and historical value. In this paper, we demonstrate that the molecular mass distributions of celluloses that have molecular masses of over 100 kDa can be successfully and quantitatively determined by means of micro-derivatization and SEEC with UV detection using acetone as the mobile phase.

EXPERIMENTAL SECTION

Chemicals. *N*,*N*-Dimethylformamide (DMF), acetone, methanol, phenyl isocyanate, and acetonitrile were obtained from Acros Organics (Geel, Belgium). Pyridine (Seccosolve), tetrabutylammonium tetrafluoroborate (TBATFB), naphthalene, and tetrahydrofuran (THF) were obtained from Merck (Darmstadt, Germany). Cellulose fibrous, medium, came from Sigma (St. Louis, MO). The narrow polystyrene (PS) and the poly(methyl methacrylate) (PMMA) standards were purchased from Merck and the narrow pullulan (PL) standards from Polymer Labs (Church Stretton, Shropshire, U.K.).

Six different types of paper were used in this study. These were a Whatman No. 1 qualitative filter paper made of purified cotton linters (Whatman, Springfield Mill, U.K.) (denoted WH), an additive-free bleached sulfite softwood cellulose paper (Papierfabriek Schutt, Heelsum, The Netherlands) (NS), regular copy paper and recorder paper. In addition, two rag papers dating from 1796 (R1) and from the 19th century (R2, exact date unknown) were analyzed.

Two types of bare silica particles were tested as the stationary phase. These were Nucleosil 100-5 and Nucleosil 300-5 (Macherey Nagel, Düren, Germany). Their respective nominal pore diameters are 10 and 30 nm, and both have a particle diameter of 5.0 μm.

Artificial Aging of Samples. Ultimately the SEEC method is to be applied to track the degradation of cellulose from objects and collections of cultural and historical value in time. To verify this with our method, the cellulose and the paper samples were artificially aged.

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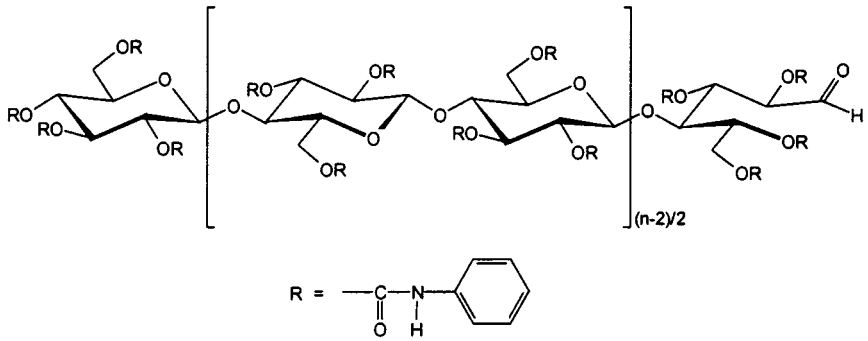

Figure 1. Molecular structure of the cellulose tricarbanilate derivative (CTC).

The fibrous cellulose sample, as well as the WH and the NS papers, was artificially aged at 90 °C and under relative humidity cycles alternating between 80% and 35%, each step having a duration of 3 h. Aging periods of 0, 3, 6, 12, and 18 days were used in these experiments. The paper sheets (~15 × 15 cm) were hung inside a Vötsch Industrietechnik (Balingen-Frommern, Germany) VC 0020 programmable climate chamber, and the fibrous cellulose sample was placed on a PTFE "watch glass" positioned at the bottom of the aging chamber. Heraeus TSI software (Hanau, Germany) was used to program the aging cycles.

Carbanilation of the Cellulosic Samples and the Pullulan Standards. The paper and cellulose samples and the pullulan standards were dried in an oven at 105 °C for 4 h prior to chemical derivatization. Approximately 5.0 mg of the dried product was weighed into a glass container, and 1 mL of water-free pyridine and 0.1 mL of (PIC were added. The mixture was allowed to react for 48 h at 80 °C in a closed vial. The reaction was terminated through the addition of 0.1 mL of methanol after which the mixture was allowed to cool to room temperature. The viscous, slightly yellowish reaction mixture was then diluted with the mobile phase. The modified pullulan standards were diluted 1:14 with acetone to obtain concentrations of 1.0 mg/mL reaction product (0.33 mg/mL native pullulan). The paper and cellulose samples were diluted to give concentrations of 5.0 mg/mL (1.6 mg/mL native cellulose).

Isolation and purification of the tricarbanilate derivatives by precipitation and redissolution was not done here, since it has been shown that this may lead to a preferential loss of the low-mass fragments of the derived cellulose.[22]

The structure of the cellulose tricarbanilate derivative is shown in Figure 1. It is important to note that the mass of the cellulose and the pullulan after PIC modification is ~3-fold higher as compared to the natural polymer. The relationship between the mass of the polysaccharide M_{cel} and the derived product M_{pic} is given by

$$M_{pic} = [3(M_{cel}/m_{mn}) + 2](m_{pic}) + M_{cel} \qquad (1)$$

where m_{mn} is the mass of the repeating unit (monomer) of the polysaccharide and m_{pic} is the mass of the derivatizating agent.

Derivatization of Single Paper Fibers. The procedure to prepare the CTC derivative of cellulose was slightly modified for use with single fibers. A single paper fiber was tweezed from a paper sample and placed into a 100-μL glass sample vial, and 50 μL of water-free pyridine and 10 μL of PIC were added. The mass of a single paper fiber was on the order of 10 μg. The sample vial was capped and placed into an oven at 80 °C for 48 h. The reaction mixture after derivatization was ~50 μL as some of the reaction solution was lost, probably as a result of evaporation during reaction. Next, 20 μL of methanol was added to remove the excess of PIC. This solution was injected onto the SEEC columns without prior dilution in order to obtain concentrations well above the detection limit of the SEEC system. Since the volume injected on the column is in the order of 10 nL, multiple injections could be performed from a single microvial.

Conventional Pressure-Driven SEC. A Waters 2690 Alliance separation module (Waters, Milford, MA) was used for mass distribution characterization of the cellulose samples by conventional SEC. The system was equipped with a model 410 differential refractometer detector (Waters). The mobile phase for pressure-driven SEC was stabilized THF pumped at a flow rate of 0.35 mL/min, and the sample injection volume was 40 μL. The columns used in series were a Styragel HR4E and a HR5 (Waters). Both columns have an i.d. of 7.8 mm and lengths of 30 cm and were thermostated at a temperature of 35 °C. Mass calibration was performed using derivatized pullulan standards which were dissolved in the mobile phase. The samples were run in duplicate, and the average values were taken. The data were analyzed using the Waters Millenium software V 3.2.

Capillary Size Exclusion Electrochromatography. The columns were prepared according to a method described previously[8,11] with a minor modification. In this study, the columns were packed to a length of 50 cm. Furthermore, the use of acetone as the mobile phase in SEEC gave some additional practical difficulties. During installation into the SEEC instrument, the column often dried at the ends thereby inhibiting electrical contact and electroosmotic flow (EOF) development. This could be circumvented through flushing the column with a less volatile mobile phase after packing of the column. Before installation, the column was flushed with an acetonitrile/water mixture (80/20 v/v) containing 1.0 mM TBATFB. After installation, the column was flushed and preconditioned with the acetone mobile phase through the application of a relatively low field strength of 100 V/cm for 30 min. When the flush solvent was exchanged with the acetone mobile phase, the electric field strength was ramped to a maximum of 400 V/cm (20 kV) in 1 h to complete the preconditioning procedure. When both a stable current and a stable UV signal were obtained, the column was ready for use.

All SEEC experiments were performed on a HP[3D]CE system (Hewlett-Packard, Waldbronn, Germany). For separation, a voltage of up to 30 kV (600 V/cm) was applied to the column and a pressure of 10 bar was applied to both ends of the column. The SEEC system was thermostated at 20 °C.

Injections were performed electrokinetically through the application of 5 kV for 10 s. All experiments were performed in duplicate, and the mean values were used for further calculations. Migration times and plate numbers were calculated using the Chemstation software (Hewlett-Packard). Calculations of the molecular mass distributions of the celluloses were done using a home-written spreadsheet program.

RESULTS AND DISCUSSION

Solvent Selection for SEEC. Proper solvent selection is critical for successful application in SEEC.[5−8,11] The solvent has to meet several requirements: the solvent should allow the generation of a sufficiently high EOF, and due to a lack of other suitable detectors for polymers, sensitive UV absorbance detection should be possible. Furthermore, the solvent should also be capable of fully dissolving all the sample constituents, which may not always be the case with polymers. Solvents that have been reported for CTC include pyridine, acetone, THF, and DMF, and these were tested first.

With THF, or with THF containing small amounts of water, only low EOF velocities could be obtained, while both pyridine and DMF were not UV transparent at appropriate wavelengths. On the basis of these results and other physicochemical properties (dielectric constant and viscosity) of the solvents, acetone was selected as being the most appropriate mobile phase for the present application.

The highest absorbency for the PIC-modified polysaccharides in acetone was measured at a wavelength of 210 nm. Acetone is almost UV transparent at this wavelength, allowing sensitive detection of the PIC-modified polysaccharides. Interestingly also, other polymers, for some of which narrow standards are available, dissolve in acetone and can be UV detected at this wavelength. The UV cutoff of DMF, which is known to produce a suitable EOF and has been used previously in SEEC,[6−8,11] was too high to get sufficient sensitivity for the PIC modified samples.

The electroosmotic mobility of acetone containing 1.0 mM TBATFB was determined on a 50-cm-long (effective) column packed with the 30-nm pore particles using naphthalene as the t_0 marker. The measured electroosmotic mobility was 1.2 10^{-8} $m^2/(V\ s)$, which is quite high compared to the electroosmotic mobilities of other low-dielectric solvents (ϵ_r of acetone, 20) in packed column capillary electrochromatography. This high mobility is related to the low viscosity of acetone ($\eta = 0.33$ mPa s).

Mass Calibration of the SEEC System. The most elegant and accurate way to perform mass calibration is to use narrow standards of the same polymer that is characterized. Unfortunately, narrow cellulose standards are not (commercially) available. Usually mass calibration is then performed according to the principles of universal mass calibration,[24] or a mass/size-specific detector is employed such as multiangle light-scattering detector (MALS).

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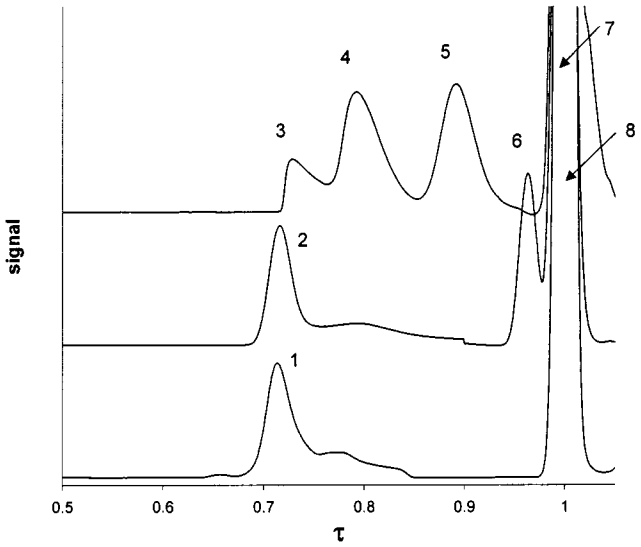

Figure 2. Size exclusion electrochromatographic separation of some PIC-modified pullulan standards. Column: 500 × 0.1 mm Nucleosil 300-5. Applied field strength: 20 kV (400 V/cm). Mobile phase: acetone containing 0.1 mM TBATFB. Peaks: (1) PL 380 kDa; (2) PL 186 kDa; (3) PL 100 kDa; (4) PL 23.7 kDa; (5) PL 12.2 kDa; (6) PL 5.8 kDa; (7) PL 738 Da (stachyose); (8) MEOH−PIC. Other conditions: see text.

Narrow polysaccharide standards are available as pullulan standards. Pullulan is structurally similar to cellulose as both types of molecules are linear chains of linked D-anhydroglucopyranose. However, pullulan (polymaltotriose) differs from cellulose in that the D-anhydroglucopyranose units of maltotriose are linked by α-1,4-glycosidic bonds and the maltotriose units are α-1,6-linked, while in cellulose, all D-anhydroglucopyranose units are β-1,4-linked. Such small differences may have important consequences for the behavior of the polymer in solution and affect its hydrodynamic volume and other physicochemical properties. Still, despite these subtle differences in structure, mass calibration using pullulan standards is considered to be the best alternative to cellulose standards.

The pullulan standards were subjected to chemical derivatization by phenyl isocyanate and were diluted to a concentration of ∼1.0 mg/mL. After derivatization and dilution with acetone, mixtures of the pullulan standards (PIC−PL) were separated on 50-cm-long columns packed with either the 10- and 30-nm pore particles using acetone as the mobile phase. Typical separations by SEEC on the 30-nm porous particles are shown in Figure 2.

It is seen that at an applied separation voltage of 20 kV the PIC−PL standards are well resolved using acetone containing 0.1 mM TBATFB as the mobile phase. The high peak at a relative retention ratio (τ) of 1 is due to the methanol-reacted phenyl isocyanate (MEOH−PIC). In further calculations, the migration time of this component was used as the totally permeating marker to determine the relative retention ratios of the standards and the cellulose. Pyridine, present in the samples at a high concentration of ∼10%, always eluted 3−4 min after MEOH−PIC as a tailing triangular peak and did not interfere in the retention window for the polymers. The relatively fast elution of pyridine eliminated the need for extended flushing and equilibration times of the column between runs or the removal of the pyridine from the reaction mixture after the derivatization procedure.

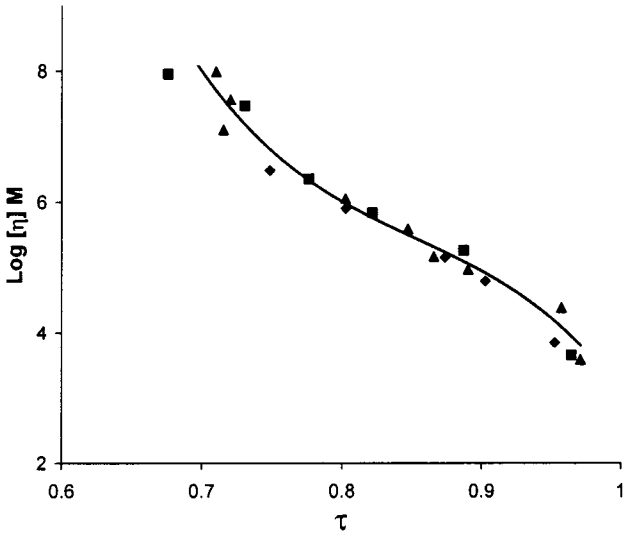

Figure 3. Universal mass calibration curve for columns packed with the 30-nm pore particles in SEEC mode. Column: 500 × 0.1 mm Nucleosil 300-5. Applied field strength: 20 kV (400 V/cm). Mobile phase: acetone containing 0.1 mM TBATFB. Standards: (◆) polystyrene; (■) PIC−pullulans; (▲) PMMA. Other conditions: see text.

Mass calibration curves were determined by measuring the retention time of the PIC-modified pullulan standards relative to the migration time of the MEOH−PIC. The migration times of the standards were calculated from the top of the peaks. Mass calibration curves were constructed for columns packed with the 10- and 30-nm pore particles.

A lower selectivity of the SEEC system at high ionic strength was observed. This can be explained by the occurrence of pore flow at these conditions.[6−8,10−11] The upper mass limit for the columns packed with the 10-nm pore particles is ∼100 kDa and with the 30-nm pore particles the upper mass limit is ∼500 kDa (PL units). The latter type of particles provides an adequate mass range for the cellulose samples. At a concentration of 0.1 mM TBATFB in the mobile phase, the separation conditions were found to be optimal with respect to selectivity and efficiency.

For the PIC-derivatized PL standards, relatively broad and multimodal peak shapes were observed. Therefore, narrow standards of PS and PMMA were injected and separated by SEEC at otherwise identical experimental conditions. With these standards, naphthalene was co-injected and served as the totally permeating marker to calculate the relative migration times of the standards. The PMMA and the PS standards were dissolved in acetone at a concentration of 1.0 mg/mL. Narrow and symmetrical peaks were observed for these standards. Apparently, the anomalous peak shapes seen for the derivatized PL standards were due to their relatively high polydispersity. Characterization by conventional PD-SEC also indicated the heterogeneity of these standards. Through multiplication of the molecular mass of the standards with their respective intrinsic viscosities (η) and plotting these values against their relative elution times, an universal mass calibration curve was calculated[24] (Figure 3). The Mark−Houwink constants required to calculate the intrinsic viscosity (η) of the respective standards were taken from a reference book[25] and are given in Table 1. For polystyrene in acetone, the Mark−Houwink constants were not known and here the values for polystyrene in butanone were taken.

Table 1. Mark−Houwink Constants for Polymers in Acetone[24]

polymer	K	a
PIC−pullulan	5	0.73
polystyrene[a]	17	0.69
PMMA	4.66	0.79

[a] For polystyrene in butanone.

It is seen that when the molecular mass (MM) of each individual mass standard is multiplied with its intrinsic viscosity (η), the mass calibration curves for the three different polymers may be superimposed on each other. The principles of universal calibration may thus be applied in this SEEC application. It can be seen that the upper mass limit for the 30-nm pore size particles is up to a specific viscosity of $\sim 10^8$. For the PIC-derivatized PL, this equates to a molecular mass of ∼1.5 MDa, which equates to 500 kDa of native pullulan. The upper mass limit for PMMA on the 30-nm pore-sized particles of the SEEC method corresponds to a mass of ∼400 kDa. Polystyrene with a mass higher than 70 kDa did not dissolve in acetone. It may be possible to extend the mass range by the use of particles with larger pore size or through the use of columns packed with a mixture of particles with different pore sizes. The use of such mixed-bed columns, as is common practice in pressure-driven SEC, may also provide linear mass calibration curves in SEEC. This requires further study.

The universal mass calibration curve was used for calculating the mass distributions of the samples with the SEEC experiments.

Mass Distribution Characterization of the Fibrous Cellulose Sample. Using the optimized sample preparation and separation conditions, the molecular mass distribution of the fibrous cellulose sample was determined. A typical electrochromatogram as obtained for CTC is shown in Figure 4. It is seen that the separation can be performed in well under 20 min at an applied field strength of 20 kV (400 V/cm), which is relatively fast compared to PD-SEC. The chromatogram shows two distinct peaks that are due to MEOH−PIC and pyridine, respectively. The cellulose derivative elutes as a broad peak in front of the MEOH−PIC peak.

The MMD of the cellulose was calculated from the chromatograms and the mass calibration curve as obtained on the same column. The MMD parameters of the cellulose sample was also determined by classical PD-SEC using THF as the mobile phase (Table 2).

The MMDs as determined by the different techniques are very similar. However, the SEEC separation was performed in under 20 min, while the PD-SEC separation took 1 h. Clearly SEEC may provide a significant decrease of the analysis time compared to PD-SEC. Furthermore, the injection volume for the SEEC system is < 10 nL, which is a factor $\sim 10^4$ smaller than the injection volume used with the PD-SEC system.

Mass Distribution Characterization of Celluloses from Paper. All paper samples, including the artificially aged papers, were reacted with PIC. The recorder and copy paper were not completely dissolved after the derivatization procedure. The

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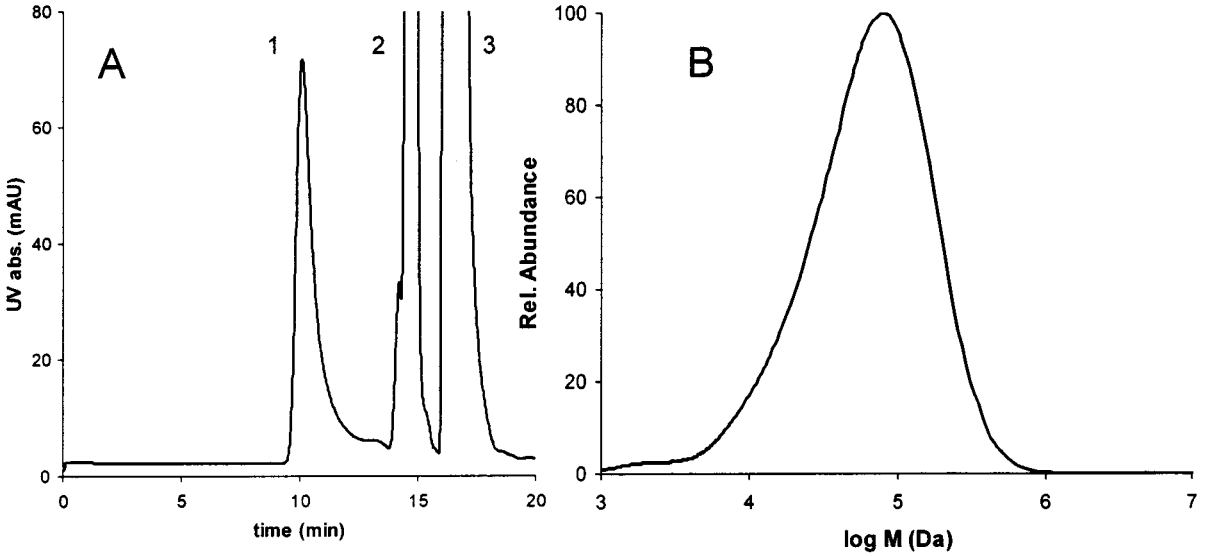

Figure 4. Size exclusion electrochromatographic separation of PIC-modified fibrous cellulose (A) and the calculated mass distribution of the native cellulose (B). Column: 500 × 0.1 mm Nucleosil 300-5. Conditions as in Figure 3. Peaks: (1) CTC; (2) MEOH−PIC; (3) pyridine.

Table 2. Comparison of Mass Distribution Parameters (Mass of the Most Abundant Polymer M_{top}, the Number-Average M_n, the Weight-Average M_w, and the Polydispersity P of the Native Fibrous Cellulose Sample) As Determined by Size Exclusion Electrochromatography and Conventional Pressure-Driven SEC

	technique	
distribution parameter	SEEC	conventional SEC
M_{top} (Da)	80.400	78.200
M_n (Da)	26.400	26.000
M_w (Da)	85.000	86.800
P	3.22	3.34

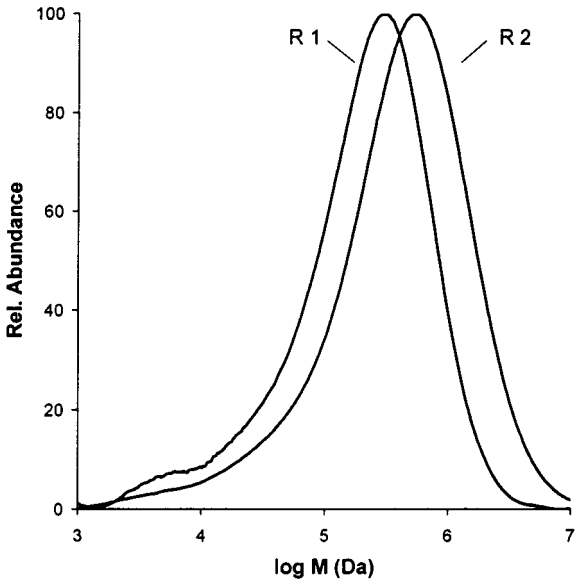

Figure 5. Mass distributions of the native cellulose from rag paper samples dating from 1796 (R1) and from the 19th century (R2) as determined by SEEC. Conditions as in Figure 3.

reason for the incomplete solubilization of these papers may be the presence of inorganic fillers such as pigments that are added to improve opacity, etc. The insoluble fraction of these samples was allowed to settle, and only the supernatant was diluted and injected. The other samples, including the naturally aged rag papers, were completely dissolved after treatment with PIC. Using the optimized SEEC system, the MMDs of the samples were determined. The chromatograms that were obtained for most of the paper samples were similar to the one obtained for the fibrous cellulose.

The mass distributions obtained for the naturally aged rag papers are shown in Figure 5. The molecular mass of the oldest rag paper sample R1 is clearly lower than that of the other natural aged rag paper R2. This may be related to degradation since sample R1 may be over a century older. When significant degradation of the paper has occurred, an increase of the relative abundance in the low molecular mass region of the cellulose may be expected. Indeed, a higher relative abundance in the low-mass region is seen for paper sample R1 compared to sample R2. However, since both paper samples originate from distinctive sources, the difference in MMD may also be due to a variance in the cellulose used in the manufacturing of the paper. The probable presence of gelatin, a typical surface-sizing agent used in early occidental papermaking, in the analyzed rag papers is also a factor to be considered as a possible source of variability in the lower mass region. Evidently, the differences in molecular mass distributions between different cellulose and paper samples needs further study.

Some of the paper samples analyzed contained celluloses with a molecular mass above the calibrated range of the present SEEC system. Quantitative data on the MMD of such samples may therefore not be reliable. Still, differences between samples in the 10^6-10^7 Da mass range can be observed clearly in the constructed MMD curves (see, for example, Figure 5). This indicates that the actual workable mass range of the system may be wider than the range that could be calibrated in this study.

Ultimately, the SEEC method is to be applied to track the degradation of cellulose of objects and collections of cultural and historical value in time. To verify this with our method, the NS paper was artificially aged. After SEEC, characterization of the

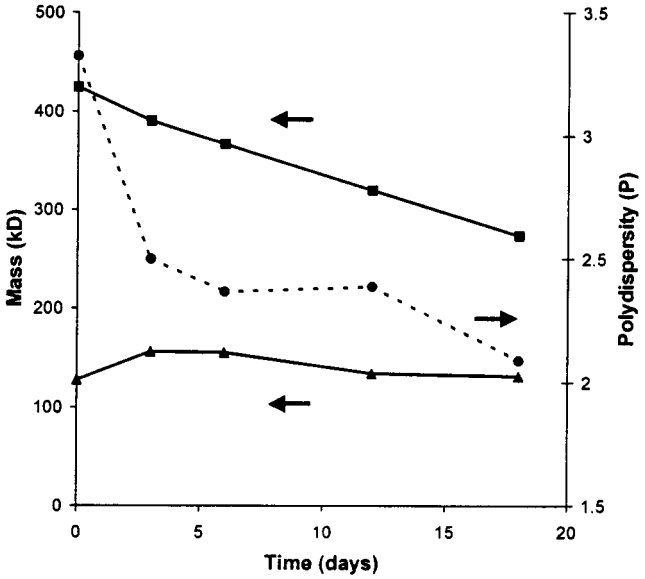

Figure 6. Results from SEEC characterization of the MMD parameters of an additive-free bleached sulfite softwood paper (NS) following accelerated aging. Conditions as in Figure 3. Symbols: (▲) M_n; (■) M_w; (●) polydispersity.

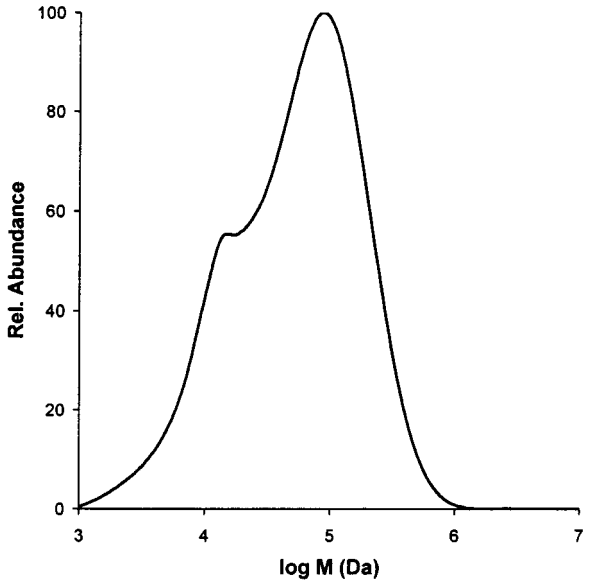

Figure 7. Mass distribution of a single fiber tweezed from regular copy paper as characterized by size exclusion electrochromatograpy. Conditions as in Figure 3.

MMD, the parameters M_w, M_n, and the polydispersity were calculated. In Figure 6, these values are plotted with respective aging time. Although only small differences in the chromatograms were found, it could clearly be observed that upon aging both the M_w and the M_n of the cellulose are reduced. Also, the polydispersity decreases. Even subtle changes in these MMD parameters can thus be traced by SEEC.

SEEC Characterization of Cellulose from Single Paper Fibers. When objects of high value are characterized, the amount of paper that is allowed to be sampled from such an object is limited. Certainly, when the state of degradation in time is being diagnosed, repetitive sampling is required and it is highly desirable to be capable of analyzing still smaller samples in order to minimize the damage inflicted to the object or collection. For this reason, we attempted to obtain molecular mass distribution data on single fibers of paper.

The sample preparation procedure was slightly modified for the derivatization of single fibers. Dilution of the mixture after reaction with the mobile phase was not done here in order to obtain concentrations well above the detection limit of the SEEC system. A molecular mass distribution as obtained from a single fiber of a regular copy paper is shown in Figure 7. It is seen that for this fiber two distinct peaks are observed in the MMD. This was also observed when larger sample amounts were processed and analyzed by either SEEC or PD-SEC. Other authors also observed two distinct peaks in the mass distribution of wood pulp celluloses and ascribed the low-mass component to hemicelluloses.[21]

When the same sample from a single fiber was injected multiple times, identical mass distributions were obtained, indicating that the repeatability of the SEEC system was satisfactory. However, the mass distribution obtained for this fiber was considerably different from those obtained when other fibers of the same paper sample were analyzed. The observed differences in the mass distributions of the single fibers may be a consequence of inhomogeneities of the paper at the small dimensions sampled. Clearly, possible local heterogeneities need to be taken into account when working with such small samples. The capability of SEEC to determine the MMDs at these small dimensions is highly promising for other types of applications where microheterogeneity is an issue.

ACKNOWLEDGMENT

The authors thank Ding Feng from the Evidence Identification Group, Liaoning Criminal Scientific and Technical Research Institute (Liaoning, PR China) and Paul le Comte (ITS, University of Amsterdam) for their assistance with parts of the experimental work. The work of R.S. was financially supported by the Dutch Organisation for Scientific Research (NWO) under Project 79.030.

Received for review October 30, 2001. Accepted March 13, 2002.

AC0111309