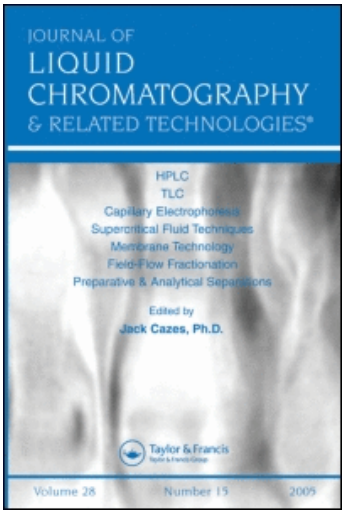

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SIZE EXCLUSION CHROMATOGRAPHY OF CELLULOSE DISSOLVED IN LiCl/DMAC USING MACROPOROUS MONODISPERSE POLY(STYRENE-*CO*-DIVINYLBENZENE) PARTICLES

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ABSTRACT

Two types of hydrophilic macroporous monodisperse particles (MMP) based on poly(styrene-*co*-divinylbenzene) have been investigated for use as column material for size exclusion chromatography (SEC) analysis of cellulose dissolved in lithium chloride/*N*,*N*-dimethylacetamide (LiCl/DMAc). The particles appeared inert to the mobile phase and no adsorption of cellulose could be detected. Favorable chromatographic properties associated with monosized particles such as low back pressures and high flow rates were obtained. Two MMP columns of different pore size distributions coupled in series, allowed separation of

cellulose in the molecular weight range 10^4-10^7 g/mole. The particles were suitable for SEC of cellulose samples dissolved in 0.5% LiCl/DMAc and the reproducibility and long term stability were superior to that of a comparable commercial SEC column.

INTRODUCTION

As probably the most abundant polymer in the world, cellulose is a compound of great industrial importance. Cellulose has a number of different applications both as a fiber, a chemical substance, and as a raw material for the synthesis of cellulose derivatives. The molecular and supramolecular structure of cellulose seems to be well described in the literature, but due to its extremely low solubility in most known solvents, the analysis of some molecular properties, such as, molecular weight (MW) and molecular weight distribution (MWD) of high molecular weight celluloses is still not a trivial task.

Cellulose is a simple homopolymer of β-D-glycopyranose units linked by (1-4)-glycosidic bonds. The equatorial conformation of the glycosidic bond and the presence of only equatorial substituents on the glycosidic ring, makes cellulose a polymer with a very large degree of stereoregularity. Crystalline structures stabilized by inter- and intramolecular hydrogen bonds are found in cellulose samples, and the dense crystalline structure and the high number of intramolecular hydrogen bonds makes cellulose virtually insoluble. However, a solvent for cellulose, lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc), has been described by McCormick.[1] This non-degrading solvent for cellulose has opened the possibility of size exclusion chromatography (SEC) analysis of cellulose.[2-5] However, in practice, cellulose has limited solubility in this solvent, especially at high molecular weights. Possible problems in SEC[6-9] may be eliminated by choosing more suitable column materials (presented here), while the former requires improvements in the procedure and conditions for the dissolution of cellulose (presented in another work).[10]

In this work, we report on the application of a new type of macroporous, monodisperse polymer particle (MMP) for SEC analysis of cellulose in LiCl/DMAc. The particle matrix consists of highly crosslinked poly(styrene-*co*-divinylbenzene) with a hydrophilic layer covalently linked to the surface of the pores. To evaluate the performance of the investigated column material, one commercial column system was included in the present study. Similar MMPs have been used for SEC analysis of polysaccharides in aqueous solutions.[11,12] In addition to extending the limiting molecular weight to about 10^8 g/mole for coil-like polysaccharides such as pullulans, and to about 10^7 g/mole for rod-like polysaccharides such as scleroglucans, favorable chromatographic properties generally associated with monosized particles (e.g. very low back pressures and fast separations) were reported.[11]

EXPERIMENTAL

Materials

Polystyrene standards, designed PS1 - PS7, with weight average molecular weight (M_w) of 4000, 20650, 28500, $2.0 \cdot 10^5$, $4.0 \cdot 10^5$, $9.0 \cdot 10^5$, and $2.0 \cdot 10^6$ g/mole, respectively, were obtained from Polymer Laboratories. Three cellulose samples, MCC (microcrystalline cellulose), CLC (cotton linter cellulose) and SSC (spruce sulfite cellulose) were obtained from Borregaard Ind. Ltd., Peter Temming, and Borregaard Ind. Ltd., respectively.

Materials used for dissolution of polystyrene and cellulose samples were *N,N*-dimethylacetamide (DMAc, p.a. grade, Burdick & Jackson) and methanol (p.a. grade, Sigma-Aldrich), both dried with molecular sieves (Type 4A, Merck), acetone (p.a. grade, Acros), ethylene diamine tetraacetic acid, (EDTA, 99%, Fluka), diethylene triamine pentaacetic acid (DTPA, 99%, Fluka), citric acid (p.a. grade, Merck), lithium chloride (98%, Baker, oven dried and stored at 150°C) and deionized water. The molecular sieve was activated by heating to 450°C for 3 days.

Column Material

The macroporous monodisperse particles were produced by SINTEF Applied Chemistry (Trondheim, Norway). Two particle types (I and II), both with a diameter of 15 μm, were prepared as described by Ellingsen et al.[13]

The pore size distribution and specific pore volume of the particles were measured by mercury porosimetry.[14] The specific surface area was measured by the Brunauer-Emmett-Teller (BET) method.[15] A commercial SEC column, PLgel 10 μm Mixed B (7.5 x 300 mm, 3 in series) was obtained from Polymer Laboratories and was included in the study for comparison.

Column I was packed with particle I and column II was packed with particle II. Column II+I is the combination of column II and column I coupled in series.

Column packing was performed essentially as described by Christensen et al.[11] An aqueous suspension of particles was washed with ethanol and packed in a vertically mounted column (Pharmacia HR 10/30, i.d. = 10 mm, l = 300 mm) at a flow rate of 1.0 mL/min. During packing, the column was vibrated at 50 Hz. Solvent exchange was performed in the following order: 0.1 M NaCl, water, methanol, DMAc, and finally, 0.5% LiCl/ DMAc.

Sample Preparation

The cellulose samples were swollen in deionized water (24 hours, room temperature) and, subsequently, washed with EDTA, DTPA, and finally citric acid.[10] The cellulose was further washed with 0.1M LiCl. An acetone extraction was then performed to remove possible extractives left in the pulp. The sample was solvent exchanged with methanol and DMAc before dissolution in 8% LiCl/DMAc. Before injection, the solution was diluted with DMAc to 0.5% LiCl/DMAc and filtered through a solvent resistant, disposable teflon filter (Millex SR 0.5 μm for the SSC and MCC samples and Millex CN 3.0 μm for the CLC sample, both filters from Millipore).

Size Exclusion Chromatography

SEC was performed at 40°C using a Perkin Elmer (Series 2000) HPLC pump with autosampler, operating at a flow rate of 1.0 mL/min. Injected samples contained 50-200 μg of dissolved cellulose (200 μL). The elution was monitored by a refractive index detector (Shimadzu RID-10A) and a multi-angle laser light scattering detector (MALLS, Wyatt Dawn DSP, 633 nm), equipped with an in-line filter holder (Millipore) with 0.2 μm PTFE-filter (Fluoropore-FG, Millipore). The refractive index increment, $(dn/dc)_\mu$, was taken to be 0.104.[10] Data acquisition and molecular weight calculations were performed using the ASTRA software (Wyatt Technologies).

RESULTS AND DISCUSSION

Physical Characteristics of the Stationary Phase

Two different types of macroporous monodisperse particles, designed I and II, were studied. Particle characteristics are given in Table 1. It may be noted that, although the materials have similar particle diameters, the specific pore volumes and specific surface areas are quite different.

Table 1

Particle Characteristics

Particle Type	Particle Diameter (μm)	Specific Pore Volume (mL/g)	Specific Surface Area (m^2/g)
I	15	1.3	125
II	15	1.94	52

The scanning electron micrographs (Figure 1) clearly demonstrate the monodispersity and the spherical shape of the particles. It is visually observed that type II particles contain larger pores than type I, something which is also seen in the pore size distribution curves. The pore size distribution curves in Figure 1 show that the particles have relatively broad pore size distributions whose maxima depend on the particle type. Particle I has a higher proportion of small pores and should, therefore, have the potential to separate at lower molecular weight than particle II. Particle II contains more pores in the higher regions than particle I, and 88% of the pore volume consists of pores larger than 500 Å, while the corresponding value for particle I is 40%. The higher surface area found for particle I is attributed to the higher amount of smaller pores.

Column Efficiency and Stability

Upon running cellulose samples continuously for more than 6 months, the number of theoretical plates (N_m) and column back pressure were monitored by regular injections of a polystyrene standard ($M_w = 2.85 \cdot 10^4$). Results for column II+I (serially coupled) are given in Table 2. N_m was initially identical for columns II+I and PLgel Mixed B, and decreased by 4% and 12%, respectively, after 6 months of use.

A back pressure of 100 psi was observed both for columns I and II using LiCl/DMAc at a flowrate of 1.0 mL/min. It increased to 150 psi when serially connected. In comparison, the commercial PLgel Mixed B column had a back pressure of 1060 psi under the same conditions.

Minor increases in the back pressure were occasionally observed in column II+I. This was attributed to accumulation of high molecular weight cellulose. However, the initial back pressure was fully recovered after washing the column with the eluent, or occasionally, with higher LiCl/DMAc concentration of LiCl (up to 6%). Generally, more pronounced increases in the back pressure, and even column plugging, was observed for the PLgel Mixed B column.

Reproducibility of the retention time for column II+I was measured by the injection of a polystyrene standard ($M_w=2.85 \cdot 10^4$). Only very small variations in retention times (typically 1%) and calculated molecular weight were observed after several hundred injections of cellulose interspersed with washing procedures.

The particles seemed to be inert towards chemical degradation, but somewhat more sensitive towards mechanical treatment, as increasing amounts of fine particle fragments were observed after storage and repacking. To remove fragments, repeated sedimentation and decantation of the particle suspension was performed.

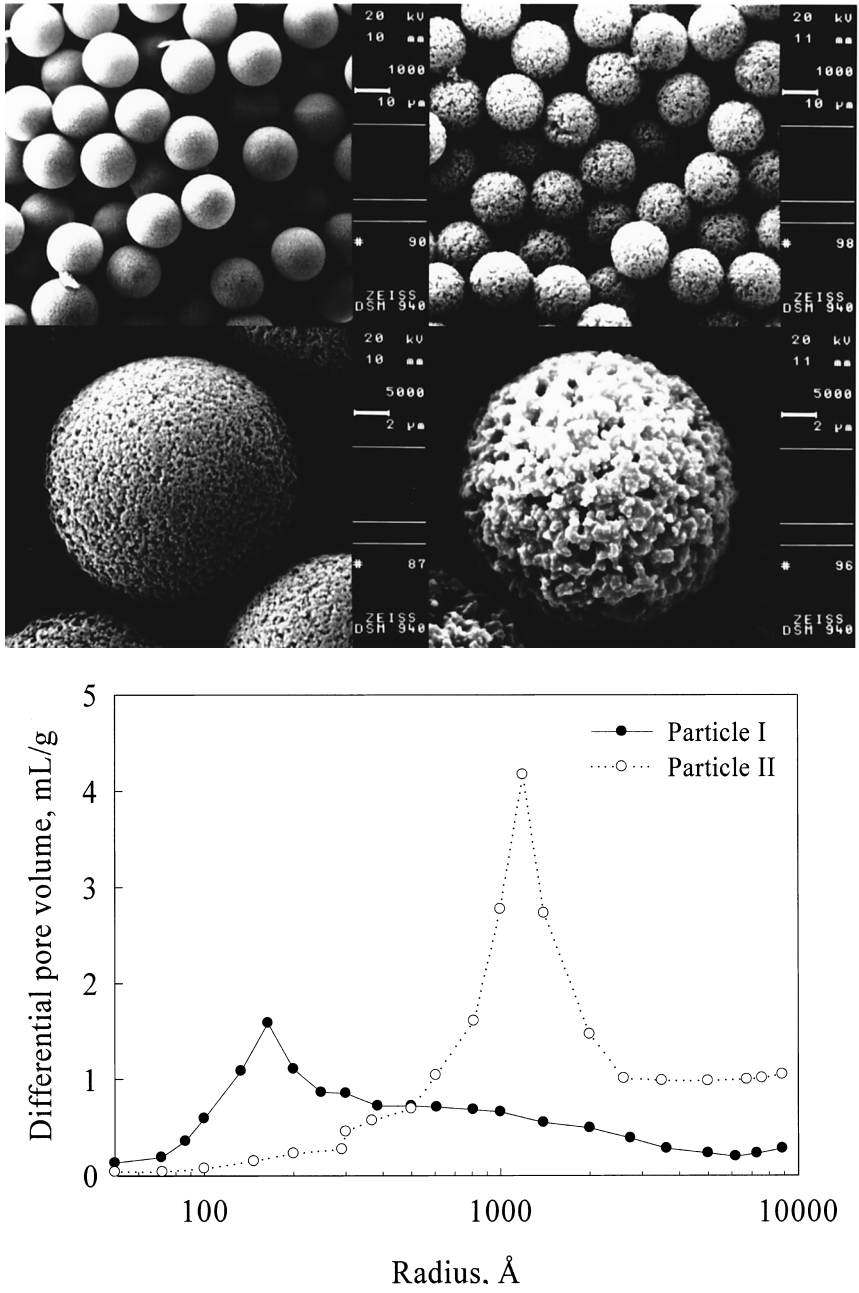

Figure 1. Scanning electron micrographs of MMP column material type I (left) and II (right) and pore size distribution of determined by mercury porosimetry.

Table 2

Stability of MMP and Commercial PL Gel Mixed B Columns

Particle Type	Nm Start	Nm After 6 Months
II + I	7160 ± 20	6850 ± 20
Mixed B	7180 ± 25	6530 ± 30

Separation Ranges (Polystyrene Standards)

Polystyrene standards were used for assessing and comparing the separation ranges of the different particle types. The selectively coefficient, α, was used, as it allows a direct comparison of selectivities of columns with different sizes. α is defined as $(V_e - V_0)/(V_t - V_0)$, where V_e is the peak elution volume, V_0 is the void volume and V_t is the total volume.[11]

Figure 2 shows SEC chromatograms obtained with columns I, II, and the serial combination of II+I for polystyrene standards with weight average molecular weights (M_w) ranging from 0.004 - $2.0 \cdot 10^6$. It is observed that high molecular weight samples are better resolved with column II as compared to column I, whereas, the opposite is the case for low molecular weight samples. As expected, the serial combination (II+I) gave good separation across the entire molecular weight range. It should be noted that polystyrene standards with high molecular weight are relatively polydisperse (M_w/M_n = 1.3), giving rise to broader peaks.

The calculated calibration curves (log M versus elution volumes at the peak maximum) are also shown in Figure 2. Also included, is data for the PLgel Mixed B column (chromatograms not shown). The curves are essentially parallel, but are shifted towards higher molecular weight when going from column I to II. The shift is in qualitative agreement with the shift in pore size distribution towards larger pores (Figure 1). The effective separation range for II+I is shifted by a factor of 3.0 in molecular weight (measured at $\alpha = 0.5$) relative to that of the commercial PLgel Mixed B column. Due to the lack of available standards with molecular weights larger than $2.0 \cdot 10^6$, the full separation potential cannot be directly assessed in the high molecular weight region for the MMP particles.

Cellulose Samples

Figure 3 shows RI signals and calibration plots (plots of log M versus elution volume as calculated using the ASTRA software) obtained for three sepa-

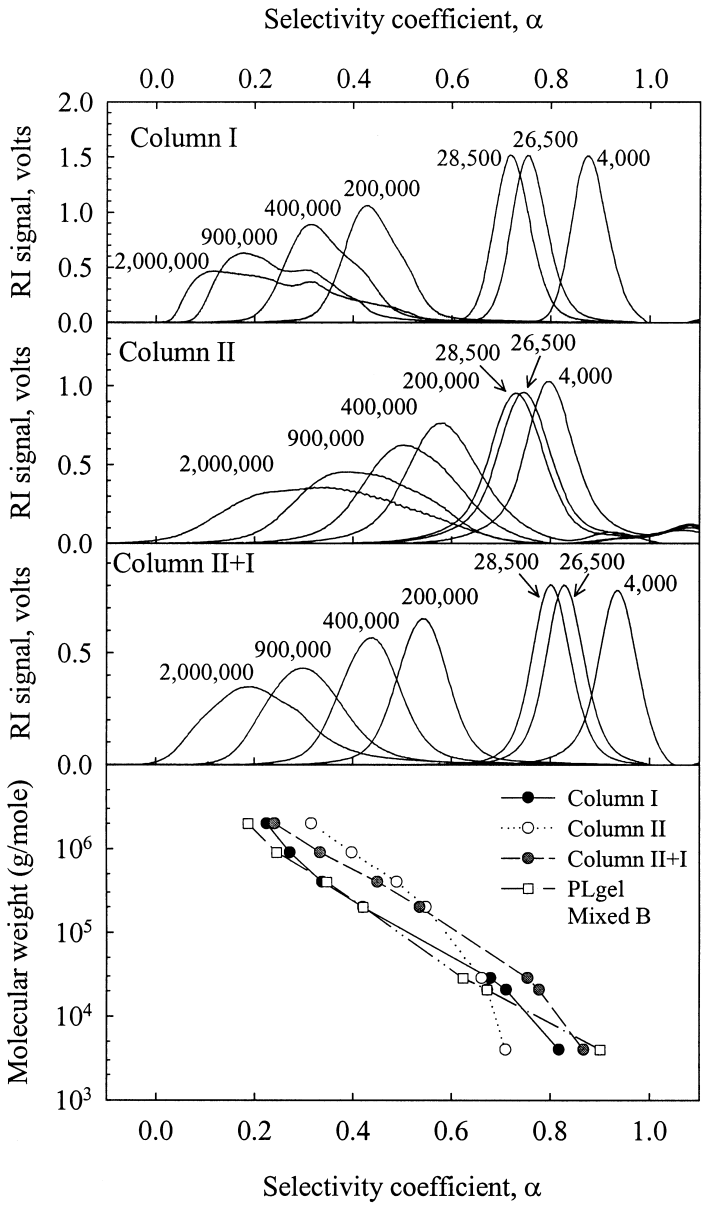

Figure 2. Chromatograms obtained for polystyrene standards (PS1-PS7) using columns I, II and II+I. The molecular weight (M_w) values are given as obtained from the supplier. Calibration plot is given at the bottom.

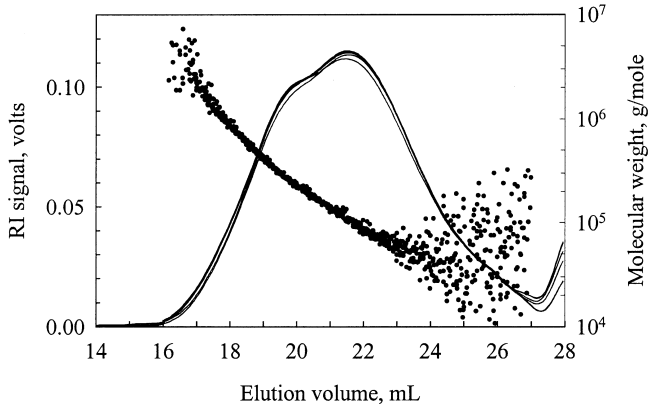

Figure 3. Chromatograms of three different preparations of the same cellulose sample (SSC). Each preparation was injected twice.

rate preparations (unfiltered) of the same cellulose stock solution (SSC, 8% LiCl in DMAc) with 2 parallels of each sample. The sample recovery was estimated to (100 ± 2) % for all 6 injections, indicating no significant adsorption of the cellulose material. The figure shows good reproducibility. The molecular weight was estimated to $(2.12 \pm 0.10) \cdot 10^5$ g/mol.

The dependence of the chromatographic behaviour on the amount of injected cellulose was tested for SSC by injecting concentrations in the range 0.2-1.0 mg/mL. The elution profiles and the corresponding calibration curves (Figure 4) are essentially independent of the amount of sample injected, although the signal-to-noise ratio of the light scattering signal decreases with decreasing sample concentration. The calculated M_w was also independent of the injected amount.

Figure 5 shows the elution curves and the corresponding plots of log *M* versus elution volume of three high purity cellulose samples of different origin and molecular weight (MCC, SSC, CLC). The difference in elution volume, compared to other results presented in this paper, is caused by repacking the columns to a different volume. The M_w values of the three samples investigated were calculated to $5.3 \cdot 10^4$, $2.0 \cdot 10^5$, and $8.0 \cdot 10^5$, respectively. The samples were well separated, as demonstrated by the linear decrease in log *M* with increasing elution volume. The column generally demonstrates good separation according to *M* in the range from $2.0 \cdot 10^4$ to $2.0 \cdot 10^6$ for cellulose. This is supported by the overlapping calibration plots.

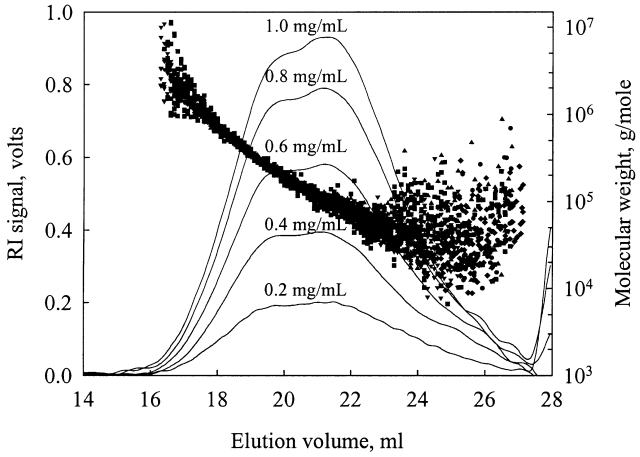

Figure 4. Calibration plots and elution curves for different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) of sample SSC. Sample volume was 200 μL.

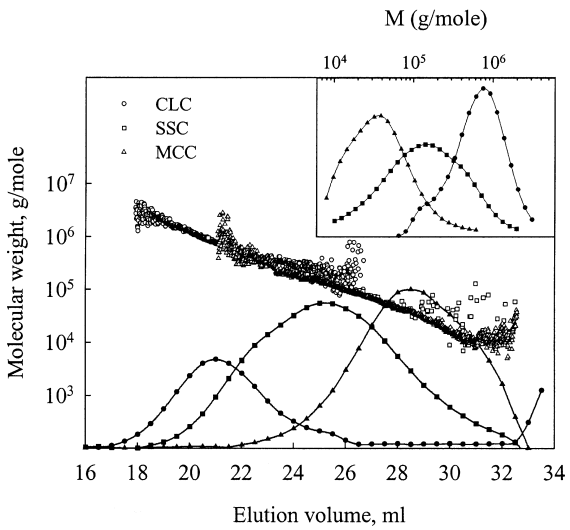

Figure 5. Calibration plots and elution curves for the cellulose samples CLC, SSC and MCC. Insert shows the calculated molecular weight distribution (MWD) of the same samples.

The differential molecular weight distribution is calculated automatically by the software. In order to obtain correct estimates of the MWD, it is necessary that a calibration curve be correctly assigned across the entire distribution. This is usually not the case at the low molecular weight tail because of decreasing signal-to-noise in the light scattering signal. In this case, a linear fit was used. The inset in Figure 5 shows the calculated MWD for the three samples. Molecular weight distributions of different wood-based cellulose samples will be further discussed in another work.[10]

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