Characterization of Nonderivatized Cellulose by Gel Permeation Chromatography

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Synopsis

The development of a gel permeation chromatography (GPC) system for the analysis of nonderivatized cellulose in the DP range of technical cellulose products is reported. In contrast to traditional GPC, where cellulose derivatives have to be prepared, in this work the cellulose solvent cadoxen was applied for the determination of the molecular weight distribution using a special analysis system. Nuclear magnetic resonance studies of cadoxen solutions indicated no complex formation between cadoxen and cellulose. Therefore, to avoid precipitation of cellulose from the prepared solution, cadoxen was also used as eluent in the chromatographic system. For this reason a stationary phase had to be found which is stable under the given strong alkaline conditions. The utilization of Fractogel TSK as column material resulted in the separation of dissolved cellulose showing a DP_v between 400 and 2000 with good reproducibility. The application of very sensitive detectors together with a GPC program allows the characterization of unknown cellulose samples of different origin within a few hours.

INTRODUCTION

Natural polymers, such as cellulose or hemicellulose, are polydisperse substances. The degree of polydispersity influences the physical properties, especially in the case of cellulose. Therefore the determination of both the molecular weight and the molecular weight distribution is of great importance. In the past, various methods have been elaborated for these investigations. The most important methods are viscosimetry, precipitation fractionation, and gel permeation chromatography (GPC).[1-6]

For the viscometric determination of the degree of polymerization (DP_v) of underivatized cellulose samples, a number of cellulose solvents can be applied (e.g., EWNN, cuen, cuoxam, cadoxen), but the results do not include information about the chain length distribution. The latter can be obtained by fractionated precipitation,[7] a very tedious and time-consuming method. In contrast to this, gel chromatography[8-12] allows the continuous fractionation of polymers. The distinct advantage of this method is that a distribution curve (chromatogram) can be obtained within a relatively short time. Most gel chromatographic results presented during recent years have dealt with the separation of modified celluloses. Derivatives, such as cellulose nitrate or cellulose tricarbanilate, which are soluble in organic solvents, were applied. The advantageous properties of these derivatives (solubility, stability) facilitate the determination of molecular weight distribution by GPC; the derivatization reaction, however, may cause an alteration of the initial cellulose. For this reason several attempts have been made to separate underivatized cellulose by means of GPC.[13-15] Very promising results were obtained by the use of cadoxen as cellulose solvent and as eluent; but the fractionation of cellulose

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solutions was complicated by the mechanical and chemical instability of the gels which were used as stationary phase.[16,17]

The rapid development of chromatography in the last few years has provided new column materials which can be used in cadoxen. In a previous work[18] a GPC system was presented that allows the separation of unmodified cellulose as well as hemicellulose and carboxymethylcellulose. In the present work the composition of the stationary phase was changed and the resulting calibration curve was investigated with respect to Benoit's universal calibration curve. Detection of the injected cellulose solutions was optimized and a GPC program was applied for the evaluation of the chromatograms.

At the same time, cellulose solutions were prepared in order to study whether the dissolution proceeds by formation of a cellulose–cadmium complex. In the literature, the question of the existence of this complex is still unanswered, owing to contradictory results reported to date.[19,20] The aim of these investigations was to verify, by means of nuclear magnetic resonance (NMR) spectroscopy, whether a complex of cellulose with cadoxen occurs or not.

MATERIALS AND METHODS

Cadoxen—Solvent and Eluent

The preparation of cadoxen (CdO) was carried out according to the method described by Henley[21] and Donetzhuber.[22] However, a variation in the preparation conditions brought better results. The application of temperatures below 0°C allows complete dissolution of CdO, which means that centrifugation of the resulting solution after the first preparation step is unnecessary. Undiluted cadoxen was used as both eluent and as cellulose solvent.

NMR Studies of Cellulose Solutions

^{111}Cd-spectra of cadoxen and cellulose solutions were recorded on a Bruker WP-80 operating in the FT mode at 16.97 MHz. As external reference a 1 M $CdCL_2$ solution was used.

GPC System

The GPC system consisted of components of different makes. The eluent was delivered with a high performance liquid chromatography pump (Model

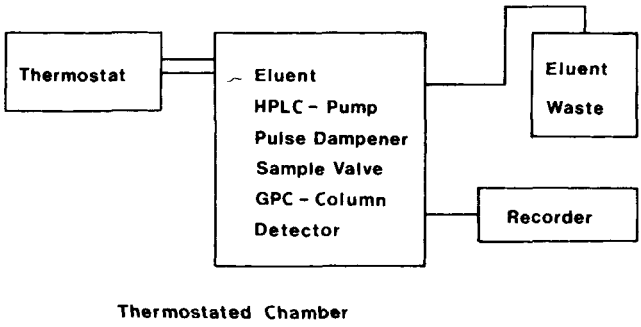

Fig. 1. Schematic representation of the GPC system constructed.

110; Altex, Berkeley, CA, USA) through a pulse dampener (Gynkotek, München, BRD). The sample valve (Valco Instruments Co., Houston, TX, USA) was fitted with a 500-μL sample loop. The dimensions of the column, which was specially made, are 50 × 1.6 cm I.D. Measurement of the eluted cellulose samples was carried out with two different detectors: (a) refractive index detector (Melz; Gynkotek); (b) interference refractometer (5902, tecator; Gynkotek). All components (Fig. 1) were kept within a thermostated chamber. As stationary phases, two gels were applied (Fractogel TSK HW 65 and 75, Merck; Darmstadt, BRD). These gels proved to be stable under the alkaline conditions given.

Sample Preparation

The cellulose samples were—if necessary—crushed with either a vibration mill or a hammer mill. Dissolution of cellulose was complete within 1–2 h; the maximum concentration was ca. 5 mg/mL. The available cellulose samples were in the DP range of 400–2000, thus covering a large part of the technical cellulose products.

Different dextrans (T10, T40, T500, T2000, Pharmacia Fine Chemicals AB; Uppsala, Sweden) were used as reference substances.

Calibration

A number of unidentified cellulose samples were analyzed viscosimetrically. Measurements were carried out with an automatic system (AVS/G, Schott GmbH; Hofheim, BRD) using EWNN (Fluka AG; Buchs, Switzerland) as cellulose solvent. The DP_v value was calculated according to SNV 95598.[23]

For investigation of the universal calibration curve, intrinsic viscosities $[\eta]$ of dextran solutions also had to be determined.

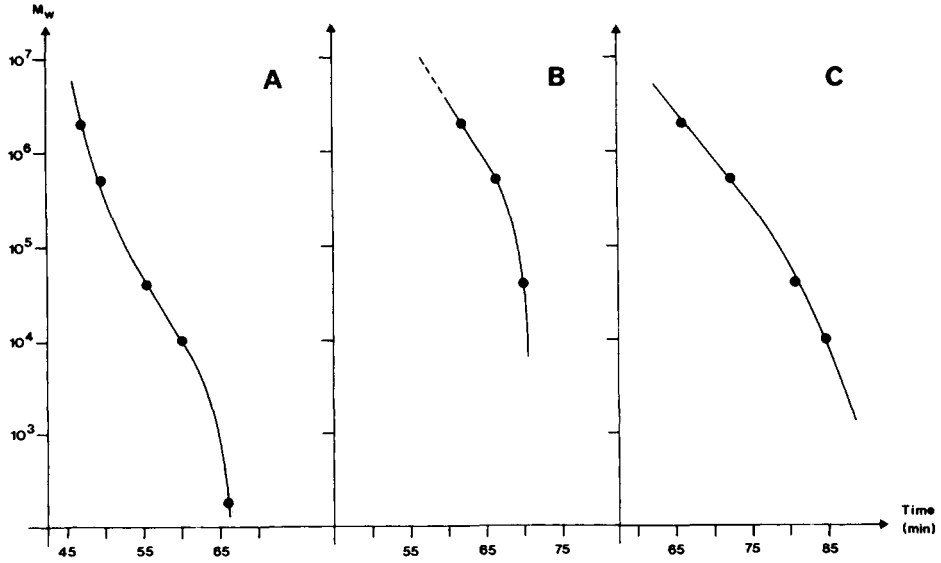

Fig. 2. Calibration curves for dextrans using three different stationary phases; Conditions: Flow rate = 1.0 mL/min; Temperature = 15°C; Eluent = Cadoxen; Detection = RI. (A) TSK HW65; (B) TSK HW75; (C) Mixture of TSK HW65 and 75 (1:1).

RESULTS AND DISCUSSION

The evaluation of NMR spectra of dissolved celluloses indicates no complex formation between cellulose and cadoxen. In comparison with the spectrum of pure cadoxen there is no effect on the chemical shift of Cd signal when cellulose is present in the solution. The coordination of cellulose to Cd would result in a change from a Cd-N_6 kernel to a Cd-N_4O_2 kernel, which is assumed to be associated with a change in the Cd chemical shift. The observed NMR data, the preferential coordination of Cd by nitrogen, and the fact that ethylenediamine is present in great excess, indicate that complexing with cellulose is not likely to take place. This observation is also supported by the fact that solutions of cellulose can only be diluted with water to a small extent without precipitation.[24] Therefore, in contrast to earlier publications,[25] it is shown that the utilization of undiluted cadoxen as eluent in gel chromatography is absolutely necessary. To avoid an interfering base line drift, the GPC system must be controlled thermostatically.[26]

A further improvement of this GPC system was possible by changing the separation characteristics of the stationary phase. In the chromatogram of dextran T2000, which was obtained on a TSK HW 65 column (exclusion

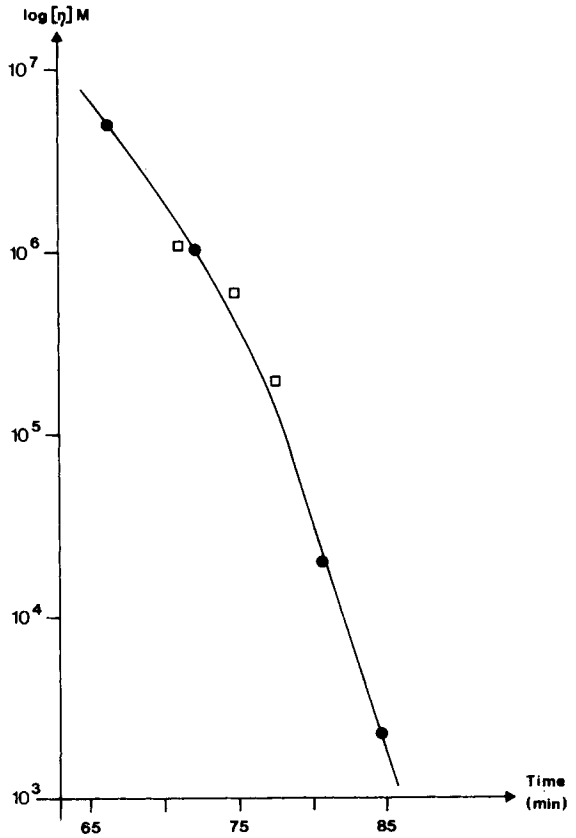

Fig. 3. Universal calibration curve for dextrans (●) and celluloses (□); Column—Stationary phase C; Conditions—see Figure 2.

limit = 1×10^6 Dalton), the high molecular portion of the polymer appears partly in the void volume. Application of a TSK HW 75 column (exclusion limit = 5×10^6 Dalton) under the same conditions also allows the separation of the high molecular component. However, TSK HW 75 has a poorer separation efficiency in the low molecular region. Therefore in this work a mixture of TSK HW 65 and 75 (1 : 1) was prepared and investigated. In Figure 2 a comparison of the calibration curves is given. Dextrans of different molecular weight were used as reference substances. It can be seen [Fig. 2(c)] that a column filled with the gel mixture clearly shows better separation properties. With this GPC system celluloses of different origin were chromatographed.

Based on these findings, it was necessary to investigate the influence of hydrodynamic volume on retention time in GPC. Benoit and co-workers[27,28] obtained a universal calibration curve, whereby different types of macromolecules can be plotted on the same curve. In this case the intrinsic viscosity $[\eta]$ multiplied with the molecular weight (M) is plotted against the retention time (time elapsed until the maximum of the elution curve appears). Figure 3 shows that the dextran and the three measured cellulose samples seem to follow this rule and may therefore be given in a single curve.

For the analysis of unknown cellulose samples, a direct cellulose calibration curve is preferred. Therefore, a number of celluloses were characterized

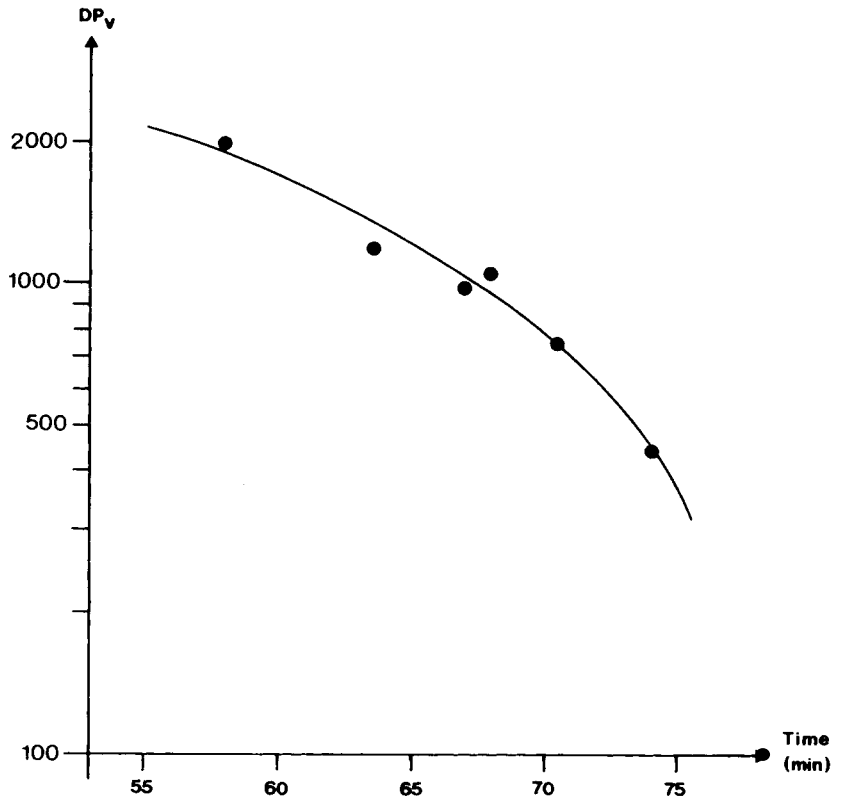

Fig. 4. Cellulose calibration curve; Column, Stationary phase C; Conditions, see Figure 2.

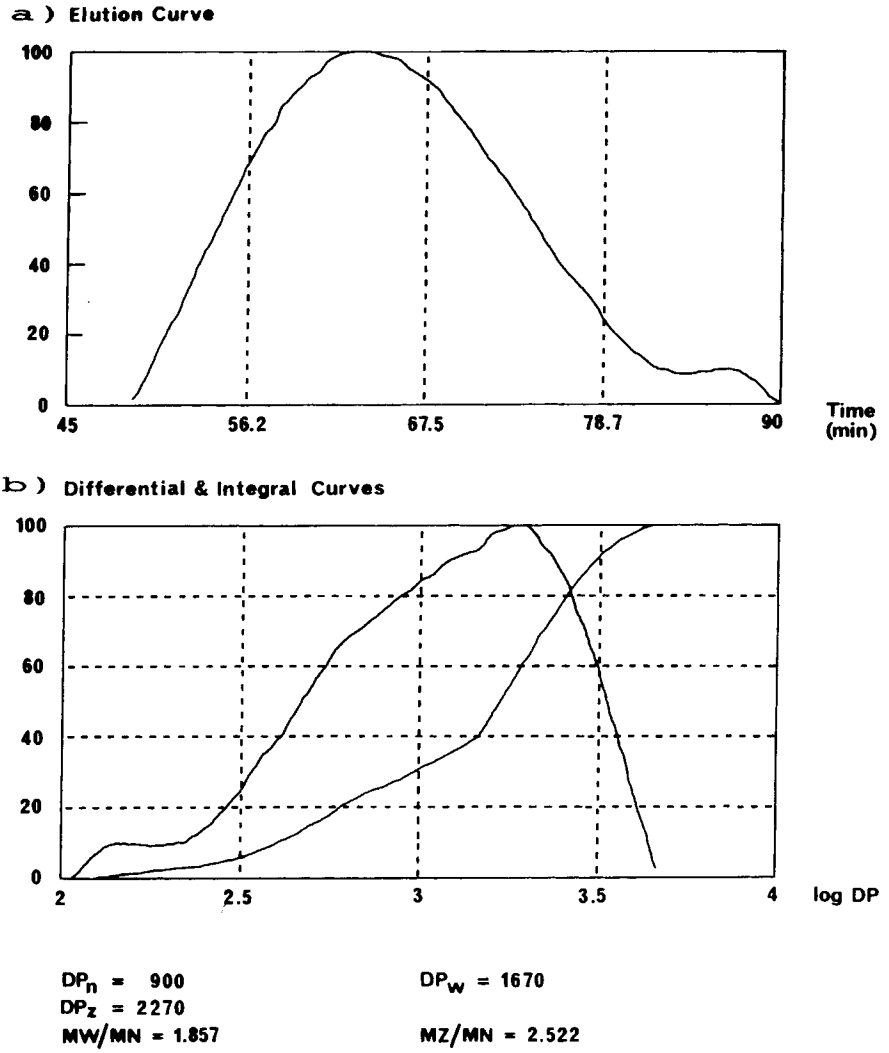

Fig. 5. Elution curve (a) and differential and integral curves (b) of a cellulose isolated from pretreated cotton linters; $DP_n \times 162.1$ = MN...number average molecular weight; $DP_z \times 162.1$ = MZ...Z average molecular weight; $DP_w \times 162.1$ = MW...weight average molecular weight; (162.1 = molecular weight of the anhydroglucose-unit).

viscometrically. EWNN served as cellulose solvent, and the evaluation of the degree of polymerization (DP_v) was carried out according to SNV 95598.[23] DP_v values are theoretically not identical with DP_w (although of very similar magnitude), but can be used for the corresponding calibration curve (Fig. 4).

After establishing the calibration curve, cellulose samples were analyzed using the described GPC system in combination with a GPC program (C-R3A, Shimadzu Corp.; Tokyo, Japan). In Figure 5 the elution curve as well as the differential and integral molecular weight distribution curves are shown. In addition, DP_w and the degree of dispersion are automatically calculated. The

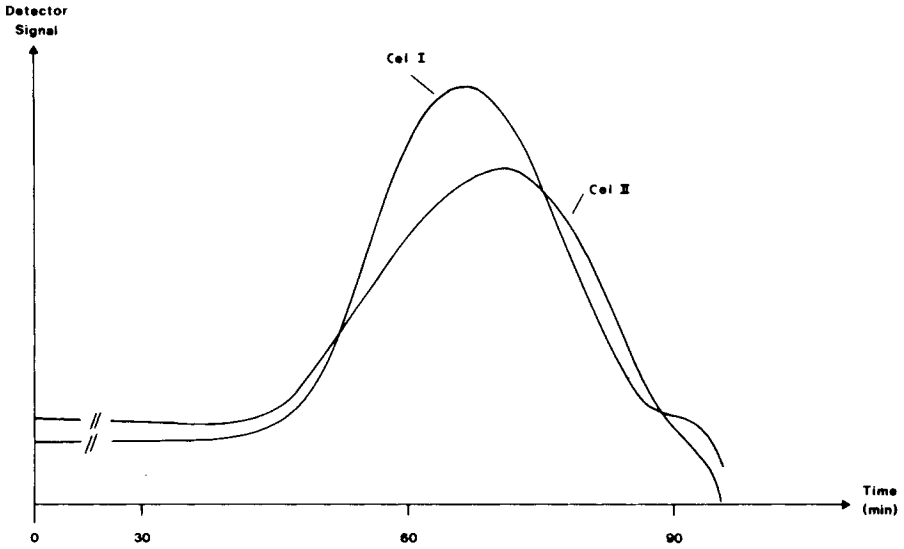

Fig. 6. Elution curves of two celluloses; Column, Stationary phase C; Conditions, see figure 2; Detection, interference refractometer; Cel I, pretreated wood cellulose; Cel II, pretreated linters.

cellulose of the given example was isolated from the cellulosic residue of a pretreated cotton linter.

The eluent of the chromatogram shown above was monitored with a sensitive refractive index detector (Melz). Even though the concentration of the cellulose solution is only ca. 5 mg/mL, the resulting solution is rather viscous. For this reason, a reduction of the concentration is desirable. To achieve this, detection sensitivity had to be increased markedly. For this purpose an interference refractometer (tecator) was tested with good results. As can be seen in Figure 6, this type of detector enabled monitoring of cellulose solutions with an initial concentration of only 1–2 mg/mL. This decrease in concentration affects the dissolution of cellulose positively (quantitative dissolution within less than one hour). Furthermore, the low viscosity of the solution facilitates sample injection on the GPC column.

CONCLUSION

Cadoxen was used as cellulose solvent because the resulting solutions are not only clear and colorless (which allows detection by refractive index measurement), but also stable and insensitive to oxidation. From NMR investigations of cellulose solutions it can be deduced that complex formation between cellulose and cadoxen does not occur. This fact refers to the observation that cadoxen solutions can be only slightly diluted with water. To avoid precipitation of dissolved cellulose, undiluted cadoxen must also be used as eluent.

In the investigated range of DP, the described GPC system and the gels chosen as stationary phase proved to be well suited for the determination of molecular weight distribution of unmodified cellulose with cadoxen as eluent.

The application of an interference refractometer showed that a reduction of the cellulose concentration to 1–2 mg/mL is possible.

The development of this analytical method allows the characterization of unknown celluloses within 3–4 h. This is a distinct improvement in comparison with other methods. It is, therefore, suitable for routine checks of molecular weight averages and molecular weight distributions of technical cellulose products.

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