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Short Communication

Size-exclusion chromatography of cellulose and chitin using lithium chloride-N,N-dimethylacetamide as a mobile phase

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ABSTRACT

Distributions of molecular mass of cellulose and chitin were determined by size-exclusion chromatography (SEC) using 5% (w/w) lithium chloride-N,N-dimethylacetamide as an eluent. The peak positions in SEC patterns of the cellulose samples used corresponded well to their molecular masses, which were measured by a viscometric method using cupriethylenediamine. Thus, the packed gel consisting of styrene-divinylbenxene copolymer was found to be applicable to the SEC analysis of cellulose and chitin, when 5% (w/w) lithium chloride-N,N-dimethylacetamide was used as the solvent for polysaccharides and as the eluent. The elution patterns obtained in this SEC system indicated that the molecular mass of commercial chitins prepared from crab shell is much higher than that of cotton, and that the chitins have two peaks of distribution of molecular mass.

INTRODUCTION

Many kinds of aqueous and non-aqueous solvent systems for cellulose or chitin have been reported so far. Although depolymerization of the polysaccharide molecules occurs to a greater or lesser extent in most systems during and/or after the preparation of cellulose or chitin solutions, some of the solvent systems may be applicable to the media for determining molecular mass and distribution of molecular mass of cellulose or chitin. Cupriethylenediamine (CED, cu. 0.5 *M*) is generally used as an aqueous solvent for cellulose samples to determine their molecular mass by the viscosity method, and carbanilation of all hydroxyl groups of cellulose samples followed by size-exclusion chromatography (SEC) using tetrahydrofuran as an eluent is well accepted as the method for obtaining their distribution of molecular mass [1,2]. On the other hand, a few solvent systems have been found for chitin, but only the lithium chloride-N,N-dimethylacetamide (LiCl–DMAc) system is applicable to viscosity measurements of chitin samples. However, so far no papers have reported the measurement of distribution of molecular mass of chitin samples.

Since the LiCl–DMAc system can dissolve cellulose, chitin, and other many polysaccharides with little depolymerization [3–8], this system may be used as the solvent for cellulose and chitin samples for size-exclusion chromatography without any derivatizing steps for the polysac-

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charides. The molecular masses and distributions of molecular mass of some cellulose samples have been estimated by SEC using 0.5% (w/w) LiCl-DMAc as an eluent [9]. In this study, the LiCl-DMAc system was applied to SEC of cellulose and chitin, using an SEC column of copolymer gel consisting of styrene–divinylbenzene.

EXPERIMENTAL

Chemicals and solvents

N,N-Dimethylacetamide and lithium chloride of analytical-reagent grade were purchased from Wako (Osaka, Japan).

The 10% (w/w) LiCl-DMAc solution was prepared by the addition of a predetermined amount of LiCl to DMAc at 80°C with stirring, and the LiCl-DMAc solution thus prepared was kept at 80°C in order to avoid partial precipitation of LiCl.

Cellulose and chitin samples

The cellulose samples used were cotton, regenerated cellulose prepared from ramie [10], microcrystalline cellulose powder (Avicel; Asahi Chemical, Tokyo, Japan) and a low-molecular-mass cellulose prepared by homogeneous hydrolysis of microcrystalline cellulose powder with phosphoric acid [11] (Table I). The viscosity-average degrees of polymerization ($\overline{DP}_v$) of the cellulose samples were evaluated from their intrinsic viscosities, which were measured using 0.5 *M* CED as the cellulose solvent, by the following equation [12]:

TABLE I

VISCOSITY-AVERAGE DEGREES OF POLYMERIZATION ($\overline{DP}_v$) OF CELLULOSE SAMPLES

$\overline{DP}_v$ values were calculated from intrinsic viscosities measured in 0.5 M CED, according to $[\eta] = 0.581 \times DP^{1.00}$ [12].

Cellulose sample	$\overline{DP}_v$
Cotton	1690
Regenerated ramie	1190
Microcrystalline cellulose powder	240
Low-molecular-mass cellulose	9

$$[\eta] = 0.571 \times DP^{1.00}$$

Two commercial chitin samples with powder and particle forms prepared from crab shell were used (PSH and CLH, respectively; Yaizu Suisan Kagaku, Shizuoka, Japan).

Preparation of cellulose and chitin solutions

Cellulose solutions in 5% (w/w) LiCl-DMAc were prepared as follows: (1) a dry cellulose sample of 10 mg was suspended in 10 ml of 10% (w/w) LiCl-DMAc; (2) the mixture was heated at 80°C until the cellulose sample was sufficiently swollen (*ca. 0-24* h, depending on the molecular mass of the cellulose samples); (3) 10 ml of DMAc were added to the mixture to adjust the concentration of the solution to 5% (w/w) LiCl–DMAc; and (4) a clear cellulose solution was obtained after the mixture was kept at room temperature for several days with occasional swirling.

Chitin solutions were prepared by stirring a mixture of a dry chitin sample of 10 mg and 20 ml of 5% (w/w) LiCl-DMAc at room temperature, as described by Terbojevich et *al.* [7].

Apparatus and conditions

A high-pressure pump of the single-plunger type (Milton Ray, New York, USA) with a pressure reservoir and a differential refractometer (R401; Waters, Milford, MA, USA) was used for the SEC system. A packed SEC column consisting of styrene-divinylbenzene copolymer gel (TSK-gel GMHXL; 10 μm particle size, 300 × 7.8 mm I.D.; Tosoh, Tokyo, Japan) was used at room temperature. The column was originally packed with acetone, because it was specially designed for SEC with dimethyl sulphoxide as an eluent. Thus, the solvent in the column was exchanged stepwise from acetone to 5% (w/w) LiCl-DMAc, using DMAc and 0.5%, 1.0% and 3.0% (w/w) LiCl-DMAc, and 5% (w/w) LiCl-DMAc was used as the eluent for the column. The solvents for SEC were degassed using an ultrasonic apparatus. The injection volume and the flow-rate for SEC were 0.26 ml and 0.1 ml/min, respectively. The concentrations of cellulose or chitin in 5% (w/w) LiCl-DMAc for SEC were ca. 0.05% (w/w). Before injection, all samples were filtered through a membrane

tec PTFE, average pore diameter of 0.50 μm; Toyo Roshi Kaisha, Tokyo, Japan).

RESULTS AND DISCUSSION

Since standard samples of neither polystyrene nor polyethylene oxide for SEC were soluble in the LiCl–DMAc system, a calibration curve for the column with the LiCl-DMAc system could not be obtained by the usual method. Then cellulose samples with various $\overline{DP}_v$ values were subjected to column chromatography in order to obtain the following information: (1) the possibility of using the column system described in this study for SEC analysis of polysaccharides and (2) distribution of molecular mass of cellulose samples.

The size-exclusion chromatograms of cellulose samples dissolved in 5% (w/w) LiCl-DMAc showed that those cellulose samples with higher $\overline{DP}_v$ values (Table I) had SEC patterns with higher molecular mass (Fig. 1). Thus, the elution patterns of celluloses in this system are probably governed by their molecular mass, and therefore this system may be applicable to SEC of polysaccharides without any derivatixing steps. Furthermore, the distribution of molecular mass of cotton was relatively narrow in comparison with that of regenerated ramie, and the low-molecular-mass cellulose also had a sharp distribution pattern. These results are very consistent with those obtained by SEC analysis of carbanilates of cellulose samples [13].

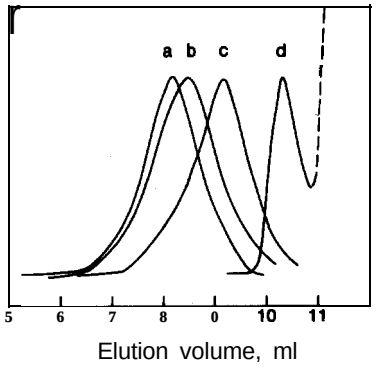

Fig. 1. SEC patterns of celluloses in 5% (w/w) LiCl-DMAc. Cellulose samples; (a) cotton; $\overline{DP}_v$ = 1690, (b) regenerated ramie; $\overline{DP}_v$ = 1190, (c) microcrystalline cellulose; $\overline{DP}_v$ = 240; and (d) low-molecular-mass cellulose; $\overline{DP}_v$ = 9. A large peak due to the solvent components of cellulose samples always appears at the end of the SEC patterns, and a part of the elution pattern of low-molecular-mass cellulose is overlapped by the large peak.

As shown in Fig. 2, although two chitin samples had similar elution patterns, the chitin PSH had slightly higher molecular mass than that of the chitin CLH. Since 1 and 2 days were required for complete dissolution of the chitins PSH and CLH, respectively, in LiCl-DMAc at room temperature, the longer dissolution treatment may bring about the slight depolymerization of the chitin CLH.

The void volume and the total volume of the same type of column, which were obtained using tetrahydrofuran as the eluent, were 5.3 and 11.5 ml, respectively. However, the values using 5% (w/w) LiCl-DMAc as the eluent could not be obtained, because the usual standard samples suitable for determining these values were insoluble in the LiCl-DMAc system. The peak positions of the chitin samples indicate that they had DP values much higher than those of the cellulose samples used. Furthermore, the elution patterns of chitins clearly showed two peaks with relatively narrow distributions. Judging from the elution patterns, the peak at the smaller elution volume is not due to the void volume of this column, but is probably due to the original distribution of molecular mass of chitins. Since the expansion parameters must be different to some extent between cellulose and chitin molecules in LiCl–DMAc, DP values of chitins may not be directly comparable with those of cellu-

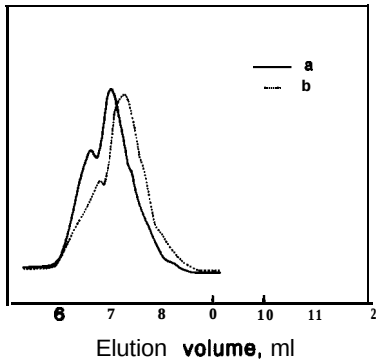

Fig. 2. SEC patterns chitins in 5% (w/w) LiCl–DMAc. Chitin samples: (a) chitin PSH and (b) chitin CLH.

loses on the basis of the elution volumes obtained in this system. Nevertheless, chitin samples seem to have molecular mass much higher than that of cotton ($\overline{DP}_v$ = 1690), on the assumption that both chitin and cellulose molecules have similar molecular expansion states, which are probably governed by their β-1,4 glycoside bonds. Since light-scattering analysis of chitins in LiCl-DMAc revealed that they had DP values of cu. 2500 [7], the relationship between the molecular masses of chitins and those of celluloses, obtained in the SEC analysis, is consistent with the report. SEC analyses using LiCl–DMAc will be further studied in order to establish the best method for determining the molecular mass and distribution of molecular mass of cellulose, chitin and other polysaccharides.

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