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Note

# Molecular mass distribution of chitin and chitosan

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Molecular mass distributions of chitosan are generally calculated from size-exclusion chromatography (SEC) elution patterns obtained using a chitosan–aqueous acid buffer solution and an SEC column for aqueous solvents [1–3]. However, since the degrees of deacetylation (ddAc) of chitosan soluble in aqueous acid buffer solutions vary in the range of ca. 60–100%, SEC elution patterns obtained by the above method may be influenced by these ddAc values of chitosan [4]. Methods for determining molecular mass distributions of chitin have not yet been established, because neither convenient solvents for chitin nor derivatization methods suitable for SEC have been found.

In the previous paper [5], on the other hand, we reported that SEC elution patterns of cellulose and chitin could be obtained using a column consisting of styrene–divinylbenzene copolymer gel and 5% (w/w) lithium chloride–*N*,*N*-dimethylacetamide (LiCl–DMAc) as the solvent of the polysaccharides and as the eluent. Although chitosan is insoluble in LiCl–DMAc, it is known that *N*-acetylchitosan can be prepared from chitosan with acetic anhydride under homogeneous and mild conditions [6], which would cause little depolymerization in chitin. Thus, *N*-acetylation of chitosan and dissolution of the *N*-acetylated chitosan in LiCl–DMAc enable the SEC analysis of chitosan. In this study, therefore, the SEC method is applied to chitin samples of different origins and to chitosan samples with different ddAc.

Fig. 1 shows elution patterns of pullulan standards. Their peak positions were completely governed by their $\overline{dp}$ reported by the manufacturer, although the

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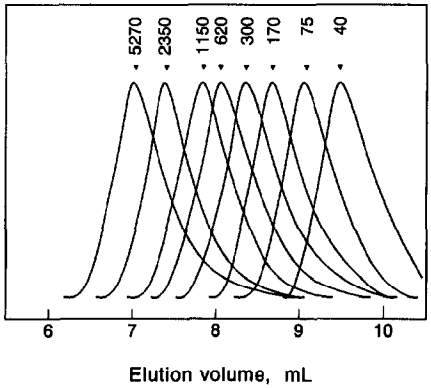


Fig. 1. Size-exclusion chromatograms of pullulan standards. Numbers are the $\overline{dp}$ reported by the manufacturer.

patterns had some tailings. When $\overline{dp}$ of pullulan standards were plotted as a function of the elution volume at their peaks, nearly linear relationship was obtained as shown in Fig. 2. This relationship may be applicable to determination of $\overline{dp}_w$ and $\overline{dp}_n$ of chitins from their SEC elution patterns, where the hydrodynamic volume of chitin dissolved in LiCl–DMAc was assumed to be similar to that of pullulan at equal $\overline{dp}$. When $\overline{dp}_w$ and $\overline{dp}_n$ of pullulan standards were calculated from their SEC elution patterns shown in Fig. 1, using the relationship obtained in Fig. 2, their $\overline{dp}_w/\overline{dp}_n$ values or dispersities were in the range of 1.5–2.1, whereas those reported by the manufacturer were ca. 1.1. The conditions for preparing pullulan–LiCl–DMAc solutions may bring about partial depolymerization of pullulan.

As shown in Fig. 3, the chitin samples used in this study had characteristic SEC elution patterns, depending on their origins, and each pattern consisted of a main

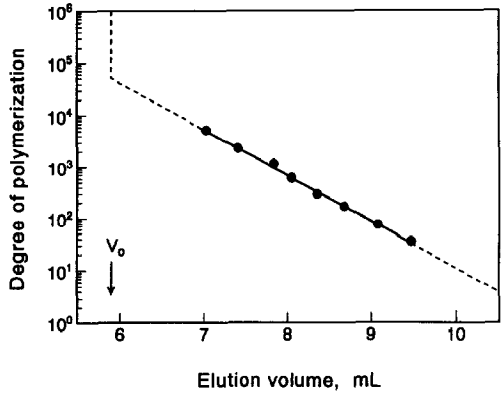


Fig. 2. Calibration curve obtained from the relationship between $\overline{dp}$ of pullulan standards and their elution volume.

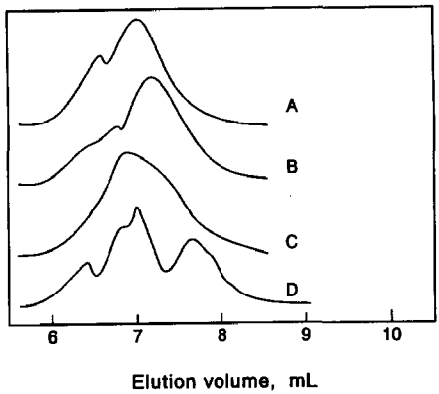


Fig. 3. Size-exclusion chromatograms of: A, crab shell chitin in powder form; B, crab shell chitin in flake form; C, shrimp shell chitin; D, squid pen chitin.

peak with shoulders or small peaks. Squid pen chitin, in particular, had a pattern consisting of several peaks clearly separated from each other. Two crab shell chitins in flake and powder forms had similar elution patterns, although the peak positions were slightly different. All these elution patterns were reproducible by repetition of SEC injection, thus indicating that the characteristic elution patterns shown in Fig. 3 reflect molecular mass distributions of the chitin samples.

The $\overline{dp}_w$, $\overline{dp}_n$, $\overline{dp}_w/\overline{dp}_n$, and $\overline{dp}$ values at the maximum peak ($\overline{dp}_{peak}$) of these chitins were then calculated from their elution patterns on the basis of the calibration curve obtained in Fig. 2, and are summarized in Table 1. These $\overline{dp}_w$ values, ca. 6300–9000, were higher than those of the usual celluloses of higher plants, ca. 800–2500 [5]. In this SEC study, $\overline{dp}_w$ and $\overline{dp}_n$ were calculated using the calibration curve obtained for pullulan standards, on the assumption that pullulan and chitin with equal $\overline{dp}$ had hydrodynamic equal volumes. Chitin is a relatively rigid polysaccharide consisting of $\beta$-(1 → 4)-linked *N*-acetylglucosamine residues, whereas pullulan consists of $\alpha$-(1 → 4)- and $\alpha$-(1 → 6)-linked glucose residues. Thus, this structural difference between chitin and pullulan may lead to a higher $\overline{dp}_w$ of chitin in this study, and some correction may be necessary for obtaining a

Table 1
Degrees of polymerization of chitin samples

| Chitin sample | ddAc [a] | $\overline{dp}_w$ | $\overline{dp}_n$ | $\overline{dp}_w/\overline{dp}_n$ | $\overline{dp}_{peak}$ |
|---|---|---|---|---|---|
| Crab shell (powder form) | 6 | 9050 | 4280 | 2.1 | 5350 |
| Crab shell (flake form) | 6 | 6190 | 1860 | 3.3 | 3640 |
| Shrimp shell | n.d. [b] | 6280 | 2290 | 2.7 | 6840 |
| Squid pen | n.d. [b] | 7510 | 2650 | 2.8 | 6780 |

[a] Degree of deacetylation.
[b] Not determined.

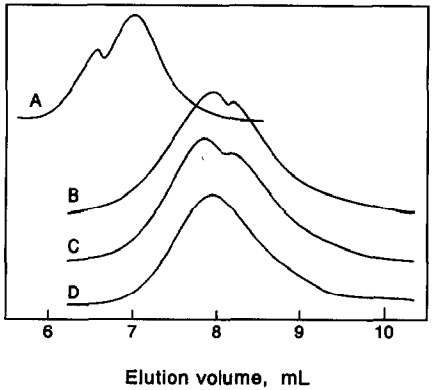


Fig. 4. Size-exclusion chromatograms of: A, crab shell chitin in powder form; B, commercial chitosan with ddAc of 75% prepared from chitin A; C, deacetylated chitosan with ddAc of 95% prepared from chitosan B by alkali-treatment; D, deacetylated chitosan with ddAc of 95% prepared from regenerated chitosan B gel by alkali-treatment.

more accurate $\overline{dp}_w$ of chitin by this SEC method, because of their different hydrodynamic volumes.

Molecular mass and molecular mass distributions of chitin and chitosan cannot be compared in the same SEC systems, because no solvent systems which dissolve both chitin and chitosan have been reported so far. Therefore, it is noteworthy that changes in molecular mass and molecular mass distributions during the process of deacetylation of chitin for preparing chitosan may be studied with respect to ddAc or other conditions of deacetylation, by using our method. As shown in Fig. 4, the commercial chitosan with ddAc of 75% (powder form) had lower molecular mass than that of the original chitin, thus indicating that depolymerization occurred to some extent during the manufacturing process for preparing chitosan. Although a shoulder peak appeared in the elution pattern of this chitosan, the pattern was different from that of the original chitin. The elution pattern of chitosan with ddAc of 95% (C in Fig. 4), which was prepared from the chitosan with ddAc of 75% by the conventional method, was very similar to that of the original chitosan. On the other hand, when chitosan with ddAc of 95% (D in Fig. 4) was prepared from regenerated chitosan gel with ddAc of 75% by deacetylation with 40% NaOH, the shoulder peak disappeared in the elution pattern. In Fig. 5, the elution pattern of the commercial chitosan with ddAc of 85% (flake form) is presented with that of the original chitin. The elution pattern of this chitosan in flake form was roughly similar to that of the original chitin in terms of shoulders or small peaks, although the molecular mass of the chitosan was lower than that of the chitin. Further deacetylation of the chitosan with ddAc of 85 to 95% by the conventional method resulted in a smoother SEC pattern, indicating that partial depolymerization of chitosan occurred during the deacetylation.

In Table 2, $\overline{dp}_w$, $\overline{dp}_n$, $\overline{dp}_w/\overline{dp}_n$, and $\overline{dp}_{peak}$ of these chitosan samples are summarized together with those of the original chitins. The manner of decrease in

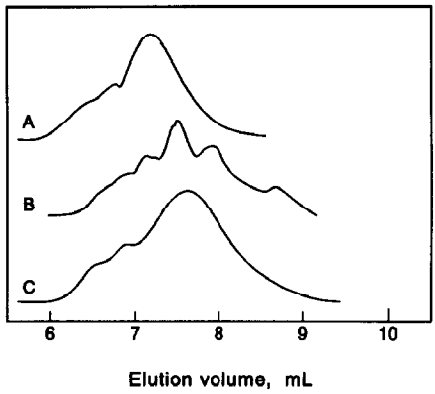


Fig. 5. Size-exclusion chromatograms of: A, crab shell chitin in flake form; B, commercial chitosan with ddAc of 85% prepared from chitin A; C, deacetylated chitosan with ddAc of 95% prepared from chitosan B by alkali-treatment.

$\overline{dp}$ during deacetylation of chitin was observed. It is noticeable that $\overline{dp}_w/\overline{dp}_n$ values or dispersities increased as deacetylation of chitin or chitosan proceeded; the original chitins had narrower molecular mass dispersities than chitosan.

## 1. Experimental

*Chitin, chitosan, and pullulan samples.*—Two commercial chitins in powder and flake forms (Chitins PSH and CLH, respectively; Yaizu Suisan Kagaku, Shizuoka,

Table 2
Degrees of polymerization of chitosan samples

| Sample | ddAc [a] | $\overline{dp}_w$ | $\overline{dp}_n$ | $\overline{dp}_w/\overline{dp}_n$ | $\overline{dp}_{peak}$ |
|---|---|---|---|---|---|
| Crab shell chitin (powder form) | 6 | 9050 | 4280 | 2.1 | 5350 |
| Chitosan | 75 | 1270 | 330 | 3.9 | 820 |
| Chitosan [b] | 95 | 1090 | 200 | 5.4 | 1080 |
| Chitosan [c] | 95 | 990 | 170 | 5.9 | 760 |
| Crab shell chitin (flake form) | 6 | 6190 | 1860 | 3.3 | 3640 |
| Chitosan | 85 | 2910 | 800 | 3.6 | 1920 |
| Chitosan [b] | 95 | 3310 | 565 | 5.9 | 1500 |

[a] Degree of deacetylation.
[b] Further deacetylated product prepared from the commercial chitosan by repetition of heating with aq 40% NaOH at 95°C for 4 h.
[c] Further deacetylated product prepared from regenerated gel of the commercial chitosan with aq 40% NaOH at 85°C for 2 h.

Japan) derived from crab shell (*Chitonoecetes japonicus*), and one commercial chitin (C-368; Katokichi, Kagawa, Japan) derived from shrimp shell (*Penaeus japonicus*) were used without further purification. A chitin sample derived from squid pen (*Loligo bleekeri*) was purified with 0.5 M NaOH at 80°C for 24 h under $N_2$. Two chitosan samples in powder and flake forms (chitosans PSH and CLH, respectively, Yaizu Suisan Kagaku) derived from the above crab shell chitins were commercially available. The chitosan sample in powder form was further deacetylated by the following two methods: (*a*) repeated (2–3 times) heating in aq 40% NaOH at 95°C for 4 h; and (*b*) a regenerated chitosan gel was prepared by dissolution in dil aq AcOH followed by regeneration in aq NaOH, and this gel was treated once with aq 40% NaOH at 85°C for 2 h. Degrees of deacetylation (ddAc) of chitosan were calculated from the N content. A series of pullulans (Shodex standards P-82; Showa Denko, Japan) were used as standards for SEC.

*Preparation of LiCl–DMAc solutions of chitin, chitosan, and pullulan.*—A chitin solution in 5% (w/w) LiCl–DMAc was prepared according to the previous paper [5]. Chitin samples used in this study were dissolved within 3 days by this method. In the case of chitosan samples, first the amine group of chitosan was acetylated with $Ac_2O$ under homogeneous conditions according to the reported method [6], and *N*-acetylated chitosan was obtained by freeze-drying. This *N*-acetylated chitosan was then dissolved in 5% LiCl–DMAc according to the method described above for the dissolution of chitin. Pullulan solutions in 5% LiCl–DMAc were prepared from a mixture of a dry pullulan sample (2 mg) and 20 mL of 5% LiCl–DMAc by heating at 60–90°C for 3–6 h.

*Measurement of molecular mass distribution by SEC.*—Chitin and *N*-acetylated chitosan solutions were subjected to SEC using a TSK GMHXL column [7.5 (i.d.) × 300 mm; Tosoh, Tokyo, Japan], according to the method described in the previous paper [5]. The injection volume was 0.26 mL, and the flow-rate of the eluent was 0.106 mL/min. The polymer concentrations were 0.1 mg/mL for the pullulan standards and 0.25 mg/mL for chitin and *N*-acetylated chitosan. The solutions were filtered through a membrane filter (Advantec PTFE with an average pore diameter of 0.5 $\mu$m; Advantec Tokyo, Tokyo, Japan), and were then degassed prior to injection. The elution patterns were detected by using a differential refractometer (R401; Waters, Milford, USA). Weight- and number-average degrees of polymerization, $\overline{dp}_w$ and $\overline{dp}_n$, respectively, of chitin and *N*-acetylated chitosan were calculated from their elution patterns on the basis of a calibration curve obtained using pullulan standards.

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