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### High-speed aqueous gel permeation chromatography of cationic polymers

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Although substances are separated on the basis of their molecular sizes in gel permeation chromatography (GPC), they are sometimes eluted earlier or later than predicted due to their interactions with the gel matrices. The solute–gel matrix interactions are especially prominent in aqueous GPC<sup>1–6</sup>. Electrostatic adsorption and exclusion effects are well-known with cationic and anionic substances. This is because negatively charged groups are present in many gel matrices. Aromatic substances also have a tendency to adsorb on many gel matrices according to charge-transfer interactions<sup>7</sup>. In addition, hydrophobic interactions and hydrogen bonding interactions sometimes occur with hydrophobic and hydrophilic substances, respectively.

Although these solute–gel matrix interactions can be exploited in the analyses of low-molecular-weight compounds or in the purifications of biological substances such as proteins, they are undesirable in the determination of the molecular weight distributions of polymers. Unfortunately, however, it seems to have been rather difficult to eliminate the interactions for polycationic and polyaromatic samples, in spite of much effort so far. Talley and Bowman<sup>8</sup> succeeded in measuring some cationic polymers on quaternized porous beads. However, since these column packings are not commercially available, their method is easy to reproduce.

Thus, we attempted to measure cationic or aromatic and yet aromatic polymers on commercially available TSK-GEL PW type columns (Toyo Soda, Tokyo, Japan) currently used extensively in high-speed GPC of water-soluble polymers<sup>9–11</sup>. Aqueous acetic acid solutions of low pH were employed. This is based on the findings with Sephadex that the adsorption of cationic substances can be reduced in eluents of low pH where ionization of carboxyl groups on the gel matrices is suppressed<sup>2</sup>, and that the adsorption of aromatic substances can be reduced in eluents containing acetic acid, pyridine, phenol or urea<sup>1</sup>. These eluents are expected to be effective also on TSK-GEL PW which like Sephadex contains many hydroxyl groups and small amounts of carboxyl groups.

#### EXPERIMENTAL

Six types of cationic polymers were studied. DEAE-Dextran, dextran containing diethylaminoethyl groups, was purchased from Pharmacia (Uppsala, Sweden). According to the manufacturer, the weight average molecular weight is *ca.* 500,000

and the nitrogen content is ca. 3.2%. Glycol chitosan for colloid titration, with a degree of polymerization higher than 400, was obtained from Wako (Osaka, Japan). The iodide salt of poly(trimethylaminoethyl methacrylate) was prepared by polymerizing dimethylaminoethyl methacrylate with  $\alpha, \alpha'$ -azobisisobutyronitrile and then quaternizing the obtained polymer with iodomethane in methanol. Two poly(*N*-methyl-2-vinylpyridine) iodide salts were kindly supplied by Dr. Fukuda of our research laboratory. They were prepared by living polymerization of 2-vinylpyridine and quaternization of the obtained polymer with iodomethane in methanol. The molecular weights of the two samples calculated from the molar ratios of monomer and initiator in the living polymerizations are 90,000 and 230,000. Two poly(4-vinylbenzyltrimethylammonium chloride) samples with number average molecular weights of 40,000 and 120,000 were kindly supplied by Mr. Higo of Nagoya University. Polyamines, SP-200 (20,000) SP-018 (1,800) and SP-003 (300), were purchased from Japan Catalyst Chemicals (Osaka, Japan). The molecular weights are shown in parentheses. Ethylenediamine was purchased from Wako.

GPC measurements were performed at 25°C with a Toyo Soda HLC-803C liquid chromatograph equipped with a refractive index detector RI-8. Three column systems of different separation ranges were employed; that used in any given case depended on the molecular weight ranges of the sample. DEAE-Dextran, glycol chitosan and poly(trimethylaminoethyl methacrylate) iodide salt were measured on a column system consisting of G6000PW and G3000PW, poly(*N*-methyl-2-vinylpyridine) iodide salt and poly(4-vinylbenzyltrimethylammonium chloride) G5000PW and G3000PW and polyamine on two G3000PW columns. Each column was 60 cm  $\times$  7.5 mm I.D. The eluent was 0.5 *M* aqueous acetic acid containing 0.3 *M* sodium sulphate (pH = 2.9) for all the measurements, except for polyamine. Because SP-200 was insoluble in this eluent, polyamine was measured in 0.5 *M* aqueous acetic acid containing 0.5 *M* sodium acetate (pH = 4.8). The flow-rate was 0.7 ml/min. 0.1–0.2% solutions of each sample were prepared and 0.1 ml and 0.5 ml were injected in the measurements of polyamine and all other samples, respectively.

## RESULTS AND DISCUSSION

The elution curves obtained are shown in Figs. 1–6. When polymer samples adsorb on the gel matrix, severely distorted elution profiles (sharp start-up and tailing) are usually observed. However, since these features are not seen in all the elution

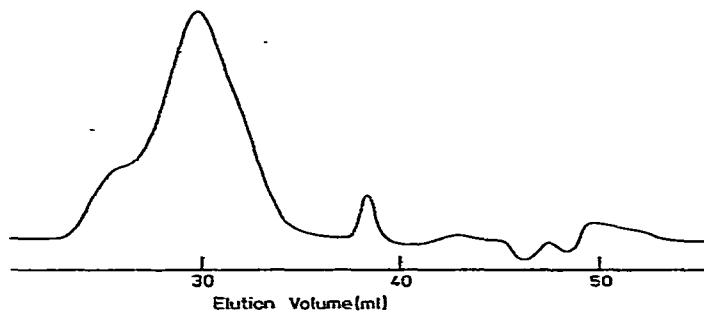


Fig. 1. Elution curve of DEAE-Dextran.

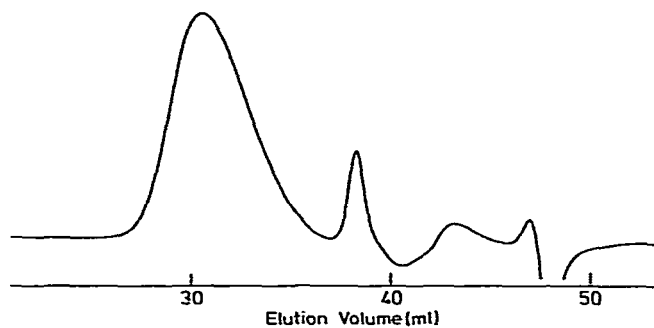


Fig. 2. Elution curve of glycol chitosan.

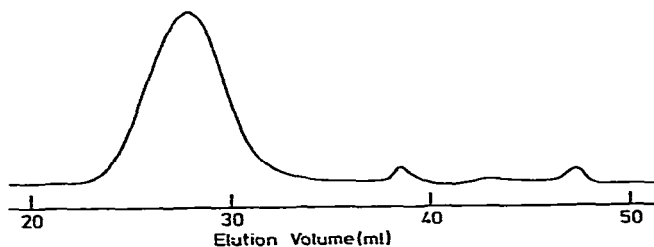


Fig. 3. Elution curve of poly(trimethylaminoethyl methacrylate) iodide salt.

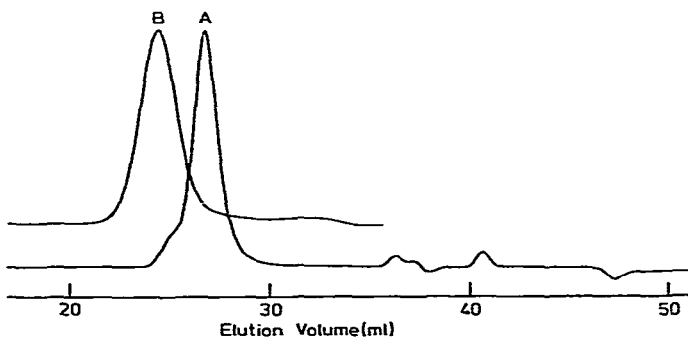


Fig. 4. Elution curves of poly(N-methyl-2-vinylpyridine) iodide salts with molecular weights of 90,000 (A) and 230,000 (B).

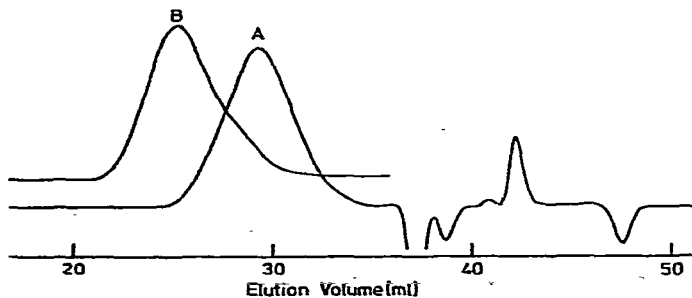


Fig. 5. Elution curves of poly(4-vinylbenzyltrimethylammonium chloride) with molecular weights of 40,000 (A) and 120,000 (B).

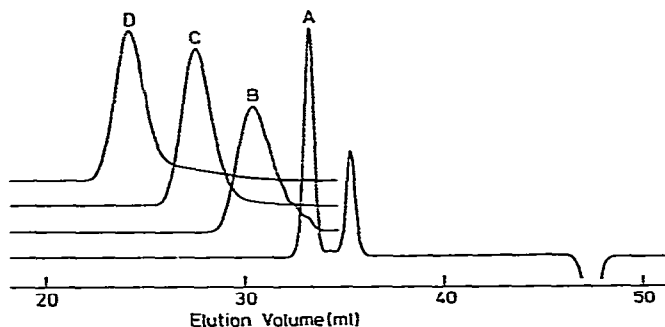


Fig. 6. Elution curves of ethylenediamine (A) and polyamines SP-003 (B), SP-018 (C) and SP-200 (D).

curves in Figs. 1–6, all samples may be considered to be separated in terms of their molecular size without adsorption. The shoulder in the elution curve of DEAE-Dextran at low elution volume can probably be attributed to the molecular weight distribution of this sample. Narrow elution curves accompanied by slight tailing or small humps were obtained with both the poly(*N*-methyl-2-vinylpyridine) iodide salts, indicating that living polymerizations of these samples proceeded almost ideally.

In the measurements of polyamine, aqueous acetic acid solutions containing sodium nitrate or sodium acetate were examined as eluents because the highest-molecular-weight sample, SP-200, was insoluble in aqueous acetic acid solutions containing sodium sulphate. Sodium nitrate was more effective in preventing adsorption of the samples. However, peaks of this salt and ethylenediamine overlapped as shown in Fig. 7, and could not be separated by adjusting the concentrations of salt and acetic acid. Although the injected solutions were prepared from the eluents, the salt peak always appeared. This is believed to be a result of ion inclusion<sup>3</sup>. At a sodium acetate concentration of 0.5 *M*, on the other hand, the peaks of the salt and ethylenediamine were separated completely, as shown in Fig. 6. However, as the sodium acetate concentration was decreased from 0.5 to 0.3 *M*, the separation became incomplete

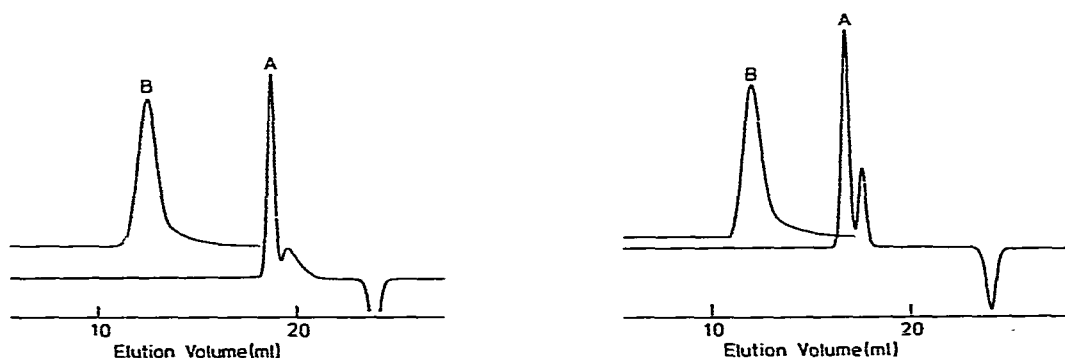


Fig. 7. Elution curves of ethylenediamine (A) and polyamine SP-200 (B) obtained on a G3000PW column with 0.5 *M* aqueous acetic acid solution containing 0.5 *M* sodium nitrate (pH = 2.5).

Fig. 8. Elution curves of ethylenediamine (A) and polyamine SP-200 (B) obtained on a G3000PW column with 0.5 *M* aqueous acetic acid solution containing 0.3 *M* sodium acetate (pH = 4.6).

and there was also a sharp start-up, which is a sign of adsorption, in the elution curve of SP-200, as shown in Fig. 8.

As shown above, aqueous acetic acid solutions containing appropriate salts were found to be very effective in GPC measurements of cationic polymers. An acetic acid concentration of 0.5 *M* was sufficient for all the samples examined. However, since the type and concentration of the salt added seem to affect the adsorption of some samples and the degree of separation between the salt and low-molecular-weight samples, they must be properly selected; 0.3 *M* sodium sulphate, which causes little corrosion of stainless steel at low pH, is recommended as a first choice.

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