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Note

Comparison of TSK-GEL PW type and SW type in high-speed aqueous gel-permeation chromatography

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Several new column packings for use in aqueous gel-permeation chromatography (GPC) have been developed and made commercially available in recent years. Many of them are mechanically stable and can be operated under high pressure, which made it possible to perform high-speed chromatography in aqueous systems just as in organic solvent systems. Moreover, some of them give excellent resolution in the high-speed chromatography of various kinds of samples. These mechanically stable and high-resolution column packings are made from either cross-linked hydrophilic polymers or silica gel chemically bonded with hydrophilic compounds. TSK-GEL PW type[1–3] and SW type [4] (Toyo Soda, Tokyo, Japan) are typical of the former and the latter, respectively. In this paper, the resolutions of TSK-GEL PW type and SW type are compared in the separation of low-molecular-weight oligomers, proteins and polymers with molecular weight distributions.

EXPERIMENTAL

GPC measurements were carried out on an HLC-802UR commercial liquid chromatograph (Toyo Soda) at 25 °C and a flow-rate of 1 ml/min.

For comparison in the low-molecular-weight range, commercial polyethylene glycols (Wako, Osaka, Japan) were measured on G2000PW (PW type) and G2000SW (SW type) of small pore size grades. The column systems for both types were 7.5 mm I.D. and 120 cm long, the solvent was distilled water and a differential refractometer was used as a detector.

For comparison in the separation of proteins, a mixture of bovine serum albumin (Wako) and myoglobin (Sigma, St. Louis, Mo., U.S.A.) was measured on G3000PW (PW type) and G3000SW (SW type), which are considered to have the best pore size distributions for this type of sample. The column systems for both types were 7.5 mm I.D. and 120 cm long, the solvent was 0.1 *M* phosphate buffer containing 0.3 *M* sodium chloride (pH 7) and UV detection at 280 nm was used.

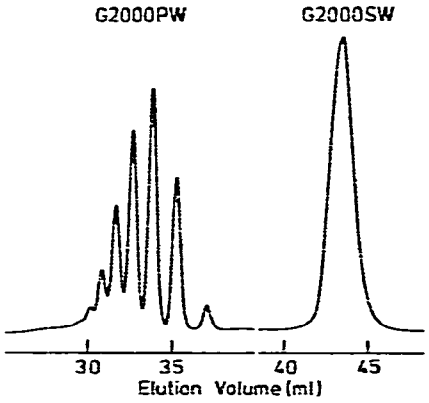
For comparison in the separation of polymers with molecular weight distributions, dextran with an average molecular weight of 234,000 (Sigma) was measured on column systems consisting of, respectively, G5000PW (7.5 mm I.D. × 60 cm) and G3000PW (7.5 mm I.D. × 30 cm) (PW type) and G4000SW (7.5 mm I.D. × 60 cm)

and G2000SW (7.5 mm I.D. × 30 cm) (SW type). Dextran standard T2000 with a weight-average molecular weight of about 2,000,000 (Pharmacia, Uppsala, Sweden) and monodisperse polyethylene glycols (Toyo Soda and Wako) were also measured on G3000PW and G4000SW. The column systems were 7.5 mm I.D. and 120 cm long, the solvent was distilled water and the detector was a differential refractometer.

RESULTS AND DISCUSSION

Separation of low-molecular-weight oligomers

Elution curves for polyethylene glycols with molecular weights of 200 and 400 are shown in Figs. 1 and 2. Components with low degrees of polymerization were separated on G2000PW but could not be separated on G2000SW, because the



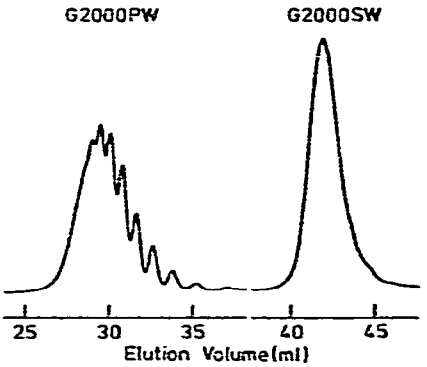


Fig. 1. Elution curves for polyethylene glycol 200 measured on G2000PW and G2000SW.

Fig. 2. Elution curves for polyethylene glycol 400 measured on G2000PW and G2000SW.

calibration graph for G2000SW is very steep in the molecular weight range under 500, as illustrated in Fig. 3. Therefore, a grade of smaller pore size is necessary. However, as G2000SW is a grade of the SW type with the smallest pore size, the SW type is not suitable for the separation of samples containing components with molecular weights lower than a few hundred and the PW type should be employed.

Separation of proteins

Fig. 4 shows the elution curves for a mixture of bovine serum albumin and myoglobin. Two components were better separated on G3000SW than on G3000PW. This is also due to the difference in the slopes of their calibration graphs, that for G3000SW being lower than that for G3000PW, as shown in Fig. 5. (As minor peaks appearing before bovine serum albumin in the elution curves in Fig. 4 are assumed to correspond to bovine serum albumin dimer, plots were also made for these peaks in Fig. 5.) This means that the difference in the elution volumes of given components is greater when the measurements are performed on the SW type. Moreover, many proteins conveniently have molecular weights between 10,000 and 1,000,000, which are in the separation range of the SW type[5]. Therefore, the SW type is better than the PW type for the separation of proteins.

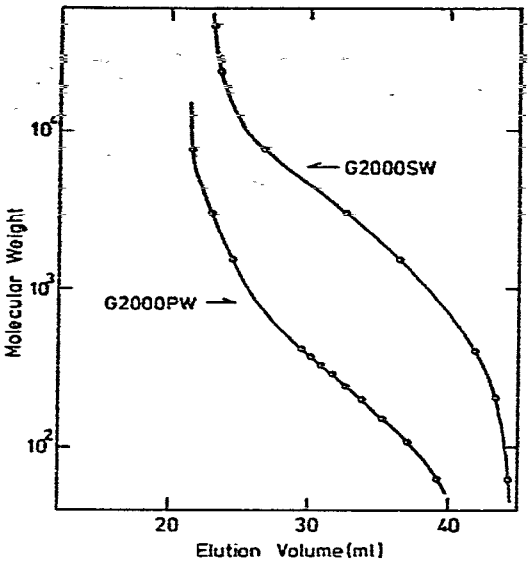


Fig. 3. Calibration graphs for G2000PW and G2000SW using polyethylene glycol.

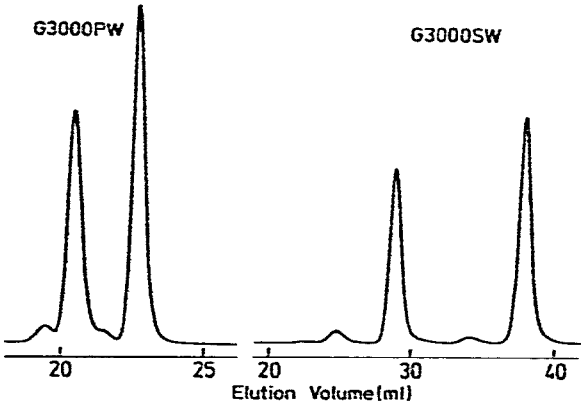


Fig. 4. Elution curves for a mixture of bovine serum albumin and myoglobin measured on G3000PW and G3000SW.

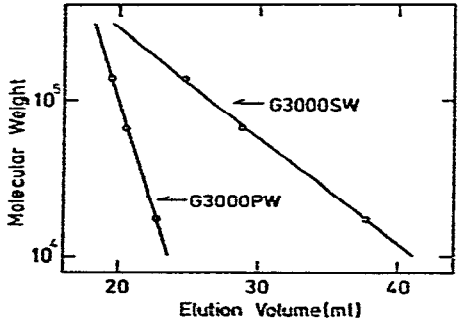


Fig. 5. Calibration graphs for G3000PW and G3000SW using protein.

Separation of polymers with molecular weight distributions

Elution curves of dextran with an average molecular weight of 234,000 are shown in Fig. 6. The sample was successfully separated on the PW type, but on the SW type high-molecular-weight components were totally excluded and a minor peak was observed in the elution curve at the void volume of the column system (about 15 ml). Therefore, a grade of larger pore size of the SW type is necessary in order to obtain the exact molecular weight distribution of the sample. However, G4000SW is a grade of the SW type with the largest pore size.

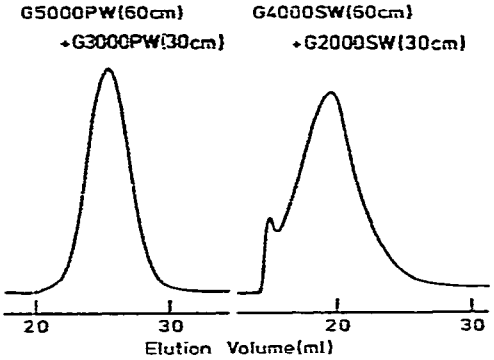


Fig. 6. Elution curves for dextran (average molecular weight 234,000) measured on G5000PW + G3000PW and G4000SW + G2000SW.

This problem of total exclusion was more severe for dextran standard T2000, as shown by the elution curves in Fig. 7. Most of the components in the sample were totally excluded when the SW type was used. In contrast, the sample was successfully measured without total exclusion on the PW type. Fig. 8 shows the calibration graphs for the column systems for polyethylene glycol. The exclusion limit of

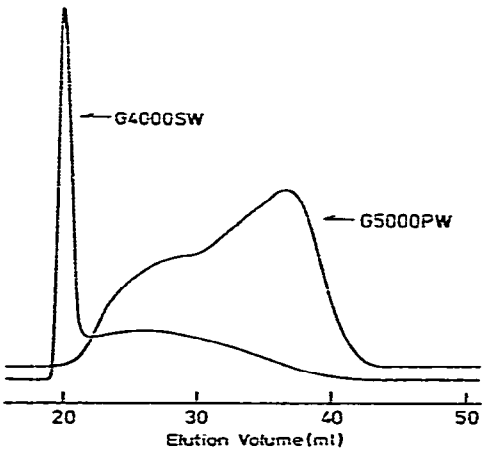


Fig. 7. Elution curves for dextran standard T2000 (weight-average molecular weight 2,000,000) measured on G5000PW and G4000SW.

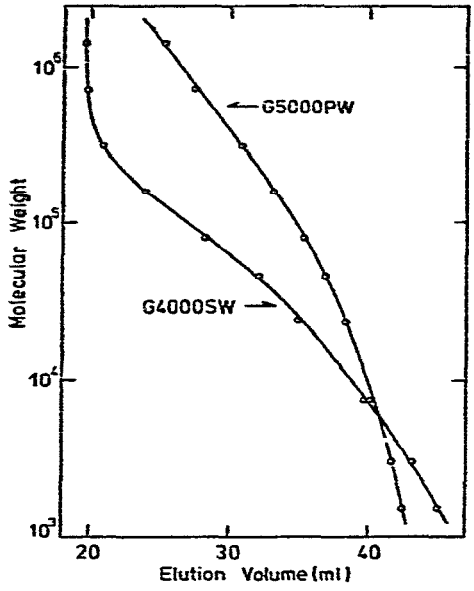


Fig. 8. Calibration graphs for G5000PW and G4000SW using polyethylene glycol.

G4000SW is 300,000–400,000. Inconveniently, however, many polymers contain some components with molecular weights higher than this exclusion limit. Consequently, the SW type is not suitable for the separation of polymers with molecular weight distributions. On the other hand, the exclusion limit of G5000PW is presumed to be several million, which should be sufficiently high for common polymers.

CONCLUSIONS

The PW type should be used for the measurements of samples containing components with molecular weights lower than a few hundred. The SW type is more efficient for the separation of proteins. The PW type is more suitable for the measurements of polymers with molecular weight distributions. The separation range of the SW type is more limited than that of the PW type, although the SW type exhibits a higher resolution than the PW type within its separation range.

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REFERENCES

1 T. Hashimoto, H. Sasaki, M. Aiura and Y. Kato, *J. Polym. Sci. Polym. Phys. Ed.*, 16 (1978) 1789.
2 Y. Kato, H. Sasaki, M. Aiura and T. Hashimoto, *J. Chromatogr.*, 153 (1978) 546.
3 T. Hashimoto, H. Sasaki, M. Aiura and Y. Kato, *J. Chromatogr.*, 160 (1978) 301.
4 S. Rokushika, T. Ohkawa and H. Hatano, *J. Chromatogr.*, 176 (1979) 456.
5 Y. Kato, K. Komiya, H. Sasaki and T. Hashimoto, *J. Chromatogr.*, 190 (1980) 297.