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NEW GEL PERMEATION COLUMN FOR THE SEPARATION OF WATER-SOLUBLE POLYMERS

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SUMMARY

A new column, TSK gel GMPW, has been evaluated for use in gel permeation chromatography of water-soluble polymers. It has a wide separation range, and the molecular weight calibration curve for polyethylene glycol is almost linear over the range 10^2 – 10^6 . A variety of water-soluble polymers have been fractionated according to molecular size, with no evidence of adsorption providing an appropriate eluent was employed. Therefore, GMPW should be very useful in the determination of molecular weight distributions of water-soluble polymers.

INTRODUCTION

Gel permeation chromatography (GPC) has been widely used for determining molecular weight distributions and average molecular weights of polymers, owing to its rapidity, ease of operation and good reproducibility. GPC in organic solvent systems has developed rapidly since its introduction by Moore in 1964¹, and the use of high-performance GPC columns, packed with very small particles, was introduced in advance of other modes of liquid chromatography^{2,3}. Columns packed with a mixture of sorbents differing in pore size were also developed at an early stage². Such columns have wide separation ranges and hence are very versatile. Their benefits have been discussed in detail^{4,5}.

On the other hand, high-performance mixed-bed columns, suitable for aqueous GPC, were not available until very recently. For this purpose, we have prepared columns packed with a mixture of TSK gels G2500PW, G3000PW and G6000PW, which have different pore sizes. Recently, these columns have become commercially available under the trade-name of TSK gel GMPW (Toyo Soda, Tokyo, Japan). In this report, the results of an evaluation of the separation range, resolution and applications of these columns are presented.

EXPERIMENTAL

GPC was performed with a Toyo Soda HLC-803D liquid chromatograph, equipped with a differential refractometer RI-8, at 25°C and a flow-rate of 1 ml/min. The GMPW columns were packed with sorbents of particle diameter *ca.* 17 μm .

The molecular weight calibration curves were determined with polyethylene glycols of narrow molecular weight distribution in distilled water. A 0.5-ml aliquot of a 0.02–0.05% solution of each sample was injected.

The theoretical plate numbers of the columns were determined by injecting 20 μ l of 1% ethylene glycol in distilled water.

The twenty-four water-soluble polymers listed below were separated on two 600 \times 7.5 mm I.D. GMPW columns, connected in series, with distilled water, aqueous sodium nitrate, mixtures of aqueous sodium nitrate and acetonitrile or aqueous acetic acid containing sodium sulphate as eluents. The injection volume was 0.5 ml and the sample concentrations were 0.05–0.1%. Non-ionic polymers were: dextran, pullulan, soluble starch, methyl-cellulose, hydroxyethyl-cellulose, polyethylene glycol, polyvinyl alcohol, polyacrylamide and polyvinylpyrrolidone. Anionic polymers were: chondroitinsulphate sodium salt, alginic acid sodium salt, hyaluronic acid sodium salt, carboxymethyl-cellulose, polyacrylic acid sodium salt, sulphonated lignin sodium salt and sodium polystyrenesulphonate. Cationic polymers were: glycol chitosan, diethylaminoethyl (DEAE) dextran, poly(trimethylammonioethyl methacrylate) iodide salt, poly(N-methyl-2-vinylpyridinium) iodide salt and poly(4-vinylbenzyltrimethylammonium chloride). Amphoteric polymers were: Blue dextran, collagen and gelatin.

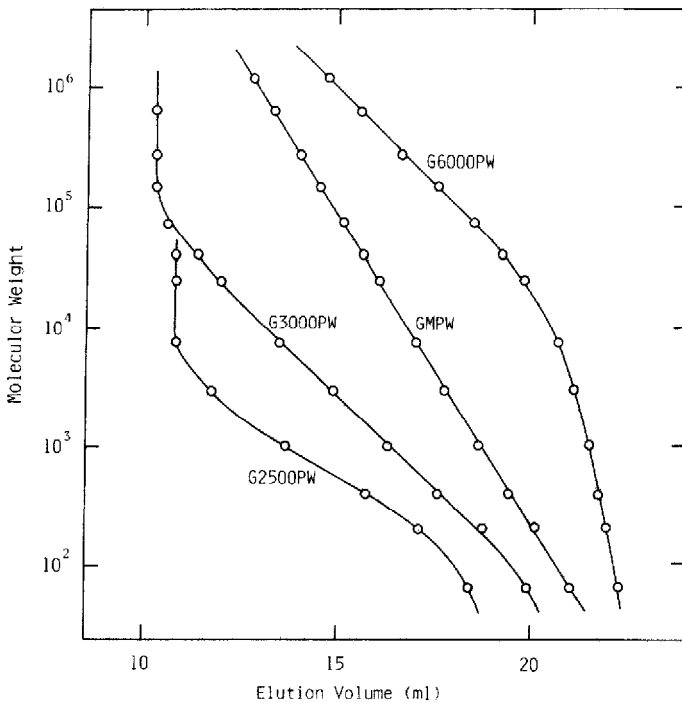


Fig. 1. Molecular-weight calibration curves of TSK gel GMPW, G2500PW, G3000PW and G6000PW columns (600 \times 7.5 mm I.D.) for polyethylene glycol in distilled water.

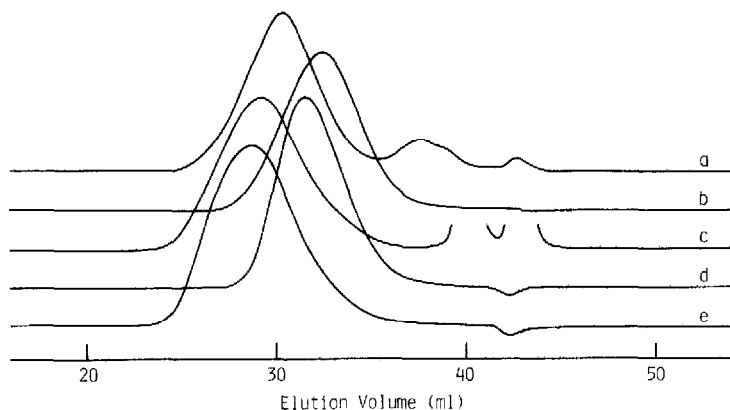


Fig. 2. Chromatograms of pullulan (a), methyl-cellulose (b), hydroxyethyl-cellulose (c), polyvinyl alcohol (d) and polyacrylamide (e) obtained with 0.1 *M* sodium nitrate.

RESULTS AND DISCUSSION

The molecular weight calibration curves of GMPW, G2500PW, G3000PW and G6000PW for polyethylene glycol are shown in Fig. 1. The separation range of GMPW is very wide, extending over a molecular weight range from less than 100 to several millions for this polymer. In addition, the calibration curve of GMPW is almost linear over this molecular weight range. This facilitates the accurate conversion of GPC chromatograms into molecular weight distribution curves⁴⁻⁷. Consequently, GMPW should be very useful in the separation of water-soluble polymers over a wide range of molecular weight.

The theoretical plate numbers of several GMPW columns (600 × 7.5 mm I.D.), as determined with ethylene glycol, ranged between 15 000 and 20 000 plates per m, which means that these columns give fairly high resolution.

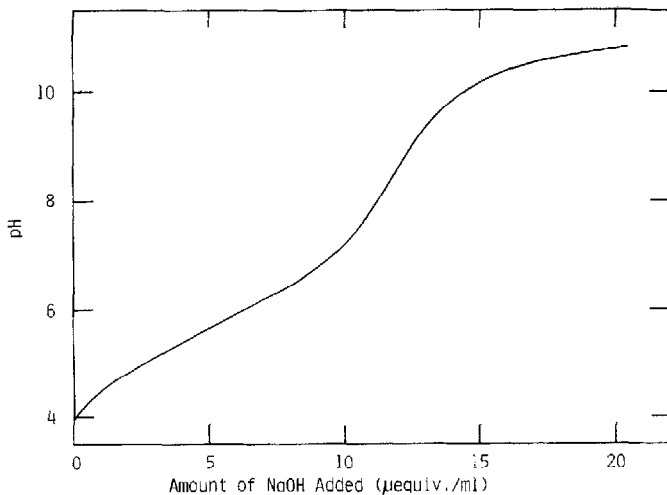


Fig. 3. Titration curve of GMPW. The sorbent was titrated in 0.5 *M* sodium chloride with 0.5 *M* sodium hydroxide.

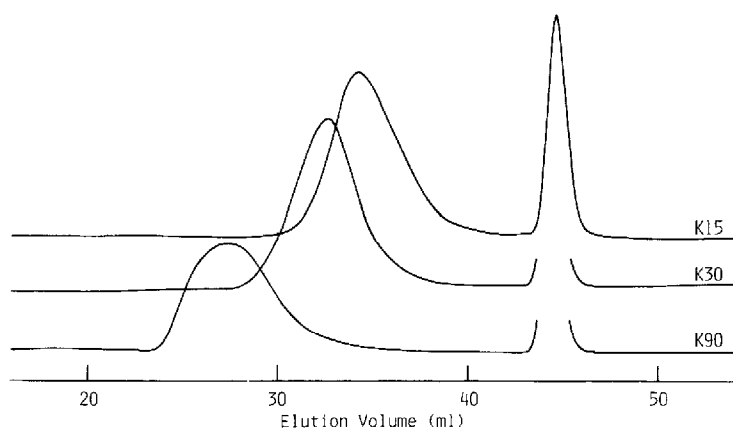


Fig. 4. Chromatograms of polyvinylpyrrolidones obtained in 0.1 *M* sodium nitrate-acetonitrile (80:20, v/v). Molecular weights: K15, 10 000; K30, 40 000; K90, 360 000.

All of the non-ionic polymers except polyvinylpyrrolidone were successfully fractionated in 0.1 *M* sodium nitrate. Under these conditions, there was no sign of adsorption (sharp leading edge followed by tailing of the peak, small peak area, retardation of elution and lack of reproducibility) nor of ionic repulsion (early elution near void volume). Fig. 2 shows some examples of the chromatograms obtained. In distilled water, however, normal chromatograms were obtained only with polyethylene glycols and dextran of low molecular weight. For the polymers in water, either minor peaks appeared near the void volume in addition to the main peaks, or chromatograms of abnormal shapes were observed. This is probably because charged components in the samples interact with the negative charges carried by GMPW. According to titration (see Fig. 3), GMPW contains *ca.* 12 μ equiv. of negative charges per ml; these are believed to reside on carboxyl groups. In the fractionation of polyvinylpyrrolidone in 0.1 *M* sodium nitrate, a slightly sharp start-up was observed, suggesting weak adsorption of the sample on the sorbent. However, normal chromatograms were obtained reproducibly by use of 0.1 *M* sodium nitrate-acetonitrile

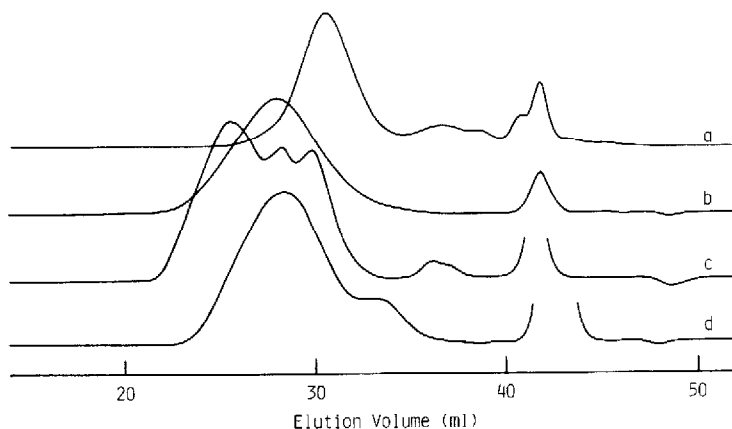


Fig. 5. Chromatograms of chondroitinsulphate sodium salt (a), alginate sodium salt (b), hyaluronic acid sodium salt (c) and carboxymethyl-cellulose sodium salt (d), obtained with 0.1 *M* sodium nitrate.

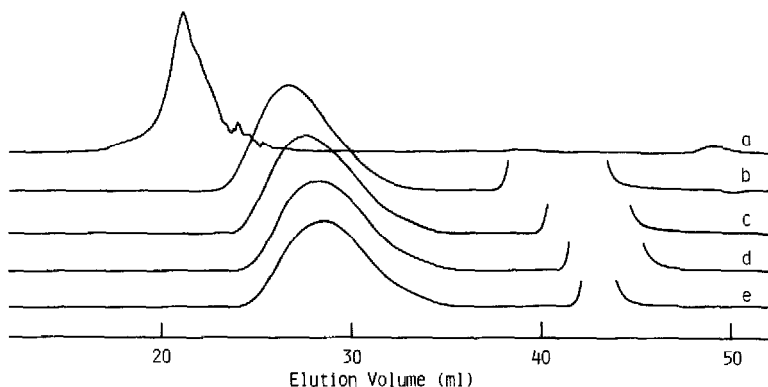


Fig. 6. Effect of eluent ionic strength on the separation of anionic polymers (polyacrylic acid sodium salt) in aqueous sodium nitrate solution. Sodium nitrate concentrations: a, 0; b, 0.01 *M*; c, 0.025 *M*; d, 0.05 *M*; e, 0.1 *M*.

(80:20, v/v), as shown in Fig. 4. It is likely that polyvinylpyrrolidone was retained by hydrophobic interaction with the sorbent in 0.1 *M* sodium nitrate and the hydrophobic interaction was eliminated by the addition of acetonitrile.

Most of the anionic polymers examined were also fractionated in 0.1 *M* sodium nitrate without interaction with the sorbent, as exemplified in Fig. 5. When the anionic polymers were fractionated in distilled water, they were eluted very early, near the void volume, owing to ionic repulsion between the samples and the sorbent. However, as shown in Fig. 6, the addition of sodium nitrate, even at a concentration of only 0.01 *M*, resulted in normal elution and peak shape. Although the peaks shifted slightly toward higher elution volumes with increasing sodium nitrate concentration, they had the same shape at sodium nitrate concentrations above 0.1 *M*. Therefore, an ionic strength of 0.1 seems to be sufficient to suppress ionic interactions between anionic polymers and GMPW. The hydrophobic anionic polymers, sulphonated lignin sodium salt and sodium polystyrenesulphonate, were not eluted by 0.1 *M* sodium nitrate. However, they were successfully fractionated after the addition of the organic solvent acetonitrile to the eluent (Fig. 7).

All the cationic polymers examined were fractionated, with no evidence of

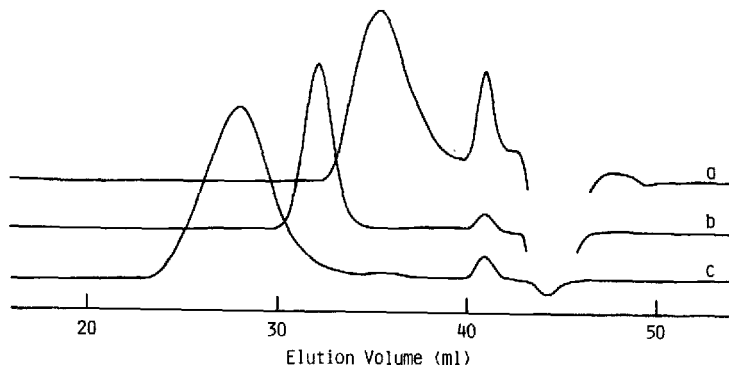


Fig. 7. Chromatograms of sulphonated lignin sodium salt (a) and sodium polystyrenesulphonate (b, 90% sulphonation; c, 100% sulphonation) obtained with 0.1 *M* sodium nitrate-acetonitrile (80:20, v/v).

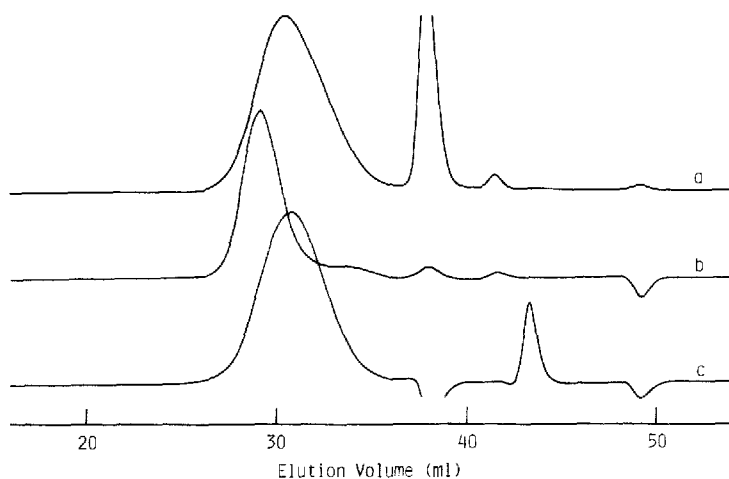


Fig. 8. Chromatograms of glycol chitosan (a), poly(*N*-methyl-2-vinylpyridinium) iodide salt (b) and poly(4-vinylbenzyltrimethylammonium chloride) (c), obtained with 0.5 *M* acetic acid containing 0.3 *M* sodium sulphate.

interactions with the sorbent, in 0.5 *M* acetic acid containing 0.3 *M* sodium sulphate. Fig. 8 shows examples of the chromatograms. When the sodium sulphate concentration was reduced to 0.1 *M*, the peak area became rather small and the results were not reproducible. The hydrophilic cationic polymers, glycol chitosan, DEAE-dextran and poly(trimethylammonioethyl methacrylate) iodide salt, were successfully fractionated also in 0.8 *M* sodium nitrate, as shown in Fig. 9. However, sodium nitrate concentrations below 0.4 *M* were not sufficient to prevent adsorption (see Fig. 10). On the other hand, the hydrophobic cationic polymers, poly(4-vinylbenzyltrimethylammonium chloride) and poly(*N*-methyl-2-vinylpyridinium) iodide salt, were not eluted from the column even in 0.8 *M* sodium nitrate, and in this case the addition of 20% acetonitrile was not effective in eliminating the adsorption.

Three amphoteric polymers were successfully fractionated in 0.1 *M* sodium nitrate-acetonitrile (80:20, v/v), as shown in Fig. 11. When acetonitrile was not included in the eluent, the peak areas were small and the results were not reproducible.

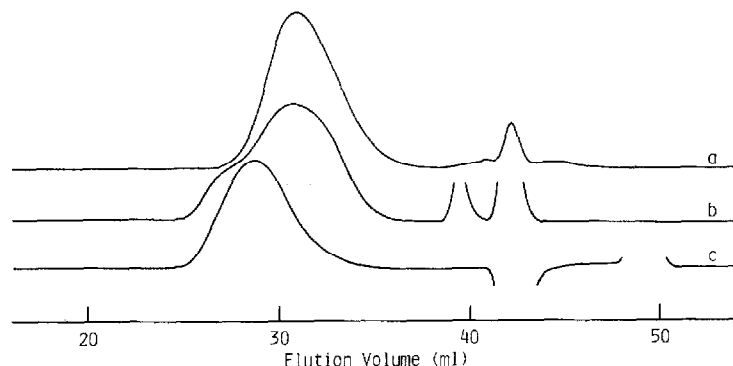


Fig. 9. Chromatograms of glycol chitosan (a), DEAE-dextrans (b) and poly(trimethylammonioethyl methacrylate) iodide salt (c), obtained with 0.8 *M* sodium nitrate.

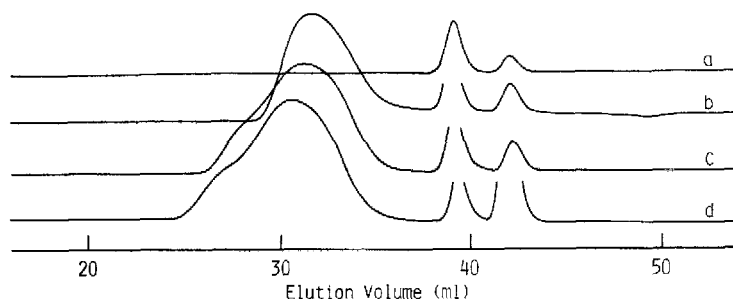


Fig. 10. Effect of eluent ionic strength in the separation of the cationic polymer DEAE-dextran with aqueous sodium nitrate solution. Sodium nitrate concentrations: a, 0.1 *M*; b, 0.2 *M*; c, 0.4 *M*; d, 0.8 *M*.

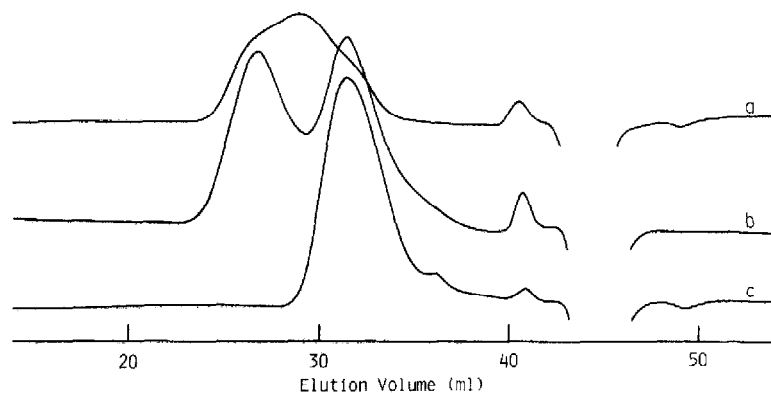


Fig. 11. Chromatograms of Blue dextran(a), collagen(b) and gelatin(c), obtained with 0.1 *M* sodium nitrate-acetonitrile (80:20, v/v).

This is probably because these amphoteric polymers contain aromatic rings and hence they tend to be adsorbed on the sorbent by hydrophobic interaction.

As shown above, twenty-four water-soluble polymers with various properties have been fractionated on TSK gel GMPW, with little or no interaction with the sorbent in an appropriate eluent. Suitable eluents are summarized in Table I.

TABLE I

SUITABLE ELUENTS FOR VARIOUS TYPES OF WATER-SOLUBLE POLYMERS

<i>Type of polymer</i>	<i>Suitable eluent</i>
Non-ionic hydrophilic polymer	Salt solution, <i>e.g.</i> , 0.1 <i>M</i> sodium nitrate (Distilled water)
Non-ionic hydrophobic polymer	Salt solution containing organic solvent, <i>e.g.</i> , 0.1 <i>M</i> sodium nitrate-acetonitrile (80:20, v/v)
Anionic hydrophilic polymer	Salt solution, <i>e.g.</i> , 0.1 <i>M</i> sodium nitrate
Anionic hydrophobic polymer	Salt solution containing organic solvent, <i>e.g.</i> , 0.1 <i>M</i> sodium nitrate-acetonitrile (80:20, v/v)
Cationic hydrophilic polymer	Salt solution, <i>e.g.</i> , 0.8 <i>M</i> sodium nitrate
Cationic hydrophobic polymer	Acetic acid solution containing salt, <i>e.g.</i> , 0.5 <i>M</i> acetic acid containing 0.3 <i>M</i> sodium sulphate
Amphoteric hydrophilic polymer	Salt solution, <i>e.g.</i> , 0.1 <i>M</i> sodium nitrate
Amphoteric hydrophobic polymer	Salt solution containing organic solvent, <i>e.g.</i> , 0.1 <i>M</i> sodium nitrate-acetonitrile (80:20, v/v)

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