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SEPARATION RANGE AND SEPARATION EFFICIENCY IN HIGH-SPEED GEL FILTRATION ON TSK-GEL SW COLUMNS

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SUMMARY

Separation ranges for gel filtration on TSK-GEL SW columns, G2000SW, G3000SW and G4000SW, were measured for globular protein, dextran and polyethylene glycol. It was possible to separate globular proteins of molecular weights from 5000 to 7,000,000, dextrans of molecular weights from 1000 to 500,000 and polyethylene glycols of molecular weights from 500 to 250,000 by using these three columns of different pore sizes. The column having the highest separation efficiency for globular proteins was also determined. The highest separation efficiency for molecular weights of less than 30,000, 30,000–500,000 and greater than 500,000 was exhibited by G2000SW, G3000SW and G4000SW, respectively.

INTRODUCTION

Since the development of controlled pore glass beads chemically bonded with hydrophilic compounds a few years ago[1], high-speed gel filtration has become possible. Several brands of columns packed with similar types of materials are now commercially available. TSK-GEL SW (Toyo Soda, Tokyo, Japan) is one such column and it has already been shown that very high resolution of proteins can be attained with this column[2]. Three columns of different pore sizes, G2000SW, G3000SW and G4000SW, are available with TSK-GEL SW. However, neither the exact separation ranges of these columns nor the best column for the separation of a particular sample is known. In this paper, we report the separation ranges of these columns for globular protein, dextran and polyethylene glycol. The relationship between molecular weight range and the best column for the separation of globular proteins is also reported.

EXPERIMENTAL

Gel filtration was carried out at 25° on a commercial liquid chromatograph HLC-802R (Toyo Soda) equipped with a variable-wavelength UV monitor. Proteins were detected at 220 nm. Dextrans and polyethylene glycols were detected with a differential refractometer. Two columns (60 cm × 7.5 mm I.D.) of each grade were

used in series. Solvents were 0.1 *M* phosphate buffer containing 0.3 *M* NaCl (pH 7) in the measurements of proteins, and distilled water for dextrans and polyethylene glycols. The flow-rate was 1 ml/min. Injection volumes were 0.1 ml in the measurements of proteins and 0.6 ml for dextrans and polyethylene glycols. The samples of commercial proteins, dextrans and polyethylene glycols are listed in Table I. The dextrans had broad molecular weight distributions, while the polyethylene glycols had narrow distributions.

TABLE I

SAMPLES USED IN GEL FILTRATION EXPERIMENTS

A, Sigma (St. Louis, Mo., U.S.A.); B, Nakarai (Kyoto, Japan); C, Wako (Osaka, Japan); D, Miles Labs. (Elkhart, Ind., U.S.A.); E, P-L Biochemicals (Milwaukee, Wisc., U.S.A.); F, Tokyo Chemical Industry (Tokyo, Japan); G, Pharmacia (Uppsala, Sweden); H, Toyo Soda (Tokyo, Japan).

Protein	*Molecular weight*	*Source*
Thyroglobulin	660,000	A
γ-Globulin (fraction II from bovine plasma)	156,000	B
Bovine serum albumin	67,000	C
Ovalbumin	43,000	C
Peroxidase (horseradish)	40,200	A
β-Lactoglobulin	35,000	D
Myoglobin (equine skeletal muscle)	16,900	A
Ribonuclease (bovine pancreas)	13,700	E
Cytochrome *c*	12,400	D
Glycylglycylglycylglycine	246	F

Dextran		M_n	M_w	*Source*
Designation	*Batch No.*			
T2000	8122			G
T500	8689	199,000	518,000	G
T250	7951	141,000	237,000	G
T150	892	105,000	167,000	G
T70	1730	42,500	70,000	G
T40	4987	29,500	39,500	G
T20	7968	15,000	22,300	G
T10	0094	6200	10,400	G

Polyethylene glycol	*Molecular weight*	*Source*
SE-150	1,400,000	H
SE-70	730,000	H
SE-30	320,000	H
SE-15	160,000	H
SE-8	80,000	H
SE-5	46,000	H
SE-2	23,000	H
PEG 6000	7500	C
PEG 4000	3000	C
PEG 1540	1500	C
PEG 1000	1000	C
PEG 600	600	C
PEG 400	400	C
PEG 200	200	C

RESULTS AND DISCUSSION

Figs. 1–3 show the calibration curves of, respectively, G2000SW, G3000SW and G4000SW for globular protein, dextran and polyethylene glycol. Since peaks believed to correspond to a dimer and trimer were observed together with the peak of monomer in the elution curve of bovine serum albumin obtained on G3000SW, plots were also made for these peaks in Fig. 2. A point for bovine serum albumin dimer is also plotted in Fig. 3. It is not easy to obtain exact calibration curves for dextran because the molecular weight distribution is broad. $(M_n \cdot M_w)^{0.5}$ was plotted against peak elution volume in Figs. 2 and 3 since the elution curves of dextrans were

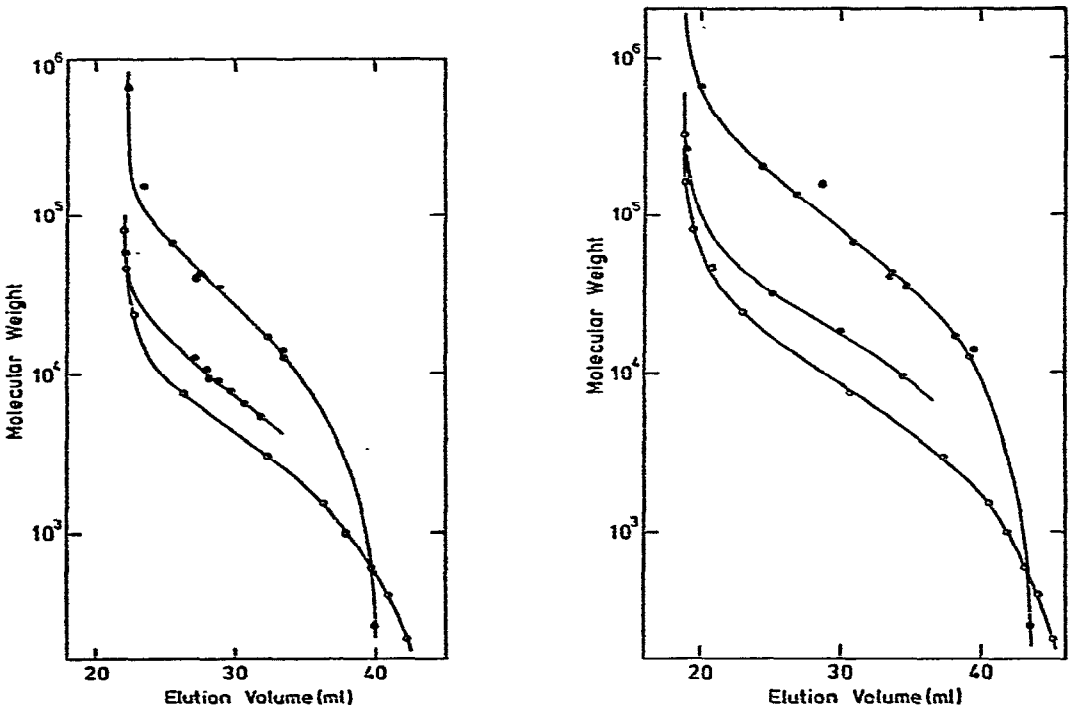

Fig. 1. Calibration curves of G2000SW for globular protein (●), dextran (◑) and polyethylene glycol (○).

Fig. 2. Calibration curves of G3000SW. Details as in Fig. 1.

nearly normal distributions[3]. In the case of G2000SW, the calibration curve was obtained using TIO by the method proposed by Abdel-Alim and Hamielic[4] and used by Van Dijk *et al.*[5]. In this method, the calibration curve is obtained by plotting molecular weights against elution volumes corresponding to the same cumulative weight fractions in the integral molecular weight distribution curve and in the integral gel filtration elution curve for the sample. The integral molecular weight distribution curve of TIO from Pharmacia (Uppsala, Sweden) was used. Points corresponding to cumulative weight fractions of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 are plotted in Fig. 1.

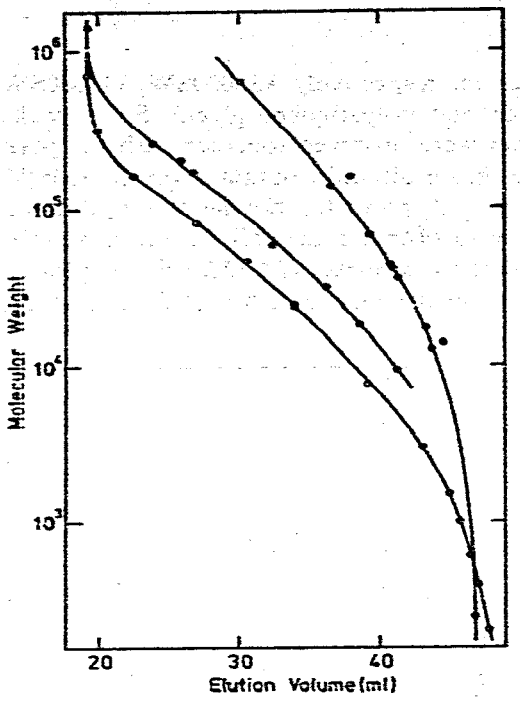

Fig. 3. Calibration curves of G4000SW. Details as in Fig. 1.

The separation ranges of the three columns evaluated from Figs. 1–3 are summarized in Table II. The exclusion limit of G4000SW for globular protein was estimated by linearly extrapolating the calibration curve up to the position of void volume. Table II indicates that globular proteins, dextrans and polyethylene glycols with molecular weights of, respectively, 5000–7,000,000, 1000–500,000 and 500–250,000 can be separated with TSK-GEL SW.

TABLE II

SEPARATION RANGES OF TSK-GEL SW

Sample	*Separation range*		
	G2000SW	*G3000SW*	*G4000SW*
Globular protein	5000–100,000	10,000–500,000	20,000–7,000,000
Dextran	1000– 30,000	2000– 70,000	4000– 500,000
Polyethylene glycol	500– 15,000	1000– 35,000	2000– 250,000

Specific resolution (R_s) was calculated for some pairs of proteins in order to find the best column for the separation of proteins of particular molecular weights, since the separation ranges of the three columns overlapped. The expression used was

$$R_s = 2(V_2 - V_1)/(W_2 + W_1)(\log M_1 - \log M_2) \quad (1)$$

TABLE III

SPECIFIC RESOLUTION FOR SOME PAIRS OF PROTEINS ON TSK-GEL SW

Sample	*Specific resolution*		
	G2000SW	*G3000SW*	*G4000SW*
Thyroglobulin γ-Globulin	*	3.37	1.84
Bovine serum albumin β-Lactoglobulin	4.31	7.76	1.95
Myoglobin Cytochrome *c*	6.28	7.52	3.30
Glycylglycylglycylglycine	7.28	7.03	3.20
	6.67	5.56	1.78
	3.40	2.24	1.13

* Thyroglobulin was totally excluded.

where V, W and M are the elution volumes, peak widths at the base and the molecular weights, respectively, of two components. The results are summarized in Table III. In Fig. 4 the specific resolutions are plotted against the average molecular weights of two components. Fig. 4 shows that G2000SW, G3000SW and G4000SW are best for the separations of proteins with molecular weights of, respectively, less than 30,000, 30,000–500,000 and greater than 500,000. However, the separation efficiencies of G2000SW and G3000SW differ only slightly for molecular weights less than 30,000, while they differ considerably in the range 30,000–500,000. Although G4000SW is useful for molecular weights above 500,000 (exclusion limit of G3000SW), proteins having such high molecular weights are rare. Consequently, G3000SW may be the

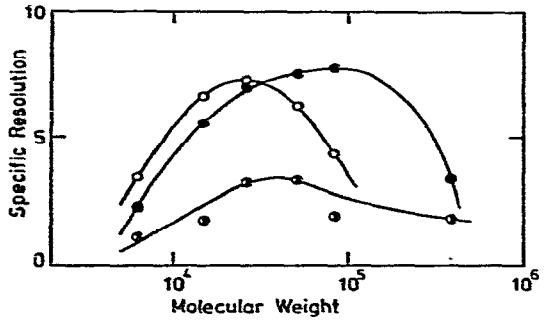

Fig. 4. Comparison of specific resolution for some pairs of proteins on G2000SW (○), G3000SW (●) and G4000SW (◑).

most useful of the TSK-GEL SW columns which were designed mainly for the separation of proteins. Fig. 5 compares the calibration curves of three columns for protein. The calibration curve of G2000SW has the smallest slope for molecular weights below 30,000. In the molecular weight ranges of 30,000–500,000 and higher than 500,000, the calibration curves of, respectively, G3000SW and G4000SW have the smallest slopes. Therefore, it can be concluded that the column whose calibration

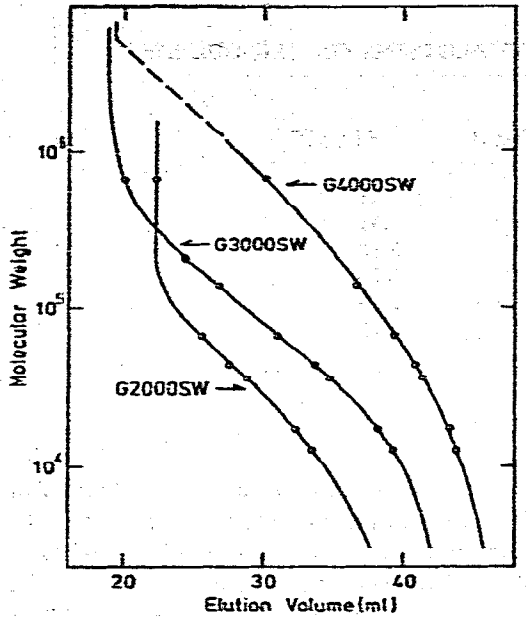

Fig. 5. Calibration curves of G2000SW, G3000SW and G4000SW for protein.

curve is least steep has the highest separation efficiency. Elution curves of a mixture of some proteins are shown in Fig. 6, those for human serum are shown in Fig. 7. These figures clearly show the relationship between molecular weight range and the best column described above.

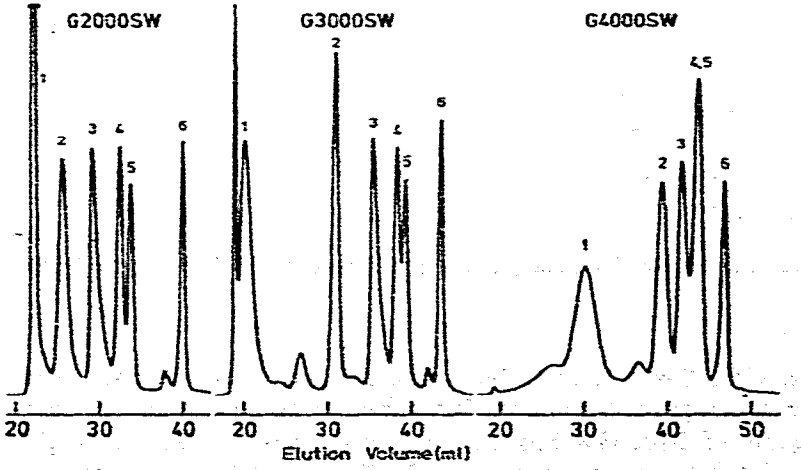

Fig. 6. Elution curves of a mixture of thyroglobulin (1, 0.03%), bovine serum albumin (2, 0.02%), β-lactoglobulin (3, 0.02%), myoglobin (4, 0.01%), cytochrome *c* (5, 0.01%) and glycylglycylglycyl-glycine (6, 0.03%) measured on G2000SW, G3000SW and G4000SW.

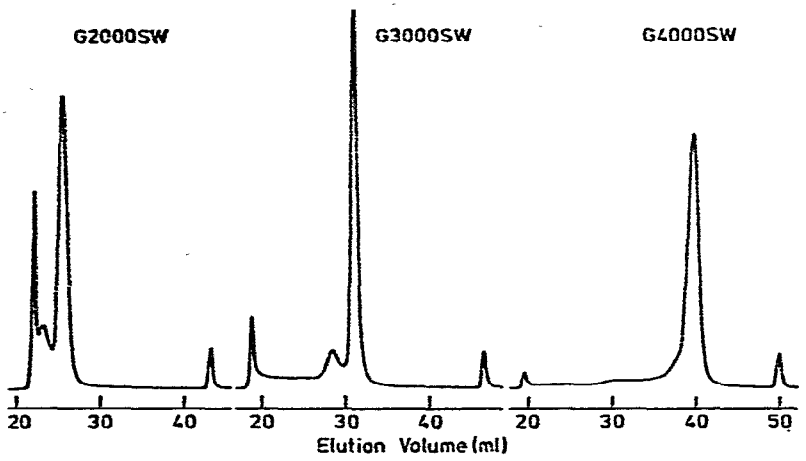

Fig. 7. Elution curves of human serum (1%) measured on G2000SW, G3000SW and G4000SW.

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