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PORE SIZE DISTRIBUTION ANALYSIS OF GEL SUBSTANCES BY SIZE EXCLUSION CHROMATOGRAPHY

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SUMMARY

A "mixed solute exclusion" method for the porous structure analysis of gel substances has been developed. A mixed solution of polymer and oligomers covering a sufficiently wide molecular weight range is brought into contact with a gel sample and the resulting differential dilution of the solute fractions is determined as a function of the molecular size by means of gel permeation chromatography (GPC). This method and two other versions of the solute exclusion technique, the single-point method (equilibrium partitioning) and the column method (inverse GPC), were applied to a cross-linked dextran gel (Sephadex G-100) using dextran and/or poly(ethylene oxide) fractions as the probe polymer. The results of the three methods were consistent with each other and demonstrated the efficacy of the mixed solute exclusion method for the rapid analysis of gel structure. An important limitation, however, is the resolution of solute exclusion methods in general, as shown by examining the assumptions in interpreting a solute exclusion curve as a pore size distribution curve.

INTRODUCTION

Information on pore size distribution is essential for understanding the physico-chemical properties of porous materials, including rigid porous solids (*e.g.*, silica gel) and swollen gels (*e.g.*, gelatin or agar gels). Three techniques with different principles have been developed for this purpose: (i) gas adsorption, (ii) mercury intrusion and (iii) solute exclusion (SE). Although (i) and (ii) are widely used for various porous materials, these are applicable only to dry samples. In contrast, the SE technique, which was developed by Aggebrandt and Samuelson[1] and Stone and co-workers[2,3] for the porous structure analysis of swollen cellulose, gives direct information on the pore structure of swollen polymeric gels without drying.

In the SE method, a polymer solution of narrow molecular weight distribution is brought into contact with the gel sample and is diluted with the imbibed liquid. The dilution ratio determined by accurate interferometry or refractometry gives the amount of "non-solvent" liquid (W_{ns}, cm^3/g dry substance) for a solute of known molecular size. Provided that the diameter or width of the smallest pore to which the

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solute can diffuse can be equated with the hydrodynamic diameter of the solute molecule ($2R$), and that specific adsorption and osmotic effects can be neglected, the SE curve, W_{ss} ($2R$), is regarded as the cumulative pore size distribution of the gel. Although this technique has been applied to some column packing materials[4–7] in efforts to elucidate the separation mechanism in size exclusion chromatography*, it seems to have been applied systematically only to swollen cellulose in studies on porous structure.

The drawbacks of the SE method are (i) many grades of a sufficiently sharp fraction of a polymer on the gram scale are neccessary and (ii) one must prepare a set of standard dilute solutions and carry out calibrating measurements on them in order to obtain a single point on an SE curve. Instead of this time-consuming procedure (single-point SE), a dynamic version of SE is possible when the gel sample is available as particles. When ordinary SE chromatography of standard solutes is carried out on a packed column of the gel particles to be examined, the calibration graph obtained and the dry weight of the packed gel give directly the SE curve equivalent to that from the single-point SE. This method has been applied to silica gels, porous glass and cross-linked polystyrene gels in non-polar liquids, with standard polystyrenes as test solute[8,9]. Although this method, designated "column SE" in this paper**, saves much time and considerable amounts of often expensive polymer standards, it is applicable only to gel samples that are available as sufficiently rigid and finely devided particles.

Recent progress in liquid chromatography has brought about the possibility of a new version of the SE technique, which is presented in this paper. This method, named "mixed solute exclusion (mixed SE)", seems to have several advantages over its precursors***. This paper gives the principle of the method and the preliminary experimental results obtained and demonstrates its efficacy as a tool for the study of the structures of gel substances. Although SE techniques can be carried out in any medium, aqueous systems (hydrogels) are of particular interest because of their importance in biological systems and in practical problems. Hence the three SE methods were applied to a typical chromatographic hydrogel, cross-linked dextran gel (Sephadex G-100). Series of dextran and poly(ethylene oxide) (PEO) or poly(ethylene glycol) (PEG) fractions were chosen as test polymers because they are available as sharp fractions of widely varying molecular weight.

PRINCIPLE OF THE MIXED SOLUTE EXCLUSION METHOD

G g of the gel sample consists of solid substance (m g), imbibed liquid (w_i g) and surplus (external) liquid (w_o g):

$$G = m + w_i + w_o \tag{1}$$

* It is referred to as "equilibrium solute partitioning" or "static experiment of polymer–gel mixing".

** The term "inverse GPC" has been proposed[8].

*** Modification of SE technique by use of GPC was first stated and attempted by El-Hosseiny *et al.*[10]. However, they tried to determine only the fibre saturation point (total amount of imbibed water) and the maximum pore size of swollen cellulose. Their results are given without either the experimental details or evaluation of the reliability of the method. The mixed SE method is based on the same concept, but it aims to determine the complete solute exclusion curve of the gel being studied.

The imbibed liquid consists of the fraction accessible to the solutes of molecular weight M, $w_a(M)$, and the remaining (non-solvent) fraction, $w_{ns}(M)$.

$$w_i = w_a(M) + w_{ns}(M) \quad (2)^{\S}$$

When X g of a mixed polymer solution, the concentration of each fraction in which is $f(M)$ g/cm^3, is added to the gel sample, equilibrated external liquid will have a modified molecular weight distribution, $h(M)$, as a result of dilution with the imbibed liquid. If the density of the solution is equal to that of the pure liquid, then

$$\frac{h(M)}{f(M)} = \frac{X}{X + w_a(M) + w_0} \quad (3)$$

From eqns. 1–3,

$$w_{ns}(M) = G - m - (f/h - 1)X \quad (4)$$

All quantities on the right-handside are measurable; $f(M)$ and $h(M)$ are determined by gel permeation chromatography (GPC). If the relationship between the molecular radius(R) and M is known, $W_{ns}(M)$ [$= w_{ns}(M)/m$] can be converted into $W_{ns}(R)$, which is interpreted as the cumulative pore size distribution of the gel sample. The experimental procedure is similar to that of single-point SE.

In this method, the use of monodisperse polymer fractions is unneccessary, and a single GPC run gives a complete SE curve, provided that the probe polymer covers a sufficiently wide molecular weight range. It is comparable to column SE in rapidity and ease, and is applicable to gel samples of any shape.

Of critical importance in this method are the resolution and the accuracy of the GPC used, because f/h is determined by absolute instead of differential measurements as in single-point SE. Open-column (gel filtration) chromatography using soft gels does not provide a sufficiently good performance for this purpose. Recently, high-pressure aqueous GPC columns covering a wide molecular size range which are well suited for the present purpose have become available[11] and these were used in this study.

EXPERIMENTAL

Gel sample

Dry Sephadex G-100 powder (Pharmacia, Uppsala, Sweden) was swollen in deionized water for several days, then thoroughly washed with deionized water by successive filtration and re-dispersion. Finally, excess water was filtered off with a tap aspirator. The gel was stored in a stoppered bottle in a refrigerator until use.

Probe polymers

The following polymer fractions and oligomers of dextran and PEO (PEG) were used as received:

(1) Dextran T fractions (Pharmacia): Dextran T2000*, T500, T70, T40 and T10.

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§ An implicit assumption involved in this expression is discussed later.

(2) Oligosaccharides: raffinose, sucrose and glucose (reagent grade).

(3) Standard poly(ethylene oxide) (Toyo Soda, Tokyo, Japan): SE-150, SE-70, SE-30, SE-15, SE-8, SE-5 and SE-2.

(4) Poly(ethylene glycol) (Wako, Osaka, Japan): PEG 20000*, 6000, 4000, 2000, 1540, 1000, 600, 400, 300 and 200.

(5) Oligo(ethylene glycol)s: Tri(ethylene glycol) (triEG), di(ethylene glycol) (diEG) and ethylene glycol (EG).

(6) High-molecular-weight poly(ethylene oxide) (Seitetsu Kagaku, Tokyo, Japan): PEO 1* and PEO 3* (nominal average molecular weight: $1.0 \cdot 10^5$–$1.5 \cdot 10^5$ and $5 \cdot 10^5$–$7.5 \cdot 10^5$, respectively).

These fractions have relatively sharp molecular weight distribution except for those marked with an asterisk.

Solute exclusion experiment

Single-point SE. The dextran–oligosaccharide series was used for this experiment. Each fraction was separately dissolved in deionized water to a concentration of about 5%, and 0.005% of Hibitane was added as an antimicrobial agent. About 10 g of the gel sample was weighed accurately (G g) in a tared stoppered bottle. An appropriate amount of the polymer solution (X g) was added so that the solution would be diluted to about 50% of the initial concentration by the accessible water in the gel. The mixture was equilibrated for several hours with occasional shaking, then the extra-gel solution was quickly filtered with a dry sintered-glass filter and collected in a glass bottle, which was stoppered immediately. The gel was washed thoroughly with deionized water, then dried and weighed (m g). The solute concentration of the extra-gel solution was determined by differential refractometry (Waters R403 refractometer) by comparing the solution with a series of calibration solutions prepared from the same stock solution. The amount of non-solvent water is given by eqn. 4, where f/h represents the dilution ratio for a single solute.

Column SE. The gel was packed in a 10 × 500 mm glass tube with plungers (Pharmacia) to form a 291-mm gel bed. Ordinary gel filtration was carried out on this column with deionized water as eluent for the sharp fractions of dextran and PEO. About 0.3 ml of a 0.2% aqueous solution of each solute was separately injected through a sampling valve. The flow-rate was kept constant at 0.140 ± 0.002 ml/min by a peristaltic pump fitted at the drain. Elution of solutes was monitored with a refractometer (Waters R403). No contraction of the gel bed occurred throughout the experiment. Finally, the gel was taken out of the column, dried and weighed.

Mixed SE. The procedure was same as that of in single-point SE except that mixed solutions of dextran or PEO fractions were used instead of solutions of single fractions, and that the extra-gel solutions were analysed by aqueous GPC. The compositions of the mixed solutions are given in Table I. These compositions were chosen so that their GPC curves give nearly flat patterns and that the output is proportional to the concentration (overloading avoided). The solutions were stored in a refrigerator, 0.005% of Hibitane being added.

The liquid chromatographic apparatus was Toyo Soda HLC 802, equipped with a refractometer. Deionized water degassed by boiling was pumped at a flow-rate of 0.550 ml/min (±1%). The sample solution was injected on to a set of aqueous GPC columns[11] (TSK-GEL, type-PW, G6000PW + G4000PW + G2000PW, each

TABLE I

COMPOSITIONS OF MIXED SOLUTIONS IN MIXED SE

Dextran		*PEO*	
Component	*Concentration (%)*	*Component*	*Concentration (%)*
T2000	1.0	PEO 3	0.30
T40	0.5	PEO 1	0.50
T10	0.2	PEG 20000	0.25
Raffinose	0.15	PEG 6000	0.10
Sucrose	0.15	PEG 4000	0.15
Glucose	0.15	PEG 1540	0.15
Total	2.15	PEG 1000	0.15
		PEG 400	0.25
		PEG 300	0.10
		PEG 200	0.15
		TriEG	0.20
		DiEG	0.20
		EG	0.30
		Total	2.80

600 × 7.5 mm I.D., connected in this order) from a 300-μl sampling loop. The loop was thoroughly flushed and filled with the sample solution contained in a glass syringe.

RESULTS

Calibration of the GPC columns

Fig. 1 shows the overall relationships between molecular weight and radius of the equivalent sphere for dextran and PEO. The hydrodynamic radius was calculated

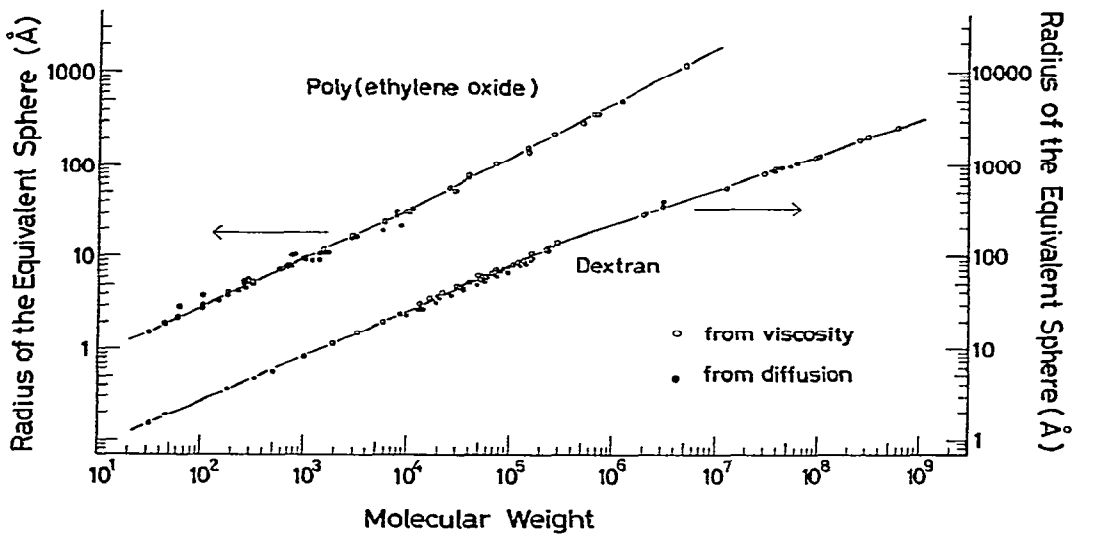

Fig. 1. Radius of the equivalent hydrodynamic sphere for dextran and PEO, calculated from the reported data for limiting viscosity numbers and diffusion coefficients[12–25].

according to the following well known relationships from the reported limiting viscosity numbers or diffusion coefficients of dextran[12–17] and PEO[18–25]:

$$R = 0.541\,(M[\eta])^{1/3} \tag{5}$$

$$R = \frac{kT}{6\pi\eta_w D} \cdot 10^8 \tag{6}$$

where R (Å) is the radius of the equivalent sphere, M is the molecular weight, $[\eta]$ (cm^3/g) and D are the limiting viscosity number and the diffusion coefficient, respectively, of the solute in water, k is the Boltzman constant, T is the absolute temperature and η_w is the viscosity of water (all in c.g.s. units, except R). In Fig. 1, the values of

TABLE II

MOLECULAR WEIGHTS AND RADII OF EQUIVALENT SPHERES OF THE POLYMER FRACTIONS USED

Polymer	*Molecular weight, M_w**	*M_w* / M_n*	*Radius of equivalent sphere, R (Å)*	*Manufacturer*
Dextran				
T2000	$2 \cdot 10^6$	—	290	Pharmacia
T500	$5.11 \cdot 10^5$	2.67	165	
T70	$6.85 \cdot 10^4$	1.70	64	
T40	$3.95 \cdot 10^4$	1.34	49	
T10	$9.4 \cdot 10^3$	1.71	24	
PEO				
SE-150	$1.20 \cdot 10^6$	1.12	500	Toyo Soda
SE-70	$6.61 \cdot 10^5$	1.10	350	
SE-30	$2.78 \cdot 10^5$	1.05	210	
SE-15	$1.48 \cdot 10^5$	1.04	150	
SE-8	$7.3 \cdot 10^4$	1.02	100	
SE-5	$4.0 \cdot 10^4$	1.03	70	
SE-2	$2.5 \cdot 10^4$	1.14	55	
PEG				Wako
6000	7500	—	27	
4000	3000	—	16	
2000	2000	—	12.7	
1540	1500	—	11.0	
1000	1000	—	8.9	
600	600	—	6.9	
400	400	—	5.6	
300	300	—	4.8	
200	200	—	4.0	
TriEG	150	1.00	3.4	
DiEG	106	1.00	2.9	
EG	62	1.00	2.2	
Ethanol	46	1.00	1.85	
Methanol	32	1.00	1.55	
Raffinose	504	1.00	6.1	
Sucrose	342	1.00	4.9	
Glucose	180	1.00	3.6	

* Given by the manufacturers.

R obtained from viscosity and diffusion agree well with each other within the scatter between the investigators. Calibration graphs for the two homologous series were drawn by connecting the points smoothly. R values of the fractions actually used were determined from these graphs (Table II).

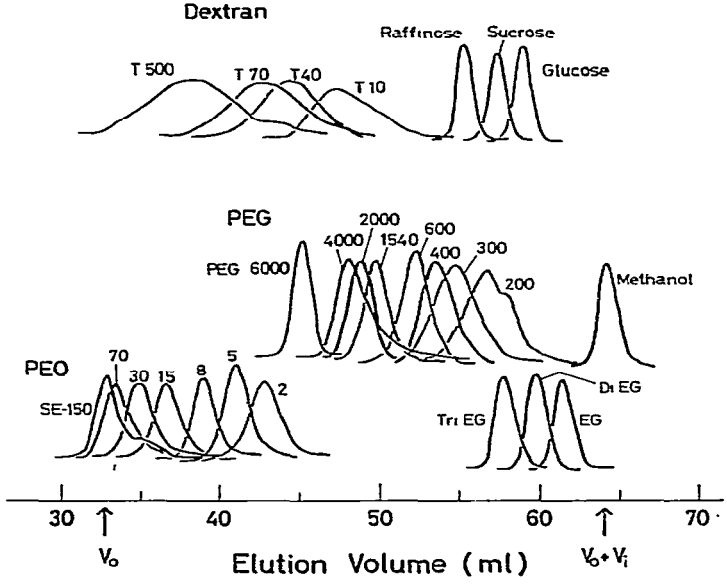

Fig. 2. Gel permeation chromatograms of the standard sharp fractions of dextran and PEO. Columns: TSK-GEL, type-PW, G6000PW+G4000PW+G2000PW, each 600 × 7.5 mm I.D. Eluent: deionized water. Flow-rate: 0.550 ml/min. Detector: differential refractometer. V_0 = Void volume; $V_0 + V_t$ = total volume.

These samples were chromatographed on the GPC columns (Fig. 2), giving the calibration graphs for the dextran and PEO series (Fig. 3). Although this set of columns seems to have an extended fractionation range above 1000 Å (in $2R$), the peak position of SE-150 was taken as the void volume ($K_d = 0$) as no sharp standard sample larger than this was available. Methanol was chosen as a total volume marker. A definite discrepancy can be seen in Fig. 3, between the curves for dextran and PEO. In absence of any specific interaction (adsorption) between the solute and the gel packing, a single calibration graph (universal calibration graph) should apply to any kind of polymer in the same solvent. Such a graph will be completely determined by the porous structure of the packed gel. The observed discrepancy, therefore, should result from either (a) specific adsorption or (b) the difference between dextran and PEO with respect to the relationships between R and the size of the smallest permeable pore.

Case (a) seems unlikely because no appreciable retardation or tailing phenomenon was observed for either polymers. As is shown later, a similar discrepancy is found in the solute exclusion curves for Sephadex G-100. This corroborates the possibility of (b), as the phenomenon seems to be general, irrespective of the porous material concerned. Thus, the relationship between the hydrodynamic radius and the

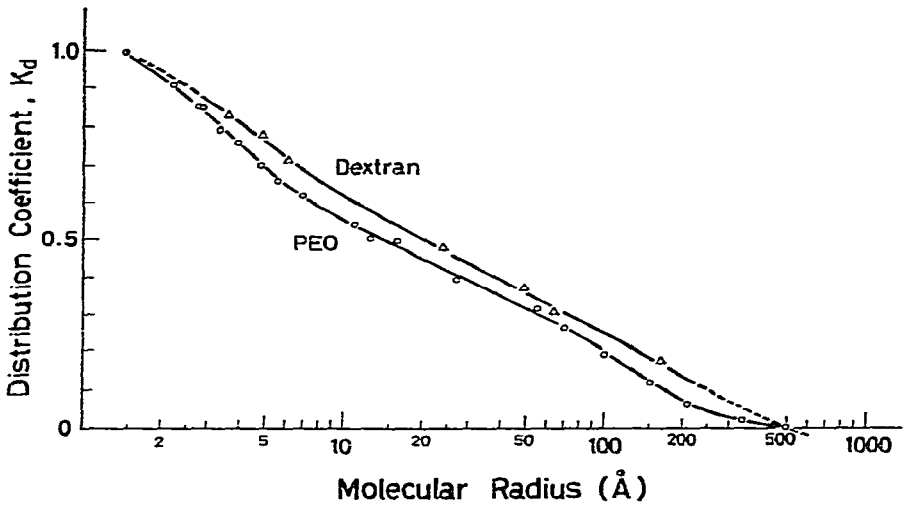

Fig. 3. Calibration graphs for the set of the aqueous GPC columns for dextran and PEO series, based on Fig. 2.

radius of the smallest permeable pore of a polymer molecule seems to depend on the nature of the polymer. This means that one of the assumptions of the SE method is not strictly fulfilled. This problem will be discussed in detail later.

Solute exclusion curves for Sephadex G-100

Fig. 4 shows the gel filtration chromatogram of the standard solutes on a packed gel bed (column SE). The peak position of the elution curve gives the distribution coefficient, K_d, which is readily converted to the accessible pore volume for the solute per gram of dry substance. The elution volume for an asymmetric curve was taken at the position of the vertical line dividing the curve into two parts of equal area. The SE curves for dextran and PEO (Fig. 5) were obtained from the chromatogram and the dry weight of the packed gel (1.143 g). Here, a similar discrepancy is observed between the curves for dextran and PEO to that in the calibration graph for the GPC columns. However, both curves show well defined plateau regions at the greatest pore diameter range. The level of the plateau gives the total amount of imbibed water, $W_{ns}(\infty) = 14.8$ cm^3/g.

Fig. 6 shows the GPC curves of the original and the diluted mixed solutions of dextran and PEO in the mixed SE method. The dilution ratio, f/h, was determined at the corresponding peaks and valleys indicated by arrows, so that errors arising from lateral deviation should be minimized. The chromatogram of the dextran mixed solution showed an anomalous peak of low reproducibility (marked by an asterisk in Fig. 6a). Because this was probably an artifact, this region was omitted from the analysis. When the surplus (external) water has been removed completely and the added amount of the test solution is nearly equal to that of the imbibed water, the dilution ratio will decrease monotonously from unity to about 0.5 as elution proceeds. In Fig. 6, however, f/h exceeds unity at the initial stage of elution. This is probably because a small amount of imbibed water had been squeezed out of the gel beads as

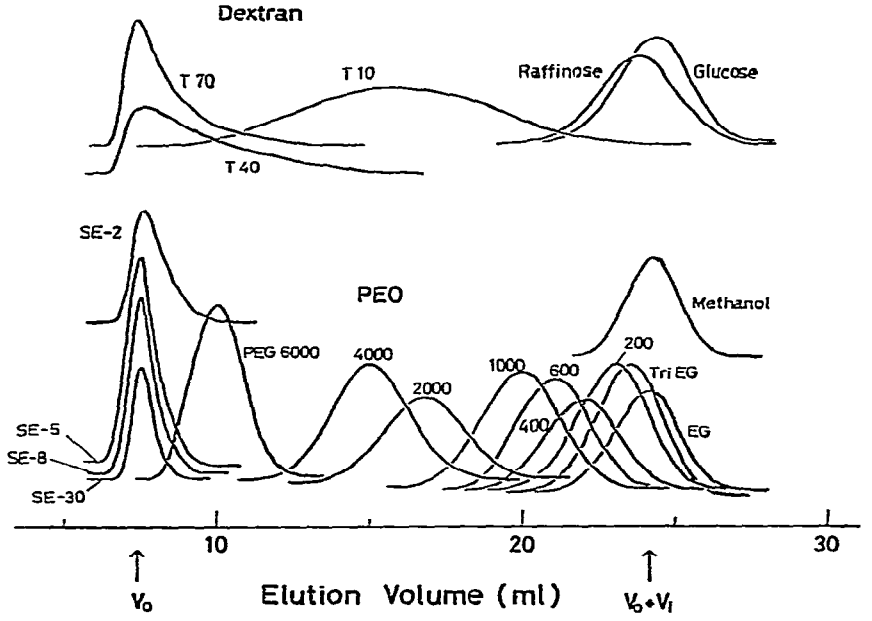

Fig. 4. Gel filtration chromatograms of dextran and PEO standards on Sephadex G-100 for the column SE method. Column: 291 × 10 mm I.D. Eluent: deionized water. Flow-rate: 0.140 ml/min. Detector: differential refractometer.

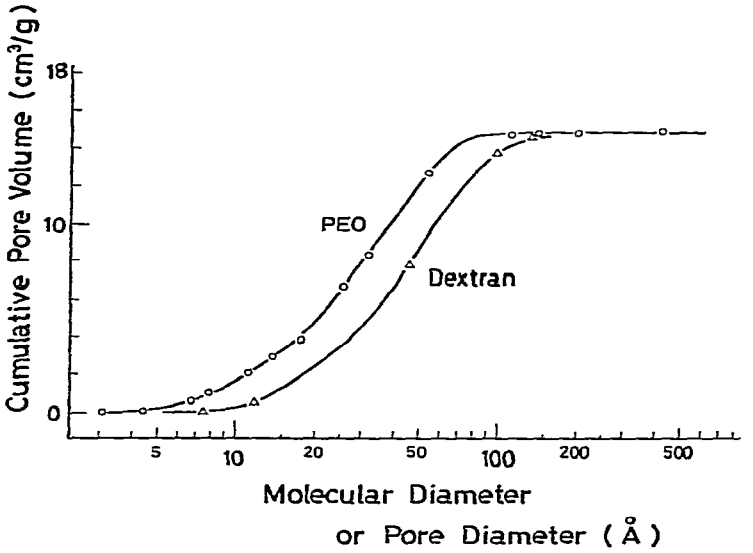

Fig. 5. SE curves for Sephadex G-100 determined by the column SE method from Fig. 4.

a result of aspirated filtration, and the solution was concentrated with respect to the completely excluded component by re-absorption of water into the gel. Even in this case, however, eqn. 4 is expected to give the correct W_{ns} value for the re-swollen state of the gel sample.

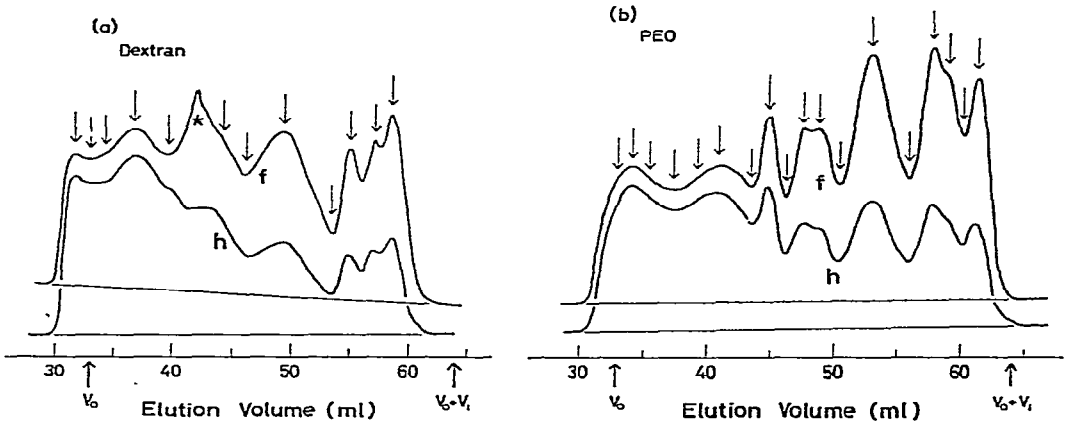

Fig. 6. Gel permeation chromatograms of the original (f) and the diluted (h) mixed solutions of (a) dextran and (b) PEO. The arrows indicate the reference positions. Conditions as in Fig. 2.

Fig. 7 shows the solute exclusion curves obtained. The results for PEO were obtained from three separate GPC runs on an identical sample solution. The points from the valleys between the sharp peaks and from the shoulder in Fig. 6 (indicated by divided circles in Fig. 7) showed greater scattering and deviation from the smoothed curve than those from the peak positions. Therefore, it is recommended that peak and plateau positions are adopted as reference positions in the mixed SE method. Again, a similar discrepancy is seen between the curves for dextran and PEO to those in Figs. 3 and 5. The values of W_{ns} (∞), however, agree with each other.

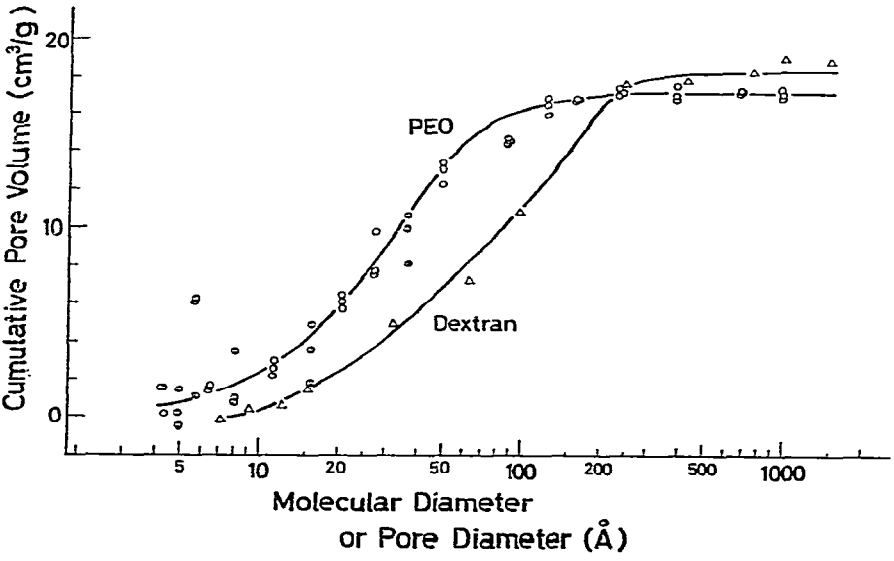

Fig. 7. SE curves for Sephadex G-100 determined by the mixed SE method from Fig. 6. Divided circles correspond to valleys in Fig. 6b.

Fig. 8 shows the result of single-point SE for the dextran series, together with those of column and mixed SE for dextran. The curves obtained by single-point and mixed SE agree well with each other over the whole range of molecular size. The curve from column SE deviates from the other two for the large pore size range (above 100 Å), and gives a markedly lower value of W_{ns} (∞). This is probably due to compression of the gel particles which are packed in a glass column with plungers. The curves for PEO obtained by column and mixed SE agree well with each other (Figs. 5 and 7), except for the values of W_{ns} (∞).

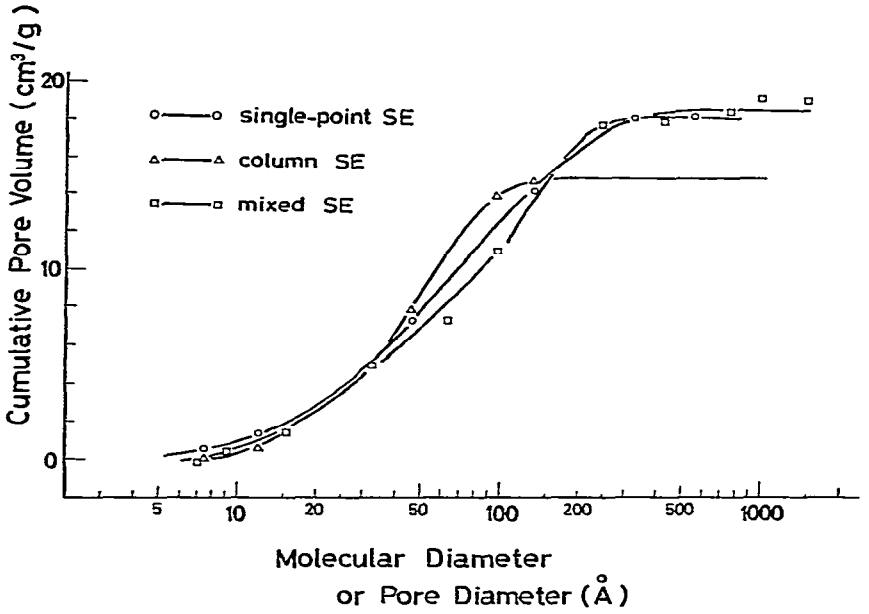

Fig. 8. Comparison of the results of the three SE methods for Sephadex G-100.

Summarizing the above results, the three SE methods examined gave reasonable and consistent results for the solute exclusion behaviour of Sephadex G-100. In particular, the mixed SE method proved effective in determining the solute exclusion curve much more rapidly and easily than single-point SE. Although column SE also has great advantages in its rapidity and ease, its applicability is rather limited. Optimization of the experimental conditions such as polymer composition and GPC instrumentation will improve the accuracy and reliability of the mixed SE method as a technique for the study of the structures of porous materials, especially of swollen polymeric gels.

DISCUSSION

The separation mechanism in size exclusion chromatography is now understood as equilibrium partitioning based on purely steric effects. However, a quantitative description of the phenomenon has not been fully established, especially for

flexible chain (random coil) molecules. Such a knowledge is required when converting the SE curves obtained above into pore size distribution (PSD) curves.

By adopting the simplest assumption that the diameter of the smallest permeable pore ("exclusion value", $\varnothing$, according to the convention by Halász and Martin[9]) is equal to the hydrodynamic diameter ($2R$), an SE curve is regarded as a cumulative pore size distribution. Other variables proportional to the radius of gyration or the root-mean-square end-to-end distance of random coil molecules have been proposed instead of $2R$[9,26,27]. As these variables are related to R by a universal numerical factor affiliated to Flory's constant Φ (ref. 28), the problem is to determine the numerical value of $\varnothing/2R$. This is carried out empirically by comparing the SE curve with the PSD curve determined by conventional porosimetry such as mercury intrusion or gas adsorption. The value of $\varnothing/2R$ was concluded to be unity in some instances[8,26], whereas a value of 2–3 was proposed by other workers[9,29]. Further investigation is necessary in order to assign the correct value. It may depend on the polymer species, thus causing a discrepancy in the SE curves such as observed here between dextran and PEO in water.

A more serious criticism may be made about the SE method with regard to the assumption which has been implicitly made hitherto in interpreting SE curves as PSD curves, namely that "all the liquid existing in the pores greater than $\varnothing$ is "available" as solvent for a solute molecule having a size ($2R$) corresponding to $\varnothing$" or, in other words, "the centre of gravity of a solute molecule can migrate through all regions within the accessible pores with equal probability". Eqn. 2 is based on this assumption, which is obviously invalid as long as the solute has a finite volume. This effect ("free volume" or "excluded volume" effect) has been treated in detail for random coil molecules[30,31] and rigid spherical or rod-like molecules[32] with respect to several simple-shaped pore models. In fact, De Vries *et al.*[33] showed that the calibration graph based on the PSD curve determined by mercury intrusion did not agree with the actual curve for a silica gel having a very sharp PSD. Similar observations have been made for porous glasses[29,34,35].

Taking these facts into account, we must regard eqn. 2 as a crude approximation of the steric exclusion of solutes from porous materials. That is, a serious limitation is imposed on the resolution of the SE method as porosimetry. However, this effect presumably becomes significant with porous materials of sharp PSD such as were examined in the studies cited above. With polymeric gels, such as cross-linked dextran and polystyrene gels, which are not likely to have such sharp PSDs, an SE curve is expected to depict the real PSD as a fairly good approximation.

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