A THEORY OF GEL FILTRATION AND ITS EXPERIMENTAL VERIFICATION

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(Received December 17th, 1963)

Gel filtration the well known method of separating substances differing in molecular size, by passing a solution through a bed of gel grains, was described by PORATH AND FLODIN[1]. These authors used mainly cross-linked dextran gels (Sephadex, AB Pharmacia, Uppsala), but other polymer gels, such as those prepared from agar, starch, polyacrylamide, polyvinyl alcohol, etc., are also capable of producing similar separations. A review of the technique has recently been published by PORATH[2]. Although gel filtration is not strictly a process of filtration in the classical sense, the term will be used in the following, because of its common acceptance.

Many investigations have shown that a substance having large molecules generally passes through the gel bed faster than one having small molecules, provided that the concentration of polymer in the gel grains lies within a suitable range. Deviations from this pattern have only been observed in cases where it seemed reasonable to believe that an adsorption of the substance to the polymer had occurred[2-4]. The possibility of adsorption during gel filtration will not be discussed here, only the process that leads to a separation of molecules according to size will be treated.

Essentially two explanations for the gel filtrations process have appeared. STEERE AND ACKERS[5] have explained it as a decreased diffusion rate of the substance in the gel grains, and LATHE AND RUTHVEN[6], PORATH[7] and PEDERSEN[8] have discussed it in terms of an exclusion of the substance from the gel. Both mechanisms certainly operate in gel filtration. It has been shown that the diffusion rate of a macromolecule is decreased in both polysaccharide gels[9] and polysaccharide solutions[10] and that this decrease is a function of the size of the macromolecule. Furthermore it has been shown, both with the aid of equilibrium dialysis[11] with osmometric determinations[12] and with solubility studies[13,14], that polysaccharides such as hyaluronic acid and dextran exclude a certain volume of solvent for other solute macromolecules.

THEORY

It has been demonstrated that the position at which a substance is eluted in a gel chromatogram does not vary appreciably with the flow rate of the eluent[1,4]. This indicates that it is the volume available for a substance in the gel grains (total volume of gel phase minus excluded volume), and not the decreased diffusion rate, that determines the position of the substance in the chromatogram, for in the latter case the

elution volume would be strongly dependent on the flow rate. In the following treatment it will therefore be tacitly assumed that the volume in the gel grains available for a particular substance will determine its position in the gel chromatogram.

The relation between the elution volume and the available volume in the gel grains

A gel bed can be regarded as consisting of two phases, one gel phase with the volume V_x and one liquid phase with the volume V_0 (void volume). The resistance towards flow of water in the gel phase is very high (compare *e.g.* the high flow resistance in polyacrylamide gels[15]) and therefore it can be assumed that all water flow takes place in the liquid phase (see Fig. 1).

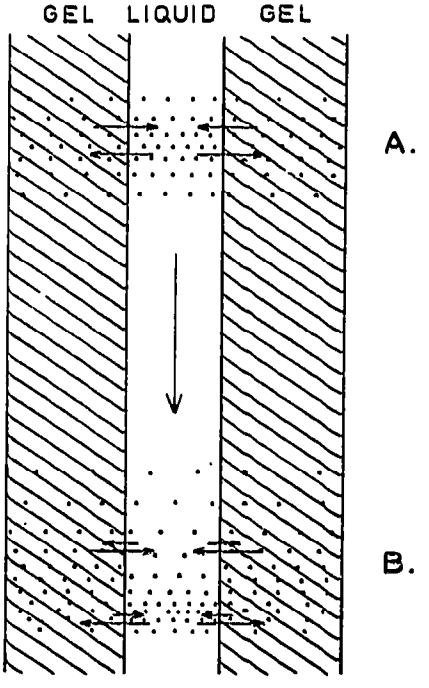

Fig. 1. Schematic diagram showing the most important mechanisms operating when a solute (dotted area) moves along a bed of gel grains. The diffusion of the solute (horizontal arrows) distributes it between the liquid phase (void volume) and the available volume in the gel phase. The flow of the liquid phase (vertical arrow) transports that fraction of the solute that at each moment is outside the gel grains. The lower density of solute in the gel phase indicates that only part of the gel phase is available for the solute. A. The ideal case of diffusion equilibrium between the gel and the liquid phases. B. The non-ideal condition, when the solute zone in the liquid phase moves ahead of that in the gel phase.

In the ideal case, a substance introduced in the system will equilibrate instantly between the liquid phase and the gel phase (Fig. 1A). Only a certain fraction, K_{av}, of the gel phase is, however, available for the substance, which means that the total available volume in the gel grains is $K_{av} \times V_x$. If a solute is introduced on the top of the gel bed and if the gel completely excludes this solute, it will be eluted after a volume, V_e, which is equal to the void volume. If, however, a volume in the gel phase is available for the solute, only that part that is in the liquid phase at each moment will move down the bed. The average speed of all solute molecules will be equal to the flow rate times that fraction of the molecules that in each instance is present in the liquid phase. In the ideal case, in which an instant equilibrium between the gel phase

and the liquid phase is assumed, the fraction of solute molecules that moves at each moment will be:

$$\frac{V_0}{V_0 + K_{av} \times V_x} \tag{1}$$

The elution volume of the substance will therefore be $(V_0 + K_{av} \times V_x)/V_0$ times larger than that of a substance that is completely excluded from the gel phase. The equation for the elution volume of the substance will thus be:

$$V_e = V_0 + K_{av} \times V_x \tag{2}$$

If the total volume of the gel bed, V_t ($V_t = V_0 + V_x$), is introduced, eqn. (2) can be rearranged to:

$$K_{av} = \frac{V_e - V_0}{V_x} = \frac{V_e - V_0}{V_t - V_0} \tag{3}$$

According to eqn. (3), it is possible to calculate K_{av} (*i.e.*, the fraction of the volume of the gel that is available for the substance) from the elution volume of the substance, the total volume of the gel bed and the void volume.

Influence on the gel filtration process of flow rate and the rate with which equilibrium between liquid phase and gel phase is attained

In the preceding section, it was assumed that equilibrium between liquid and gel will take place instantly. This is an ideal condition, which cannot be attained in practice. A study of the influence that a finite diffusion rate of the solute into the gel may have on the resolution in gel filtration is at present in progress in our laboratories and no quantitative presentation will be given here. Only a short qualitative description will follow (Fig. 1B).

The rate at which diffusion equilibrium is reached is governed by the size of the gel grains and the diffusion rate of the solute in the gel. The latter is a complicated function of the free diffusion rate, the molecular size of the solute and the concentration and structure of the gel substance[9,10].

When a solute passes through the gel bed with a finite flow rate, the diffusion between the liquid phase and the gel phase in front of the solute zone will not be sufficiently rapid to lead to an equilibrium. The result is a higher concentration in the liquid phase than in the available gel space. On the other hand, in the rear part of the solute zone, the concentration in the available space in the gel phase will be higher than in the liquid phase. The result can be regarded as if the solute zone in the liquid phase moves ahead of the solute zone in the gel phase. The effect will be more pronounced the higher the flow rate and give rise to a broadening and asymmetry of the solute band. The elution volume will, however, change relatively little, as will be shown in a subsequent paper.

Diffusion along the gel bed

If diffusion takes place along the gel bed, this will cause further dilution of the solute and broadening of the band, but the elution volume will not change.

Calculation of the available volume in a polymer network

In order to calculate the available volume in a gel, it is necessary to design a physical model of the gel network. The simplest model will be obtained by assuming that the dextran chains are straight rigid rods, which are infinitely long and distributed at random in the gel. OGSTON[16] has calculated the available volume for spherical particles in such a system. He arrives at the following formula:

$$K_{av} = \exp\left[-\pi L(r_s + r_r)^2\right] \quad (4)$$

where L is the concentration of rods in the solution, expressed as cm rod per cm^3, r_s is the radius of the spherical particles and r_r the radius of the rod.

This picture will of necessity be an approximation. The dextran molecule is branched, there are cross-linkages and a certain degree of rotation can take place at the glucosidic bonds. Also micro-heterogeneities may occur in the gel. It is, however, reasonable to make the assumption that these deviations from a linear straight polyglucose chain will essentially lead to a shortening and thickening of the chain. The molecule is still considered to have a rigid rodlike character.

The theory can then be summarized as follows: the volume available for a substance in the gel determines its position in the elution diagram; this volume can be calculated from the assumed physical model of the gel structure.

EXPERIMENTAL

Materials

Human fibrinogen, about 95 % coagulable, was kindly supplied by AB Kabi, Stockholm. Ribonuclease from bovine pancreas, crystallized five times (lot No. 32143), and cytochrome *c* from equine heart (assay 71 %, lot No. 32117) were obtained from California Corporation for Biochemical Research, Los Angeles, Calif. Horse myoglobin I was kindly supplied by Dr. A. EHRENBERG, Stockholm and violet dextran (molecular weight $\sim 2 \cdot 10^6$) by AB Pharmacia, Uppsala.

Methods

Gel filtration was performed as described in detail elsewhere[17]. All experiments were made on Sephadex G-200 with a water regain of 19.9 g/g gel, dry mesh 200–270 (U.S. sieve series). The columns had a diameter of 4.2 cm and lengths of 70.5 or 71.7 cm. The samples were applied in 14 ml volume. As buffer, 0.1 *M* Tris–HCl, pH 8.0 was used, containing 0.5 *M* sodium chloride and 0.02 % sodium azide. The transmission at 254 mμ was continuously registered in a Uvicord absorptiometer (LKB-Produkter, Stockholm). The substances were also determined in the collected fractions by spectrophotometry at their absorption maxima, and in the case of fibrinogen also as coagulable protein.

Results

The results are presented in Fig. 2. The ratio between elution volume and total volume is plotted on the abscissa. It can be assumed that the elution volume of violet dextran is equal to the void volume of the bed.

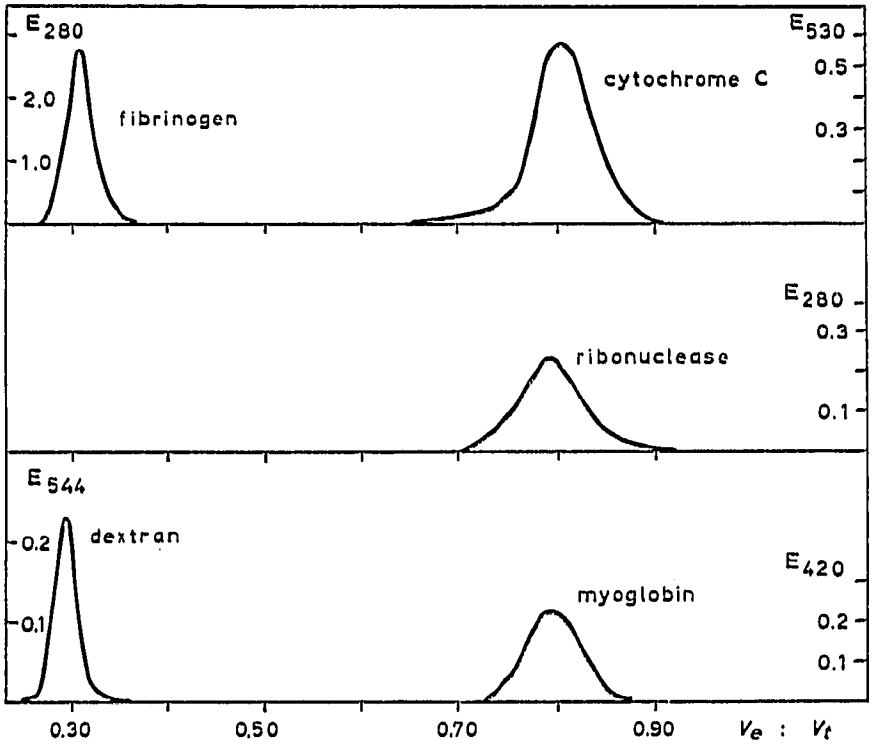

Fig. 2. Gel filtration of four proteins and violet dextran on Sephadex G-200.

COMPARISON BETWEEN THEORY AND EXPERIMENTS

The data presented above as well as data on the following substances taken from the literature have been used to test the hypothesis: oligosaccharides from cellulose[18] and dextran fractions[19] chromatographed on Sephadex G-25; dextran fractions[19] chromatographed on Sephadex G-50; dextran fractions[19] and proteins[20,21] chromatographed on Sephadex G-75; proteins[20,21] chromatographed on Sephadex G-100; and proteins[17,21,22] chromatographed on Sephadex G-200.

Calculation of K_{av}

FLODIN AND ASPBERG[18] and GRANATH AND FLODIN[19] have published data on void volumes, total volumes and elution volumes obtained in their experiments. These data were used to calculated K_{av} according to eqn. (3). It should be pointed out that K_{av} is not the same as K_d listed by GRANATH AND FLODIN, since K_d includes a correction term for the volume occupied by the dextran chains in the gel. Only those dextran fractions, collected by GRANATH AND FLODIN, that contained at least half as much material as the largest fraction, have been used for the calculations.

ANDREWS[20] has listed his data as migration rates of the substances on the gel columns divided by the migration rate of serum albumin. The elution volumes of albumin were taken from Fig. 1 in ANDREWS' paper. The total volume of each column was 226 ml and the elution volume of India ink and thyroglobulin has been assumed to be equal to the void volume. The elution volumes of four enzymes were estimated from data in Table IV and Fig. 1 in ANDREWS' paper. K_{av} has been calculated from these data. DETERMANN AND GELOTTE[21] present their data as K_d values. These

TABLE I

ESTIMATED MOLECULAR RADII AND K_{av} VALUES FOR SUBSTANCES STUDIED BY ANDREWS[20], DETERMANN AND GELOTTE[21], KILLANDER[17,22] AND THE PRESENT AUTHORS

Substance	$D_{20,w} \times 10^7$	Ref.	$r_s \times 10^8$	K_{av}					
				G-75		G-100		G-200	
				ANDREWS	DETERMANN AND GELOTTE	ANDREWS	DETERMANN AND GELOTTE	KILLANDER *et al.*[a]	DETERMANN AND GELOTTE
Sodium barbiturate			~ 4[b]					(0.94)	
Sucrose	41.6	24	5.1	0.88		0.94			
Vitamin B_{12}			~ 7.5[b]					(0.92)	
Cytochrome *c* (equine heart)	13.0	27, 28	16.4	0.43	0.38	0.59	0.66	0.72	
Myoglobin (horse)	10.3; 11.3	26, 28	20.7; 18.8	0.36		0.54		0.71	
Ribonuclease	11.1	29	19.2	0.43	0.33	0.58		0.70	0.74
Chymotrypsin	10.2	28	20.9		0.26		0.51		0.69
Trypsin	11.0	28	19.4		0.26		0.51		0.69
α-Lactalbumin (cow)	10.6	28, 30	20.1	0.39		0.56			
Chymotrypsinogen	9.5	28	22.4	0.27		0.45			
Soybean trypsin inhibitor	9.4[c]	31, 32	22.6	0.28		0.46			
Pepsin (monomer)	9.3	28	22.9		0.23				0.57
Cytochrome *c* (dimer)	9.1	27	23.4	0.25		0.42			
Ovalbumin	7.8	28	27.3	0.12		0.29	0.22		
Alkaline phosphatase (*E. coli*)	7.3[c]	33	29.2			0.17			
Peroxidase (horse radish)	7.05	34	30.2			0.27			
Hemoglobin, human	6.8	28	31.3	[d]	0.07	[d]	0.28[e]	0.49 (0.46)	0.50
Alkaline phosphatase (bovine intestine)	6.2[c]	35	34.6			0.08			
Serum albumin, human	5.9-6.1	36	36.1; 34.9	0.04		0.19	0.15	0.41 (0.37)	0.42
Heme-binding β-globulin, human	5.6[c]	37	38.0					0.36	
Transferrin, human	5.3; 5.9	38, 39	40.2; 36.1		0.00		0.10	0.37 (0.33)	0.31
Serum albumin (dimer)	4.9[c]	8	43.5			0.06			0.25
Alcohol dehydrogenase (yeast)	4.7	40	45.3			0.07			
Ceruloplasmin, human	4.7; 4.5; 3.8	41, 42	45.3; 47.3; 56.1					0.28 (0.24)	
Haptoglobin 1-1, human	4.7	43	45.3					(0.20)	
Phycocyanin	4.1	28	52.6						0.22
γ-Globulin (7-S), human	3.8	28	55.5	0.00	0.00	0.05	0.00	0.21 (0.19)	0.25
Haptoglobin 1-1–hemoglobin, human	3.4	43	62.6					(0.12)	
Fibrinogen, human	2.0	44	107		0.00		0.00	0.02	0.00

[a] Obtained on a lot of Sephadex G-200 with water regain 19.9. Figures within parentheses were obtained on lots with water regain 18.5–19.3.
[b] Approximate estimation from molecular weight, assuming the molecules to be spherical.
[c] Calculated with SVEDBERG's equation from molecular weight, $S_{20,w}$ and partial specific volume.
[d] ANDREWS reports that the elution volume for hemoglobin is dependent on concentration.
[e] The same value has been obtained by the present authors.

TABLE II

ESTIMATED MOLECULAR RADII AND K_{av} VALUES ON SEPHADEX G-25 FOR DEGRADATION PRODUCTS FROM CELLULOSE

Substance	*Estimated* $r_s \times 10^8$	K_{av}
Glucose	3.9	0.58
Cellobiose	5.1	0.52
Cellotriose	6.2	0.47
Cellotetraose	7.2	0.42
Cellopentaose	8.1	0.37
Cellohexaose	8.9	0.34

values, using 0.61 as the value of the partial specific volume of dextran[25], have been recalculated to K_{av}. Only those proteins for which reliable $D_{20,w}$ values were found have been listed.

The data of KILLANDER[17,22] and those presented above are expressed as ratios between elution volume and total volume. They have been converted to K_{av} using the elution volume of violet dextran or of human macroglobulins as the void volume The K_{av} values are listed in Tables I, II and III.

TABLE III

ESTIMATED MOLECULAR RADII AND K_{av} VALUES FOR DEXTRAN FRACTIONS OF DIFFERENT MOLECULAR WEIGHT

M_n	*Estimated* $r_s \times 10^8$	K_{av}
	Sephadex G-25	
1,230	9.5	0.34
1,320	9.9	0.29
1,610	11.0	0.24
2,240	12.9	0.19
2,630	14.0	0.14
3,570	16.2	0.09
4,550	18.2	0.04
	Sephadex G-50	
1,250	9.6	0.55
1,450	10.3	0.51
1,760	11.3	0.44
2,000	12.1	0.39
2,280	12.9	0.35
2,880	14.5	0.30
2,880	14.5	0.27
3,690	16.5	0.21
	Sephadex G-75	
1,350	10.0	0.71
1,790	11.5	0.63
2,270	12.9	0.55
2,690	14.1	0.50
3,180	15.3	0.45

Calculation of molecular radius (r_s)

The radius of the equivalent sphere was calculated from the diffusion constant, using STOKE's formula[23], to obtain a general measure of the molecular size of each substance.

Proteins. The diffusion constants ($D_{20,w}$) of the various proteins have been taken from the literature and are listed in Table I together with the calculated values of the radii. For some proteins, $D_{20,w}$ has been calculated from known molecular weights, $S_{20,w}$ and partial specific volumes using SVEDBERG's equation[23]. For a few proteins more than one value has been listed.

Carbohydrates. The diffusion constants for three monosaccharides, a disaccharide and a trisaccharide have been listed by LONGSWORTH[24]. GRANATH[25] has measured the diffusion constant of various dextran fractions (B512-Ph Dextran) with known number-average molecular weights. When the square root of the molecular weight was plotted *versus* the diffusion constant for these two sets of data, a linear relationship was obtained. This has been used for estimating the diffusion constants of the various cellulose and dextran fractions and their molecular radii (Tables II and III).

The radius of the dextran chain (r_r)

The radius of a straight polysaccharide chain is in the order of $2–3 \cdot 10^{-8}$ cm. However, the dextran in the gel is branched and flexible. The actual chain must therefore be assumed to be considerably thicker. A value for the radius of $7 \cdot 10^{-8}$ cm has been used throughout, because good agreement was then obtained between $\pi L r_r^2$ and the volume occupied by the dextran in the gel. For the estimation of L, see below.

Plotting of the data

The K_{av} values calculated from the data determined by ANDREWS on Sephadex G-75 and G-100, and by KILLANDER and the present authors on Sephadex G-200 are plotted in Figs. 3 and 4 *versus* the molecular radius. The curves are drawn according to eqn. (4), with a value for r_r of $7 \cdot 10^{-8}$ cm. The value of L was chosen for closest fit to the experimental values and is for G-75, $4.6 \cdot 10^{12}$; for G-100, $2.9 \cdot 10^{12}$; and for G-200, $1.6 \cdot 10^{12}$. The experimental values are in general close to the theoretical curves and the deviation can in most cases be explained by uncertainties in diffusion data and K_{av} values. The most notable discrepancies are shown by the alkaline phosphatases when chromatographed on G-100. They fall far below the theoretical curve. 7-S γ-globulin seems to fall above the line and so does ceruloplasmin. Sucrose and ovalbumin also deviate.

The points at r_s equal to zero are obtained by calculating the volume occupied by the dextran chains and subtracting this from the total volume of gel grains. The volume of dextran is calculated from its concentration and partial specific volume[25], 0.61.

The data given by DETERMANN AND GELOTTE are generally in agreement with the data presented in Figs. 3 and 4 (See Table I). There is, however, a larger scattering of their points, probably due to the fact that they have been obtained in two laboratories under different experimental conditions.

Fig. 5 shows the same kind of plot with the cellulose and dextran oligosaccharides chromatographed on Sephadex G-25, G-50 and G-75. The values of L chosen for the theoretical curves are $14.0 \cdot 10^{12}$, $8.2 \cdot 10^{12}$ and $4.8 \cdot 10^{12}$ respectively. The experi-

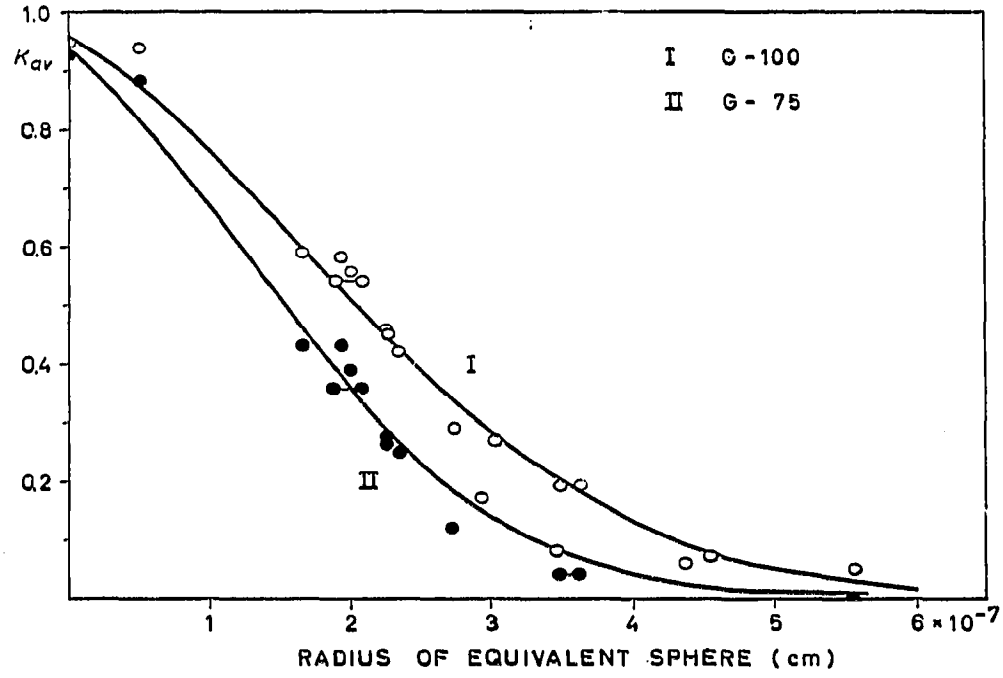

Fig. 3. K_{av} values calculated from the experimental data of ANDREWS plotted *versus* the radii of the equivalent spheres. The lines are drawn according to eqn. (4) using the values $2.9 \cdot 10^{12}$ (I) and $4.6 \cdot 10^{12}$ (II) for L. Points on the ordinate indicate calculated total volume of solvent in the gel grains.

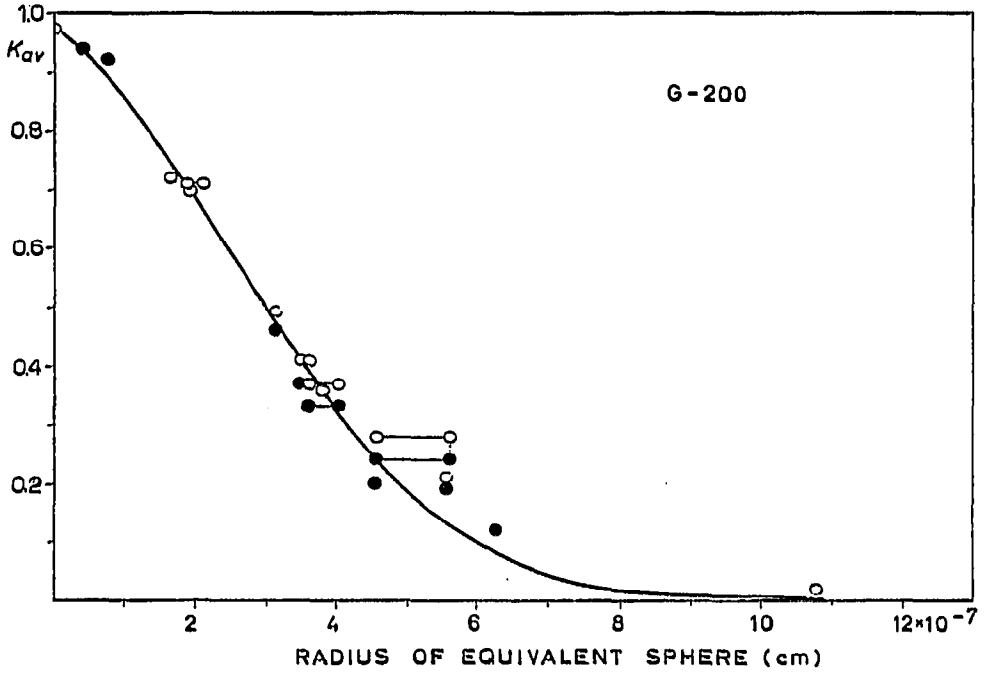

Fig. 4. K_{av} values calculated from the experimental data of KILLANDER and the present authors obtained on Sephadex G-200 plotted as in Fig. 3. A value of $1.6 \cdot 10^{12}$ for L is used. Open circles show experiments with a gel with water regain 19.9; filled circles represent experiments on gels with water regain 18.5–19.3.

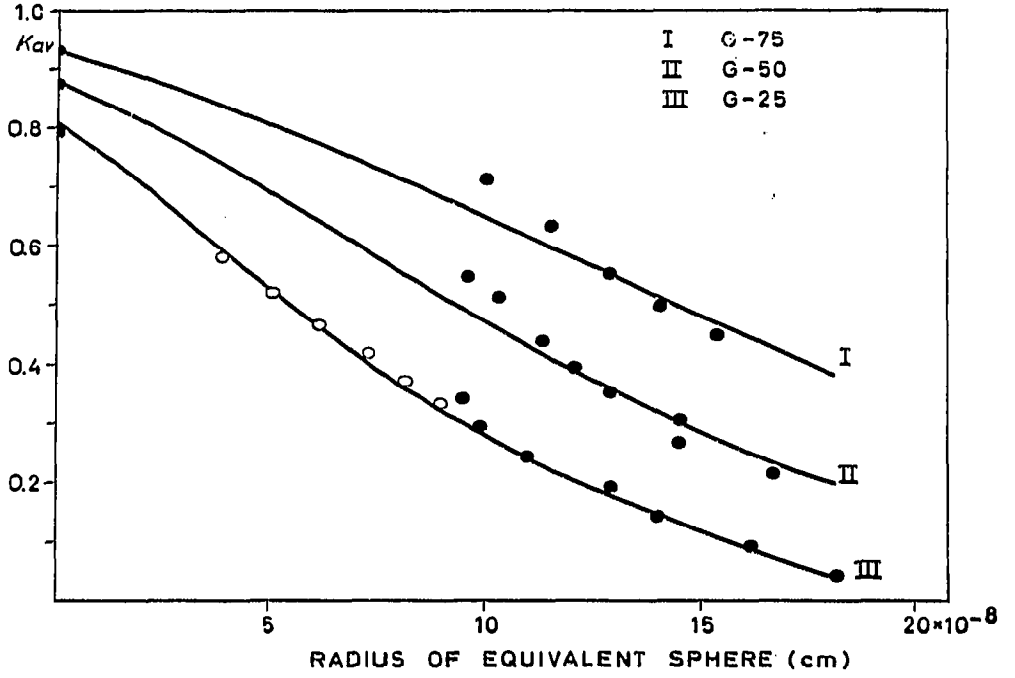

Fig. 5. The K_{av} values for degradation products from cellulose (○) and dextran (●) plotted as in Figs. 3 and 4. Lines are drawn according to eqn. (4) using the values $4.8 \cdot 10^{12}$ (I); $8.2 \cdot 10^{12}$ (II); and $14.0 \cdot 10^{12}$ (III) for L.

mental points fall somewhat above the lines for the small dextran molecules and below the lines for the larger.

Relation between L and the polymer concentration

The polymer concentration of the gels can be calculated from the water regain values (W_r) of the gels, using 0.61 for the partial specific volume of dextran[25]. The W_r value is equal to the number of ml of water held in the gel grains per gram of dextran. The values are, however, relatively uncertain due to difficulties in the determinations. The dextran concentrations can only be regarded as known within approximately 10 %.

The W_r values of the gels used by ANDREWS were 7.9 and 9.7, respectively (communication from AB Pharmacia) and those used by the present authors 19.9 and 18.5–19.3 respectively. The values obtained on the gel with $W_r = 19.9$, were used for fitting the curve in Fig. 4. FLODIN AND ASPBERG do not state the W_r of their gels. It is assumed to be the same as for the Sephadex G-25 used by GRANATH AND FLODIN. The latter authors used gels with W_r values of 2.3, 4.3 and 8.7 respectively.

Fig. 6 shows the relationship between L and the dextran concentration. An almost linear relationship is obtained in agreement with the hypothesis leading to eqn. (4).

From Fig. 6 it was found that in a dextran gel with the concentration 0.1 g/ml, L has a value of $4 \cdot 10^{12}$ cm/cm^3. If it is assumed that the length of a monosaccharide is $5 \cdot 10^{-8}$ cm, the total length of the dextran chains in 0.1 g of dextran was calculated to be $18.6 \cdot 10^{12}$ cm or approximately 4 to 5 times more than the experimental value. This ratio can be taken as an estimate of how much the dextran chain has been shortened by branching, flexibility in glucosidic linkages etc.

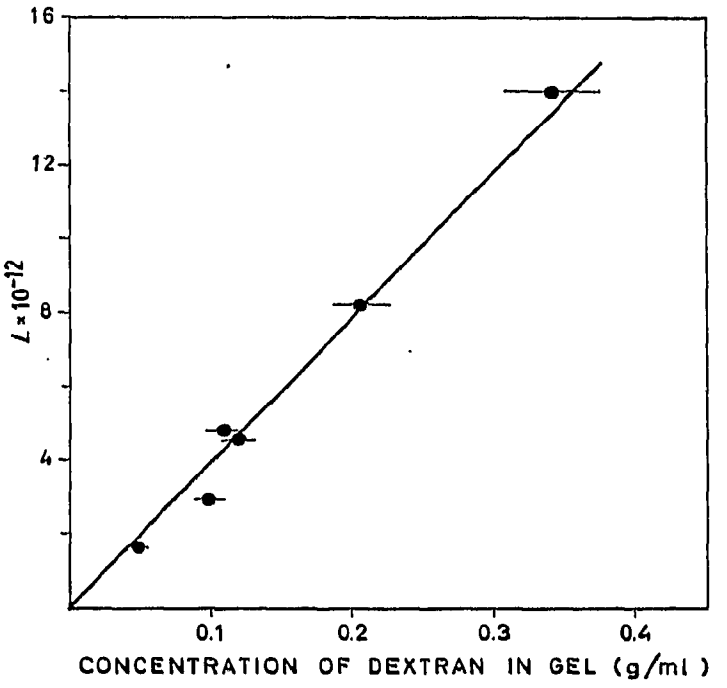

Fig. 6. The values of L used in Figs. 3, 4 and 5 plotted *versus* the concentration of dextran in the gel grains. Horizontal lines indicate ± 10% error in the calculated concentrations.

DISCUSSION

The present work was initiated in order to give a theoretical explanation of the well known observation that molecules of various sizes can be separated by chromatography on a bed of gel grains. It has been pointed out by some of the earlier authors that this effect may be due to an exclusion from the grains[6–8]. So far only PORATH[7] has tried to deduce a theoretical formula for the exclusion on the assumption that the free spaces in the gel grains are conical. He has tested his model using the dextran fractions obtained by gel filtration by GRANATH AND FLODIN[19]. PORATH gives an equation, which theoretically should only be valid for randomly coiled macromolecules and not for globular proteins. Unfortunately the test does not seem to be very sensitive, and DETERMANN AND GELOTTE[21] and ANDREWS[20] show that proteins give as good a fit as dextran fractions.

In the theory presented here, it is assumed that the gel network can be approximately treated as a three-dimensional network of randomly distributed straight fibers. The experiments using substances of a large range of molecular size and using a number of gels differing in polymer concentration show that if this model is used, most of the gel filtration data can be satisfactorily explained on the basis of an exclusion of molecules from part of the gel.

In some cases, however, there is a deviation from the expected values. Most deviations can be explained by the uncertainty in diffusion data and the approximation involved in calculating an equivalent radius from these data. A good example of the uncertainty in the values of the diffusion constants is given by ceruloplasmin, where available values in the literature disagree by as much as 20%. The diffusion constant of 7-S γ-globulin is also probably too low, since all 7-S γ-globulins studied by diffusion probably contained higher molecular weight aggregates[15]. This could explain

the deviation of γ-globulin to the right from the theoretical line in Fig. 4. The error in the determination of K_{av} is less than in that of the radius. Repeated determinations[17] on the same gel batch gave values varying only within $\pm$ 0.01.

In some instances, notably the alkaline phosphatases, the deviation is too large to be explained by errors in measurements. In this case, it can be explained by a type of aggregation of the protein, as the points fall below the theoretical line in Fig. 3. If any points should fall above the line, one has to assume a disaggregation or an adsorption of the substance to the gel matrix. Some results obtained by ANDREWS[20] are pertinent as examples of the latter. He showed that hemoglobin and β-lactoglobulin had elution volumes that were dependent on concentration and this was interpreted as indicative of a disaggregation. On the other hand this was not observed by KILLANDER[17] with hemoglobin in gel filtration or ultracentrifugal studies, and DETERMANN AND GELOTTE[21] did not report similar effects.

In a recently published report WHITAKER[46] gives data obtained by gel filtration on Sephadex G-75 and G-100. Uncertainties in reported volume have, however, made it difficult to calculate accurate K_{av} values. Rough calculations show, however, that most of his data correspond to those of earlier authors and agree closely with the theoretical curves of Fig. 3. This is also the case for hemoglobin and ovomucoid, two proteins which according to WHITAKER behaved anomalously on the columns.

The dextran fractions deviate from the theoretical functions, as can be seen in Fig. 5. This is, however, to be expected. GRANATH AND FLODIN[19] chromatographed a polydisperse dextran sample on dextran gels. They determined the molecular weights of fractions recovered at certain elution volumes. These fractions are, however, also polydisperse. A fraction eluted before the maximum of the peak is contaminated to a greater degree with low molecular weight than high molecular weight material. Conversely, a fraction which is eluted after the maximum contains proportionately more high molecular weight contaminating material. The radii calculated from the molecular weight values should therefore deviate in a way similar to what is the case in Fig. 5 from that of a monodisperse sample eluted at the same volume. This is also the reason why only the main fractions obtained by GRANATH AND FLODIN have been used for the calculations.

The observation that the exclusion effect determines the position in the gel chromatogram where a solute is eluted, opens up the possibility of treating the dynamics of gel filtration. In a subsequent contribution, we intend to show how a solute zone changes during the chromatographic process if the initial zone width, the flow rate and the rate at which the solute attains equilibrium between the liquid phase and the gel phase are known.

An important observation was made during this study. LAURENT[13,14] studied the solubilities of various proteins in the presence of dextran solutions. From the decrease in solubility the volume available for, *e.g.*, serum albumin, γ-globulin, cyanomethemoglobin and fibrinogen was determined. The values obtained in 4.9 % and 9.7 % (extrapolated) dextran of a mol. wt. of $5 \cdot 10^5$ are shown in Table IV. The K_{av} values from chromatography on Sephadex G-200 and G-100, which contain these concentrations of polymer in the gel grains, are also given in this table. In the case of gel filtration, the value of oxyhemoglobin is given instead of that of cyanomethemoglobin. Taking into account the experimental errors in the various determinations, there is good agreement between the available volumes in solution and in the gel,

TABLE IV

COMPARISON BETWEEN AVAILABLE VOLUMES OF FOUR PROTEINS IN DEXTRAN SOLUTIONS AND DEXTRAN GELS

Protein	*Available volume in*			
	4.9 % dextran		*9.7 % dextran*	
	solution	*gel*	*solution*	*gel*
Cyanomethemoglobin	0.42	—	0.25	—
Oxyhemoglobin	—	0.49	—	0.28
Serum albumin	0.40	0.41	0.19	0.19
γ-Globulin	0.26	0.21	—	—
Fibrinogen	0.11	0.02	—	—

with the exception of fibrinogen. In the latter case it should be noted that the molecular weight of the soluble dextran and the fibrinogen are of the same order and that the error in the determination of very low K_{av} values is larger than for higher values.

The results indicate that the cross-linking does not essentially change the exclusion properties of a certain concentration of dextran, at least not for moderately large proteins.

It is very probable that the unequal partition of macromolecules between two polymer phases, which has been demonstrated by ALBERTSSON[47] can partly be explained in terms of unequal exclusion properties of the two phases.

ACKNOWLEDGEMENTS

The authors are grateful to Drs. GELOTTE AND ANDREWS for providing copies of their manuscripts before publication, and to AB Pharmacia, Uppsala, for gifts of Sephadex and violet dextran. The investigation was supported by the Swedish Medical Research Council and the Swedisch Cancer Society.

SUMMARY

The separation by gel filtration of molecules varying in size is explained as a steric exclusion of solutes from the gel phase.

The volume available for a solute in the gel phase can be determined from the elution volume, the void volume and the total volume of the gel column. It has been calculated for a number of proteins and dextran fractions and for various dextran gels from data given in the literature as well as from some new data. The values were used to test the hypothesis that the exclusion takes place from a three-dimensional random network of straight polymer fibers distributed in the gel. The experimental data were found to verify the hypothesis.

The experimentally determined available volumes in the gel phase for three proteins are approximately the same as the available volumes in dextran solutions, having the same polymer concentrations as the gels. Therefore there seems to be no essential difference between the exclusion phenomenon in a polymer gel and in a polymer solution.

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