Journal of Chromatography, 292 (1984) 137–148
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,472

FUNDAMENTAL DIFFERENCES IN THE EQUILIBRIUM AND KINETIC PARAMETERS FOR PENETRATION OF GLOBULAR PROTEINS AND FLEXIBLE-CHAIN POLYMERS INTO PORES

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SUMMARY

The theory of the equilibrium distribution of macromolecules in pores based on separate consideration of conformational and steric exclusion factors has been developed and confirmed by experimental data. This theory permits a precise quantitative calculation of the distribution coefficients for flexible-chain polymers according to the data obtained for globular proteins, and allows in exclusion chromatography a unique calibration for macromolecules of various shapes. It is shown that the real structure of silica sorbents is described equally well by a model of randomly intersecting spheres and by that of an isoporous sorbent with equivalent numbers of cylindrical and slit-like pores. Also that the diffusion of globular proteins in pores as compared to that in the bulk solution is hindered to a much greater extent than for flexible-chain polymers, as manifested in the lower efficiency of steric exclusion liquid chromatography for proteins.

INTRODUCTION

Two parameters related to the penetration of macromolecules into pores are of fundamental importance in size exclusion chromatography (SEC) and membrane ultrafiltration of macromolecules, namely the distribution coefficient, K_d, characterizing the thermodynamic equilibrium between the phases and the diffusion coefficient in pores, D_s, which determines the extent of band spreading in SEC.

The theory of distribution in a porous medium and the relationship between K_d and the ratio of the radius of the macromolecule, R, to the average pore radius, $\bar{R}_p$, has been developed for rigid spherical macromolecules by Giddings et *al.*[1] and for flexible-chain polymers by Casassa[2]. However, it is not yet clear how to carry out universal calibration for macromolecules with different shapes, i.e., how to relate the calibration curves for K_d to the characteristic size of Gaussian coils or rigid spherical macromolecules. It should also be noted that the conformational theory of the equilibrium distribution of flexible-chain macromolecules in pores developed by Casassa does not ensure quantitative agreement with experimental data, in particular, at high ratios, I, of the radius of gyration of the macromolecule to the pore radius. At low

0021-9673/84/$03.00

A, agreement with experimental data has been obtained for an unrealistic geometry of the porous medium (slit-like pores). This discrepancy is usually attributed to errors in the mercury porosimetry. Correction of the pore radius" partly improves the situation, but such procedures are of somewhat artificial character. On the other hand, the theory of steric exclusion of spherical particles' has not been established by reliable experiments. Globular proteins can serve as a good model for spherical macromolecules, but until recently it has not been possible to carry out exclusion chromatography of proteins on sorbents with a known geometrical pore structure, *i.e.,* on rigid aerogels. The main obstacle to high-performance SEC of proteins is the high adsorption activity of silica sorbents and the difficulty of modifying them so as to ensure their inertness to adsorption of proteins. In recent years sorbents sufficiently inert for use with proteins have become available on the basis of a modified silica gel: they are the diol bonded phase[4] and TSK GEL SW[5].

With the appearance of this type of sorbents new possibilities become available for SEC. First, if a relationship between the exclusion behaviour of globular proteins and flexible-chain polymers is established, it is possible to calibrate chromatographic columns for globular or random-coil proteins and to use them in the analysis of other water-soluble polymers and, possibly, in the determination of molecular-weight distributions (MWDs) of many synthetic polymers using tetrahydrofuran (THF) as eluent. THF ensures the exclusion mechanism for separation on a sorbent with carbinol OH groups[6]. This would enable us to replace narrow-range fractionated polymer standards by monodisperse proteins. The Pharmacia dextrans used in aqueous SEC as standards have broad MWDs (M_w/M_n = 1.63 for T-10 and 2.32 for T-5000). Hence, to obtain reliable results they should be fractionated further by a very laborious procedure.

Secondly, experiments with high-speed SEC of proteins on diol bonded phase and, in particular, on TSK GEL SW have revealed (quite unexpectedly) that the efficiency of chromatography for rigid impermeable molecules of globular proteins on aerogels is much lower than that of SEC for flexible-chain polymers with the same

TABLE I

COMPARISON OF THE EFFICIENCY OF SEC FOR PROTEINS AND POLYSTYRENES ON VARIOUS SORBENTS, BASED ON SILICA

Sorbent	*Elution rate (cm/sec)*	*Reference substance*	K_d	N^* $(cm^{-1}\ min^{-1})$	V_p/V_0**	*Ref.*
TSK3000SW	0.033		0.61	2.9	1.35	8
TSK2000SW	0.033		0.35	1.4	0.95	8
Synchropack GPC-100	0.033	Ovalbumin (M = 45 10^3)	0.53	1.2	1.23	8
LiChrosorb-diol	0.033		0.33	0.8	0.63	8
μBondagel (linear)	0.07	PS (M = 111 10')	≈0.5	15	–	9
PSM-800	0.033	PS(M = 97.2 10^3)	≈0.4	44	0.89	7

• Efficiency (plate number) is normalized for column length and elution time of the chromatographic peak.

• * Ratio of pore volume to the interparticle volume.

equilibrium distribution coefficients. Table I presents a comparison of the efficiency of SEC for proteins and polystyrenes (PSs) on various sorbents based on silica (particle diameter, d_p, 10 μm) according to the data from refs. 7-9.

The low efficiency of SEC for proteins can also be seen from the following example. The separation of six polystyrene samples in the range of $M = 3.6 \cdot 10^3$–$7.1\,10^6$ on a column of total length 60 cm packed with microspherical silica gels PSM-50, PSM-300, PSM-800 and PSM-1500 (particle diameter 9 μm) was carried out in 15 min[7], whereas the separation of six globular proteins on a column with TSK GEL SW3000[10] of the same length and particle diameter required 50 min.

The possibility of an universal calibration of columns for SEC of proteins and flexible-chain macromolecules should be considered and the reasons for the low efficiency of SEC for globular proteins as compared to that for flexible macromolecules on sorbents with a silica framework should be elucidated. In this work we develop and compare with experimental data the theory of steric exclusion of macromolecules of various shapes, correlate the calibration dependences of globular proteins and flexible-chain polymers and compare the extent of band spreading for these two types of macromolecules.

EXPERIMENTAL

Chromatography was performed on a Model 334 Altex gradient liquid chromatograph using a Spherogel TSK SW 3000 (10 μm) pre-packed column (60 × 0.8 cm) from Altex and a LiChrosorb-diol (10 μm) pre-packed column (25 × 0.4 cm) from Merck.

Protein standards for calibration were obtained from Serva (MS-II Kit).

The elution buffer, 0.005 M K_2HPO_4, 0.005 M KH_2PO_4 and 0.2 A4 Na_2SO_4 (pH 6.8), was prepared from reagent grade salts. The total accessible column volume, V_t, was determined for sodium azide (reagent grade) and the interparticle volume, V_0, for influenza virus, which was a gift from the Institute of Analytical Instrument Making (U.S.S.R.).

The dependences of height equivalent to a theoretical plate (HETP) on the solvent velocity were obtained in the range 0.01-l cm sec^{-1} for the LiChrosorb-diol column.

RESULTS AND DISCUSSION

The equilibrium distribution of macromolecules in an inert porous medium depends on the restrictions imposed by the pore walls on the position of the centre of gravity, the conformation and orientation of the molecules as compared to those in the bulk solution'. The main difference between globular proteins and flexible-chain polymers lies in the fact that a globular protein has only one relatively stable conformation which in spite of some asymmetry may be regarded as a rigid impermeable sphere, whereas a Gaussian coil may be represented as a statistical assembly of interconvertible spherical conformations with different radii' [1].

Hence, only the steric exclusion factor is essential for globular proteins: the centre of gravity of the macromolecule cannot approach the pore wall closer than its radius. For flexible-chain macromolecules the conformational exclusion factor also

plays an important part: the pore dimensions limit the number of possible conformations as compared to that in the bulk solution. The theory of the equilibrium distribution of flexible-chain macromolecules in pores developed by Casassa[2] does not distinguish these two exclusion factors and hence cannot be used in SEC for developing a universal calibration for globular proteins and flexible-chain polymers. The present work describes a fundamentally new approach: a separate consideration of steric and conformational exclusion factors. This approach, together with the application of an adequate model for a porous medium, makes it possible to carry out precise quantitative calculations of the distribution coefficients of rigid spherical macromolecules and flexible chains and to establish the relationship between them (universal calibration principle).

For monodisperse spherical macromolecules on an isoporous sorbent the distribution coefficient, K_d, is related only to the steric factor[1]

$$K_{d_{St}}(R) = (1 - R/R_p)^n \tag{1}$$

where n = 1 and 2 for slit-like and cylindrical pores respectively. However, the modelling of the sorbent structure as an isoporous medium with a definite pore geometry (slits or cylinders) is far from real. As shown by electron microscopy, in real sorbents, pores of various geometries and various dimensions exist, although the pore size distribution can be relatively narrow as, for example, in macroporous glasses.

The porous structure of the most widely used silica sorbents may be described[12,13] by a random sphere model (RSM). Regardless of the method by which such gels are obtained, their matrices comprise spherical microglobules of hydrated SiO_2 with various extents of interpenetration. The RSM takes into account the pore size distribution and yields[3] the following expression for K_d in a purely steric exclusion mechanism

$$K_{d_{St}}(R) = \psi^{(1 + R/R_s)^3 - 1} \tag{2}$$

where ψ is the sorbent porosity (fraction of pore volume) and R_s is the radius of an elementary SiO_2 sphere in the aerogel matrix. In this case the following relationship exists between the mean pore radius, $\bar{R}_p$, and the radius of an elementary sphere[13]:

$$\bar{R}_p/R_s = -\frac{2}{3}\frac{(1.32 - \psi)}{\ln \psi} \tag{3}$$

It might be expected that the distribution coefficients of globular proteins on modified silica sorbents would obey eqn. 2.

In accordance with ref. 11, we will consider a Gaussian coil as a statistical assembly of conformations instantaneously passing into each other and having a distribution density according to the radii, f(R)

$$f(R) = 3\sqrt{\frac{6}{\pi}}\,\bar{R}_g^3\,R^2 \exp\left(-\frac{3}{2}R^2/\bar{R}_g^2\right) \tag{4}$$

where $\bar{R}_g$ is the mean-square radius of gyration. It is assumed that the polymer is

monodisperse and all its chains are of the same length but can coil to different extents. Each conformation is represented by an impermeable sphere, which is close to reality for the O-solvent as defioned by Flory[30]. The assembly is normalized:

$$\int_0^\infty f(R)dR = 1 \tag{5}$$

The main concept of this theory for K_d may be formulated as follows: for globular proteins (monodisperse spheres), K_d is described by eqns. 1 and 2 and is related only to steric exclusion, whereas for flexible-chain (fc) macromolecules K_d should be averaged over the assembly given by eqn. 4. Hence, we have:

$$K_{d_{fc}} = \int_0^{R_p} K_{d_{St}}(R)f(R)dR \tag{6}$$

Moreover, depending on the model for the porous medium, $K_{d_{St}}$ is described either by eqn. 1 or by eqn. 2.

For slit-like pores ($n = 1$):

$$K_{d_{fc}} = \mathrm{erf}(\sqrt{1.5/\lambda^2}) + 2\lambda\sqrt{2/3\pi}(e^{-1.5/\lambda^2} - 1) \tag{7}$$

For cylindrical pores ($n = 2$)

$$K_{d_{fc}} = (\lambda^2 + 1)\ \mathrm{erf}(\sqrt{1.5/\lambda^2}) + (4\sqrt{2/3\pi})\lambda e^{-1.5/\lambda^2} - 4\lambda\sqrt{2/3\pi} \tag{8}$$

where

$$\mathrm{erf}(x) = \int_0^x e^{-t^2}\,dt; \quad \lambda = \bar{R}_g/\bar{R}_p$$

In order to compare the theoretical predictions with experimental data we selected those papers in which apart from narrow pore distributions, reliable determinations of K_d, the mean pore radius of the sorbent and the size of narrow-disperse macromolecules used as standards were reported. Fig. 1 shows a comparison of the experimental[14–16] dependence $K_d(\lambda)_{fc}$ and the Casassa theoretical curves and the theoretical curves calculated from eqns. 7 and 8. Evidently, eqns. 7 and 8 are in better agreement with the experimental data than Casassa's conformational theory. An almost exact agreement was obtained for a sorbent containing equal quantities of slit-like and cylindrical pores:

$$K_d = 1/2(K_{d\ cyl} + K_{d\ slit})$$

In the framework of the RSM, the distribution coefficient of flexible-chain macro-

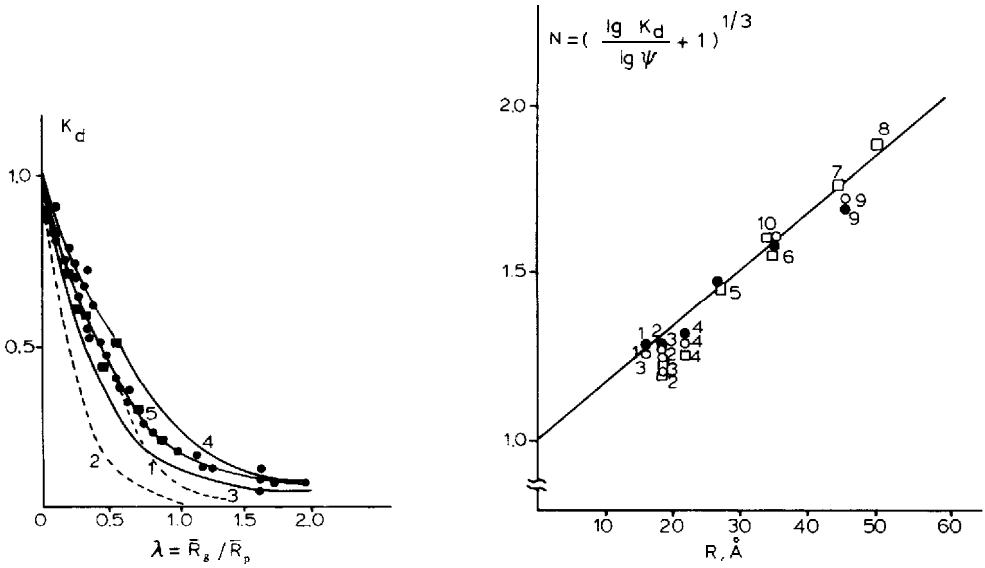

Fig. 1. Comparison of experimental and theoretical dependences of $K_d(\lambda)$ for flexible-chain polymers in SEC on porous glass. ●, Experimental points for dextrans[16]; ■, experimental points for polystyrenes[14,15]; 1 and 2, Casassa theoretical curves for slit-like (1) and cylindrical (2) pores; 3 and 4, according to eqns. 7 and 8; 5. according to eqns. 7, 8 for equivalent amounts of cylindrical and slit-like pores.

Fig. 2. Test of RSM (eqn. 2) for globular proteins on TSK GEL SW 3000, ψ = 0.738; 1 = cytochrome C; 2 = ribonuclease; 3 = myoglobin; 4 = chymotrypsinogen A; 5 = ovalbumin; 6 = bovine serum albumin (BSA); 7 = BSA dimer; 8 = BSA trimer; 9 = aldolase; 10 = ovalbumin dimer. Data from refs. 5 (0), 17 (□) and the authors (●).

molecules can be obtained as follows:

$$K_{d_{fc}} = \int_0^\infty \psi^{(1 + R/R_s)^3 - 1} f(R)dR \qquad (9)$$

It is noteworthy that when the RSM is used $K_{d_{sl}}$ does not become zero even at high R/R_s ratios but tends asymptotically to zero. Hence, infinity may be used as the upper limit of integration. The integral in eqn. 9 cannot be solved analytically, but may be approximated.

To be able to compare the equilibrium distribution of proteins and flexible-chain polymers in pores all the experiments should be carried out on a single sorbent. Otherwise the values of ψ, R_s and $\bar{R}_p$ should be known, which are not usually published in chromatographic papers. Recently, the Toyo Soda Corp. (Japan) has developed a non-swelling aerogel sorbent TSK GEL SW based on silica for the SEC of proteins. The chemical nature of this sorbent is not stated but it is known to be based on silica gel the surface of which is modified by a hydrophilic polymer to make it inert to proteins, Many papers have been published on the SEC of globular proteins[5,17] and flexible-chain polymers, polyethylene glycol (PEG)[10] and denatured proteins in 6 *M* guanidinehydrochloride[18], on this sorbent. It is noteworthy that proteins in 6 *M* guanidine hydrochloride behave as flexible-chain polymers[19].

Since all the structural parameters of TSK GEL SW 3000 are not known ($\psi = 0.738$[8]; no data on R_s and $\bar{R}_p$), we analyzed data on the K_d of globular proteins[5,17] and their agreement with the RSM. Fig. 2 shows the dependence of $N = (\log K_d/\log \psi + 1)^{1/3}$ on the Stokes radii of proteins according to the results reported in refs. 5 and 17 and our own data. According to the RSM, this dependence should be linear with an intercept of 1 and a slope of $1/R_s$. Among the proteins investigated in refs. 5, 17, those selected for plotting in Fig. 2 had reliable values of the Stokes radii available in the literature[20,21]. For aggregated proteins (points 7, 8 and 10) the Stokes radii were determined according to the model for the confluence of several spheres into one sphere, *i.e.*

$$R_{\text{aggregate}} = R_{\text{monomer}} \sqrt[3]{n}$$

where n is the degree of association. Fig. 2 shows that the RSM adequately describes the equilibrium distribution of proteins (rigid spheres) on TSK GEL SW and the slope of the straight line allows the determination of $R_s = 59.5$ Å and according to eqn. 3, $\bar{R}_p = 76$ Å. Hence, the porous structure of TSK GEL SW 3000 was characterized in the framework of RSM by using globular proteins as reference substances.

Next the equilibrium distribution of denatured proteins and PEG on this sorbent will be analyzed, *i.e.*, the conformational factor for these flexible-chain (fc) polymers will be taken into account. The theoretical curve $K_{d_{fc}} = f(\lambda)$ was calculated according to eqn. 9 by the method of successive iterations (according to the trapezium equation) using the above values of ψ, $\bar{R}_p$ and R_s determined with the aid of globular proteins. This curve is shown in Fig. 3 with experimental data from refs. 10, 18. The values of ii, were calculated from the molecular weight by using the following equations:

$$\bar{R}_g = 0.23\ \bar{M}_w^{0.555} \quad \text{for denatured proteins} \tag{10}$$

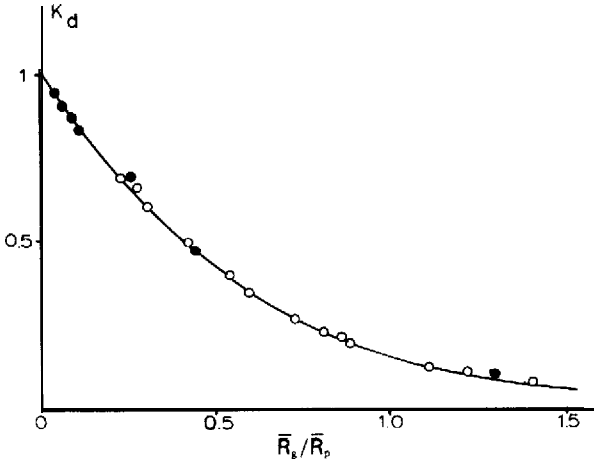

Fig. 3. Comparison of experimental and theoretical dependences of $K_{d_{fc}}(\lambda)$ obtained for flexible-chain polymers on TSK GEL SW 3000 according to the RSM: 0, experimental points for denatured proteins"; ●, experimental points for PEG[10].

$$\bar{R}_g = 0.17\ \bar{M}_w^{0.593} \text{ for PEG, } \bar{M}_w > 3.\ 10^3 \quad (11)$$

$$\bar{R}_g = 0.33\ \bar{M}_w^{0.5} \quad (12)$$

for PEG at $\bar{M}_w < 3 \cdot 10^3$

$$\bar{R}_g = 0.103\ \bar{M}_w^{0.429} \quad (13)$$

Eqns. 10–13 were obtained from the Mark-Kuhn constants reported in refs. 19, 22 with the aid of traditional conversion using the Flory constant and taking into account the thermodynamic strength of the solvent

$$[\eta]\bar{M}_w = \varphi_0\ (1 - 2.63\varepsilon + 2.86\varepsilon^2)\ (\sqrt{6}\ \bar{R}_g)^3 \quad (14)$$

where $\varphi_0 = 2.86 \cdot 10^{25}$ is the Flory universal constant, $\varepsilon = (2a-1)/3$ is the parameter of solvent strength, a is the exponent in the Mark-Kuhn equation, $[\eta] = kM^a$ where $[\eta]$ is the intrinsic viscosity and k is the empirical constant. Since, for low-molecular-weight PEG, eqns. 12 and 13 give different results, the average value of $\bar{R}_g$ obtained from both equations was used.

The results plotted in Fig. 3 show exact agreement between the experimental data on the $K_{d_{fc}}$ of flexible-chain polymers and the theoretical curve calculated from eqn. 9 according to the RSM on the basis of sorbent porosimetry carried out by use of globular proteins. Thus an universal calibration of TSK GEL SW 3000 for globular proteins and flexible-chain polymers is achieved. It is possible to determine the chromatographic radius of an equivalent sphere for a flexible-chain polymer, R_e, from eqns. 2 and 9. By R_e *we* mean the radius of a sphere which may be represented by an assembly of conformations (eqn. 4) so as to obtain at a given pore geometry the same value of $K_{d_{fc}}$ as for purely steric exclusion. In other words, it is necessary to solve for R the equation:

$$K_{d_{fc}} = \int_0^\infty \psi^{(1 + R/R_s)^3 - 1} f(R)dR = \psi^{(1 + R_e/R_s)^3 - 1} \quad (15)$$

Fig. 4 shows the dependence of $R_e/\bar{R}_p$ on $\bar{R}_g/\bar{R}_p$ for various models of the porous medium. It is clear that the chromatographic radius of an equivalent sphere of Gaussian coils depends not only on the radius of gyration but also on the pore radius. If in the measurements of diffusion coefficient or intrinsic viscosity we have $R_e = k\bar{R}_g$, then in SEC the value of k depends on $\bar{R}_g/\bar{R}_p$.

It is noteworthy that the theoretical dependence of $K_{d_{fc}}$ on λ according to the RSM shown in Fig. 3 is in good agreement with a similar dependence in Fig. 1 obtained for an isoporous sorbent with equal numbers of slit-like and cylindrical pores. In order to check whether this coincidence is accidental or the real pore geometry of a silica sorbent actually corresponds to an intermediate shape between cylinders and slits, this dependence was calculated according to the RSM at varying porosities, $\psi = 0.4$–0.75. This range exceeds that of the ψ values for most porous glasses and silica gels. Fig. 5 shows the results of these calculations. When the RSM

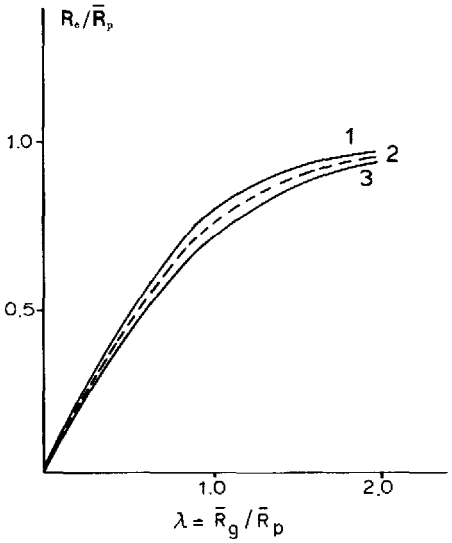

Fig. 4. Dependence of chromatographic radius of an equivalent sphere, R_e, *vs.* radius of gyration for slit-like pores (1), RSM (2) and cylindrical pores (3).

is used, the type of the dependence of K_d on λ is virtually independent of the value of ψ for $0.5 \leqslant \psi \leqslant 0.8$ and this dependence is identical with curve 5 in Fig. 1. Hence, both models; RSM and an isoporous sorbent with equivalent quantities of slit-like and cylindrical pores, describe equally well the penetration of macromolecules into the pores of a sorbent with narrow pore size distribution. However, the RSM is preferred because it ensures a more precise result for silica gels whose pore size distribution is not narrow.

We now consider the reasons for the low efficiency of high-speed SEC of globular proteins as compared to that of flexible-chain polymers. At high elution rates

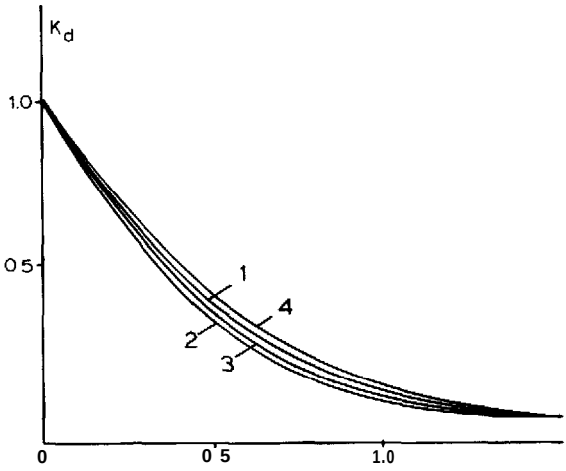

Fig. 5. Theoretical dependence of $K_{df\delta}(\lambda)$ for a flexible-chain polymer on sorbents with different porosities. 1, Model for an isoporous sorbent with equivalent amounts of slit-like and cylindrical pores; 2–4, RSM, ψ = 0.42, 0.5 and 0.75 respectively.

U, the main contribution to the HETP is provided by intradiffusion mass transfer[23]

$$H = A + \frac{1}{30} \cdot \frac{d_p^2 U}{\bar{a}} \cdot \frac{\varphi K_d}{(1 + \varphi K_d)^2} \quad (16)$$

where A is the contribution of external mass transfer at high U, d_p is the diameter of the sorbent grains, $\bar{D}_s$ is the diffusion coefficient in the sorbent and $\varphi = V_p/V_o$.

The diffusion of macromolecules is hindered in comparison to that in the bulk solution. There are at least three reasons for this; first, the tortuosity, τ, of the diffusion path in the pore volume; secondly, the above mentioned steric factor and finally, the hydrodynamic effect of interaction with the pore walls.

For rigid impermeable spherical particles the internal diffusion coefficient is given by[24]

$$\bar{D}_s = \frac{\psi D_0}{\tau} K(R) \quad (17)$$

where $D_0 = KT/6\pi\eta R$ is the diffusion coefficient in solution, η is the solvent viscosity, and $K(R)$ is the hydrodynamic hindrance. The most precise equation for $K(R)$ is valid for the axial motion of spherical particles in cylindrical pores (the so-called central approximation[25]:

$$K(R) = \frac{1 - 2.1\lambda + 2.1\lambda^3 - 1.7\ \lambda^5 + 0.73\ \lambda^6}{1 - 0.76\ \lambda^5} \quad (18)$$

Eqn. 17 containing $K(R)$ in the form of eqn. 18 should be obeyed for globular proteins, although there is little experimental evidence to confirm this.

To test the foregoing theory of diffusion at hindrance of spherical particles in pores, the internal diffusion coefficients of globular proteins were determined by chromatography on LiChrosorb-diol. The value of $\bar{D}_s$ was calculated according to eqn. 16 from the linear part of the dependence of HETP at high elution rates. The tortuosity factor (τ = 2.0) was determined from the condition $\tau\bar{D}_s/\psi D_0 = 1$ for a low-molecular-weight reference substance (sodium azide), and the pore radius $\bar{R}_p$ = 80 Å was obtained from a calibration carried out according to the cylindrical pore model (Fig, 6). The porosity ψ = 0.47 was determined from $\psi = (V_t - V_0)/(V_c - V_0)$ where V_t and V_0 are the elution volumes of sodium azide and influenza virus, respectively. The results are shown in Fig. 7 (curve 1). It is clear that even at $\lambda = 0.6$ the internal diffusion coefficient of globular proteins is lower by at least one order of magnitude than in the bulk solution.

The conformational factor may be taken into account in the calculation of the diffusion coefficient of a flexible-chain polymer by analogy with the calculation of $K_{d_{fc}}$. Since sorbent pores act as a filter for conformations exceeding a certain size, it is necessary to know the density of distribution function of conformations of a flexible-chain polymer in a pore, $f^*(R)$. This function is found from the condition:

$$K_{d_{fc}} = \int_0^{R_p} K_{d_{St}}(R) f(R) dR \equiv \int_0^{R_p} f^*(R) dR \quad (19)$$

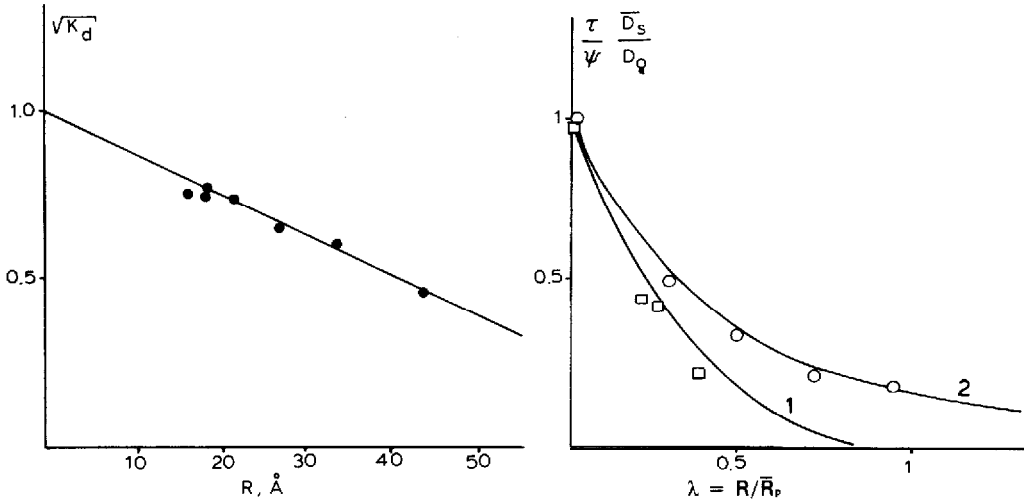

Fig. 6. Calibration of a 25 × 0.4 cm column packed with LiChrosorb-diol for globular proteins by using a cylindrical pore model.

Fig. 7. Hindrance factor of diffusion in pores $(\tau/\psi)(D_s/D_0)$, *vs.* the ratio of the sizes of the macromolecule and the pore, λ; 1, rigid spherical particles (globular proteins; solid line is theoretical curve according to eqn. 18; 2, flexible-chain macromolecules (solid line is theoretical curve according to eqn. 2 1. In all cases λ was determined according to the Stokes radius for globular proteins and according to the mean-square radius, $\bar{R}_g$, for flexible-chain polymers.

Hence, we have:

$$f^*(R) \equiv K_{d_{St}}(R) f(R) \tag{20}$$

Now the value of $\bar{D}_s$ determined by eqn. 17 will be averaged over the assembly of conformations (20):

$$\bar{D}_{s_{fc}} = \frac{\psi}{\tau} \int_0^{R_p} D_0(R)\, K(R)\, K_{d_{St}}(R) f(R) dR \tag{21}$$

When $\bar{D}_{s_{fc}}$ was calculated approximately according to eqn. 21, the expression for $K_{d_{st}} = (1 - \lambda)^2$ valid for cylindrical pores and the hindrance function K(R) given by eqn. 18 were used. The theoretical curve is shown in Fig. 7 (curve 2). To test the results, data from the only reliable paper on the experimental determination of the internal diffusion coefficients of polystyrenes[26] on Porasil silica gel were used. In this work reliable data on the porosimetry of the sorbent are reported, values of τ and ψ are determined and the intra-diffusion character of mass transfer is ensured. Other papers[21,28] in this field describe a chromatographic experiment at moderate elution rates, $U < 0.5$ cm sec^{-1}, and it is doubtful whether eqn. 16 may be used because the contribution of external-diffusion mass transfer depends on U.

Although the model of cylindrical pores is approximate, Fig. 7 (curve 2) dem-

onstrates the very good agreement between the experimental and theoretical data on $\bar{D}_s$. The results indicate that the diffusion of flexible-chain macromolecules in pores is hindered to a much lesser extent than that of globular proteins. This effect is due to the random-type distribution of the conformations of a flexible-chain polymer. A part of these conformations can acquire the size $R > \bar{R}_p$ in a pore only by extending along the pore and hence losing its mobility.

When $\lambda = \bar{R}_g/\bar{R}_p$ increases, the value of $\bar{D}_s$ (for a flexible-chain polymer) decreases according to the Stokes-Einstein law. In this case, however, the mobility in pores is retained only for strongly coiled conformations for which $R < \bar{R}_p$.

This result explains the experimentally observed higher efficiency of SEC for flexible-chain polymers. It may be concluded that high-speed SEC of proteins requires considerable optimization with respect to the parameters of the porous structure of the sorbent (porosity and pore radius). Although high SEC selectivity is attained at relatively low $\bar{R}_p$ values, it may be desirable to use a sorbent with larger pores to increase the efficiency of the process. The increase in $\bar{R}_p$ is particularly important at $K_d = 0.15$ when the efficiency of SEC is at a minimum[8,29]. This K_d value is obtained for globular proteins at $\lambda = 0.73$ and for flexible-chain molecules at $\lambda = 1$.

REFERENCES

1 J. C. Giddings, E. Kucera, C. P. Russel and M. N. Mexers, *J. Phys.* Chem., 73 (1968) 43974402.
2 E. Casassa, *J. Polym. Sci., Part B-5,* (1967) 773-778.
3 A. A. Gorbunov and A. M. Skvortsov, *Vysokomol. Soedin., Ser. A* (1980) 1137-l 145.
4 F. Regnier and R. Neel, *J. Chromatogr. Sci.,* 14 (1976) 316320.
5 S. Rokushika, T. Ohkava and H. Hatano, *J.* Chromatogr., 176 (1979) 456461.
6 J. Borák and M. Smrž, *J.* Chromarogr., 144 (1977) 57-62.
7 J. J. Kirkland, *J.* Chromatogr., 125 (1976) 231–250.
8 E. Pfannkohk, K. C. Lu, F. E. Regnier and H. G. Barth, *J.* Chromatogr., Sci., 18 (1980) 430–441.
9 R. V. Vivilechia, B. G. Lightbody, N. Z. Thimot and H. M. Quinn, *J.* Chromatogr. Sci., 15 (1977) 424433.
10 J. Kato, K. Komiya, H. Sasaki and T. Hashimoto, *J.* Chromarogr., 190 (1980) 297–303.
11 B. G. Belenkii and L. Z. Vilenchik, *Khromatografiya polimerov,* Khimiya, Moscow, 1978, p. 103.
12 W. Haller, *J. Chem. Phys., 42 (1965) 686-693.*
13 M. E. van Kreveld and N. van den Hoed, *J. Chromatogr., 83 (1973)* 111-124.
14 J. C. Moore and M. C. Arrington, *Int. Symp. Macromol. Chem., Tokyo, 1966,* Preprint 11-107.
15 W. Yau, C. Malone and H. Suchan, *Separ. Sci.. 5 (1970)* 259–271.
16 W. Haller, *Macromolecules,* 10 (1974) 83-86.
17 M. E. Himmel and P. 0. Squire, *Int. J. Pept. Protein Res., 17 (1981) 365-373.*
18 N. Ui, *Anal. Biochem., 97 (1979) 65-71.*
19 C. Tanford, *Advan. Protein* Chem., 23 (1979) 122-275.
20 P. Andrews, *Biochem. J., 96 (1965)* 595–606.
21 L. M. Siegel and K. J. Monty, *Biochim. Biophys. Acra,* 112 (1966) 346.
22 R. A. Briggs and E. E. Gruber, in *Encyclopedia of Polymer Sci., Technol.,* Vol. 6, Wiley, New York, 1967, pp. 103-209.
23 J. C. Giddings, *Dynamics of Chromatography,* Marcel Dekker, New York, 1965, p. 320.
24 C. N. Sattertield, C. K. Colton and W. H. Pitcher, *AIChE J., 18 (1973) 628-631.*
25 W. L. Haberman and R. M. Sayre, *David Taylor Model Basin Report, No.* 1143, U.S. Navy Dept., Washington, DC, 1958.
26 M. E. van Kreveld and N. van den Hoed, *J. Chromatogr., 149 (1978) 71-76.*
27 J. Klein and M. Grunberg, Macromolecules, 14 (1981) 1411-1415.
28 J. H. Knox and F. Mc. Lennan, *J. Chromatogr., 185 (1979) 289-304.*
29 L. H. Tung and J. R. Runyon, *J. Appl.* Polym. Sci., 13 (1969) 2397–2409.
30 P. J. Flory, *Principles of Polymer Chemistry,* Cornell University Press, Ithaca, NY, 1953, pp. 523, 612.