Experimental Studies of Restricted Protein Diffusion in an Agarose Matrix

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The diffusion of proteins in polymer matrices is an important step in the adsorptive and chromatographic processes used for protein purification, as it is often rate-limiting. Methods for the estimation of the intraparticle diffusivity in polymer gels have been developed and were applied to the diffusion of seven model proteins in agarose particles. The intraparticle diffusivity was not affected by particle diameter. A correlation based on the restricted diffusion model of Ogston et al. (1973) and Cukier (1984) has been proposed. This correlation allows the estimation of protein diffusivity in these matrices based on the molecular weight of the protein and the polymer concentration.

Introduction

The purification of therapeutic proteins from either natural sources or culture fluids often represents the major cost of their production. Adsorptive and chromatographic separations are the most widely used techniques in these processes; hence, there has been great interest in modeling these processes that enables biochemical engineers to design optimized purification schemes for these high-value biological products (Chase, 1984). The success of these efforts, however, has been limited in part by the difficulty in determining the kinetic parameters employed in these models (Chase, 1987).

The adsorbent matrices used in biopharmaceutical production must be able to withstand repeated cleaning and sterilization cycles that often include washing with 1-M sodium hydroxide as a depyrogenation step. Due to its great chemical stability, beaded agarose is one of the most widely used support matrices in these processes. Agarose supports also have an open internal structure that allows the penetration of large proteins and consequently, high-protein binding capacity in affinity adsorbents. Another advantage over other matrices is the extremely low occurrence of nonspecific protein binding on the agarose matrix itself. These properties have established agarose as the matrix of choice for many large-scale adsorption processes, despite its compressibility (Hodgson, 1990).

One of the major concerns in the application of agarose adsorbents is the slow diffusion of proteins and other biomolecules inside the particle. In adsorption kinetics studies, it has been reported that diffusion of proteins in the agarose matrix is rate-limiting in both ion exchange and biospecific protein adsorption processes (Horstmann and Chase, 1989; Skidmore et al., 1990). Therefore, determination of the intraparticle diffusivity is a very important step in the characterization of any adsorption processes employing agarose-based adsorbents.

Several methods have been described to estimate the intraparticle diffusivity of proteins in porous matrices. A classical approach, described by Crank (1975), involves monitoring the uptake of solute by spherical particles in a finite bath. Although this method has been applied extensively to study diffusion in systems of immobilized enzymes or cells, its use in chromatographic systems is limited due to the small size of the sorbent particles, which results in very fast approach to equilibrium (usually less than one minute). Similar procedures employ either a thin disk or a cylinder of the matrix in contact with a well-stirred solution containing the solute of interest (Itamunoala, 1987). Other more recent methods of measuring restricted diffusion in polymer matrices and solutions involve either light scattering techniques (Phillies, 1989; Lodge et al., 1989) or fluorescence recovery after photo bleaching (Hou et al., 1990). These methods, however, are not applicable to the problem of restricted diffusion in agarose gels due to the fact that these gels are supplied in beaded form for chromatographic use. Consequently, diffusion in these particles is usually studied by fixed bed methods. Arnold et al. (1985) estimated protein diffusivity in agarose particles by applying the height of an equiv-

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alent transfer plate (HETP) analysis to the pulse responses of proteins from agarose columns. Davies (1989) has described another method based on frontal column operation, which involves estimation of intraparticle diffusivity by comparison of the holdback area of the experimental breakthrough curve with values predicted by a rate model. More recently, Ernst and Hsu (1991) have shown that kinetic parameters can be evaluated by direct comparison of the experimental data with the time domain solution of the rate model obtained by the fast Fourier transform (FFT) inversion of the Laplace solution. This method can be used with either frontal or zonal analysis of the agarose column.

The drawback with all of these methods is that several experimental runs are required to adequately characterize the system and evaluate intraparticle diffusivity. Another problem is that the protein sample must be pure if the detection method is not specific for that particular protein. Consequently, these methods could prove to be time-consuming, tedious and expensive, especially if the protein is difficult or costly to isolate. For many engineering calculations, an approximate value of intraparticle diffusivity may be sufficient; thus it is clear that a quick, inexpensive method of estimating the intraparticle diffusivity of proteins in these agarose adsorbents is needed.

Each of the methods described above makes the assumption that the adsorbent particles are monodisperse; however, commercially available agarose usually exhibits a moderately wide particle size distribution. Several rate models describing fixed-bed adsorption have recently been presented which account for the particle size distribution of the adsorbent. Carta and Bauer (1990) considered the simplest case where the intraparticle diffusivity and other parameters were assumed to have a constant value that was independent of particle size. Chen and Hsu (1990) and Rasmuson (1985a) have developed more complex models in which intraparticle diffusivity and other parameters may vary with particle size. The results of these studies suggest that for the case of constant intraparticle diffusivity, adsorbents that have a Gaussian particle size distribution with a moderate standard deviation can be modeled adequately using the average particle radius in a monodisperse model. In more complex cases where the intraparticle diffusivity varies with particle size, this simplification cannot be made. Agarose has been shown to have a Gaussian particle size distribution; however, the intraparticle diffusivities of proteins in agarose beads of different size have not been investigated. Hence, the first objective of this study is to determine intraparticle diffusivity on particles of different size. These results should indicate whether simplified kinetic models based on average particle size is a valid approach for simulation of adsorption processes utilizing agarose particles.

Another aspect of this study is to identify the effect of protein size on its diffusivity in an agarose matrix. The extent of diffusion restriction of small molecular weight solutes in polymer solutions and gels has been found to be independent of the solute size (Navari et al., 1971). Davies (1989) in his study on the intraparticle diffusion of two proteins in a beaded agarose gel, however, found that the diffusion of the larger protein was retarded more severely by the matrix than that of the smaller protein. This result suggests that for these large solutes, molecular size can be expected to affect intraparticle diffusivity not only through its effects on solution diffusivity, but also by the mechanism of hindered diffusion inside the agarose matrix. Therefore, it is clear that any attempt to predict the intraparticle diffusivity of a protein must take into account both of these effects. The development of a correlation that relates intraparticle diffusivity in an agarose matrix with protein size appears to be feasible and constitutes the second objective of this study.

The first step in the development of the correlation is to consider the physical process of protein diffusion inside agarose particles. The interior structure of an agarose particle differs greatly from that of other commonly employed porous matrices such as silica, which have a rigid pore structure of known dimension. The structure of agarose and other polysaccharide gels has been described as a suspension of randomly oriented fibers (Laurent, 1967), similar to that of a polymer solution. The investigation of hindered transport of molecules in rigid pores has received considerable attention recently, as shown by Deen (1987) in his recent review. Theoretical studies of hindered diffusion of large solutes in polymer matrices, on the other hand, have been limited (Ogston et al., 1973; Cukier, 1984; Altenberger and Tirrell, 1984; Phillies, 1989). Early experimental studies showed that the diffusion of proteins in these gels empirically obeyed the expression of Eq. 1 (Laurent, 1967). Both Ogston et al. (1973) and Cukier (1984) have derived theoretical models describing restricted diffusion in polymer solutions. Although these two models were based on different mechanisms for restricted diffusion, both led to expressions of the form:

$$\frac{D_e}{D_0} = \exp(-Br_0 c_f^{1/2}) \tag{1}$$

In this expression, D_e is the effective intraparticle diffusivity, D_0 is the solution diffusivity, r_0 is the interaction radius between the protein and the polymer fibers, c_f is the polymer fiber concentration, and B is a proportionality constant. It is noted that the form of this equation corresponds to that of Phillies' "universal scaling equation for polymer self-diffusion" (Phillies, 1989). Equation 1 will serve as the starting point for the correlation. The intraparticle diffusivity of several proteins in an agarose matrix will be evaluated, and these data will be used as the basis for the correlation.

Chromatography Model and Analytical Methods

Zonal chromatography model

Consider a fixed bed of length L that consists of spherical agarose particles with an average radius R. It is assumed that the liquid flow in the column is axially dispersed, and the mass transfer processes occurring include external film diffusion and intraparticle diffusion. As previously mentioned, protein adsorption in unsubstituted agarose matrices is negligible. At time zero, a pulse of solute is introduced at the inlet of the fixed bed which was initially free of solute. Such a system can be described by the following set of equations.

Material balance on the mobile phase:

$$\frac{\partial C}{\partial t} + V\frac{\partial C}{\partial z} - D_L\frac{\partial^2 C}{\partial z^2} = \frac{-3k_F}{mR}(C - C_p|_{r=R}) \tag{2}$$

Intraparticle material balance:

$$\epsilon_p \frac{\partial C_p}{\partial t} = D_e \left(\frac{\partial^2 C_p}{\partial r^2} + \frac{2}{r} \frac{\partial C_p}{\partial r} \right) \tag{3}$$

Initial and boundary conditions:

$$C(z,0) = 0 \tag{4}$$

$$C(0,t) = \begin{cases} C_0 & 0 \le t \le t_0 \\ 0 & t_0 < t \end{cases} \tag{5}$$

$$C(\infty,t) = 0 \tag{6}$$

$$C_p(r,z,0) = 0 \tag{7}$$

$$C_p(0,z,t) \neq \infty \tag{8}$$

$$k_F(C - C_p|_{r=R}) = D_e \left. \frac{\partial C_p}{\partial r} \right|_{r=R} \tag{9}$$

In this set of equations the parameters are given as: C is solute concentration in the mobile phase, C_p is the solute concentration in the pores of the particle, C_0 is the pulse concentration, V is the mean interstitial mobile phase velocity, D_L is the axial dispersion coefficient, k_F is the film mass transfer coefficient, $m = \epsilon/(1-\epsilon)$ where ϵ is the column void fraction, ϵ_p is the intraparticle void fraction, R is the average radius of the particles, D_e is the effective intraparticle diffusivity, t_0 is the input pulse time, t is time, z is axial distance from column inlet, and r is the intraparticle radial position.

The set of Eqs. 2 to 9 can be solved by Laplace transformation. The Laplace domain solution for the mobile-phase concentration at a point z in the column is shown as follows.

$$\overline{C}(z,s) = C_0 \exp\left[\left(\frac{V}{2D_L} - \sqrt{\left(\frac{V}{2D_L} \right)^2 + \frac{s}{D_L} + \frac{3k_F}{mD_L R} \alpha(s)} \right) z \right] \tag{10}$$

The function $\alpha(s)$ in Eq. 10 is given by:

$$\alpha(s) = \frac{\sqrt{\frac{\epsilon_p s}{D_e}} \cosh \sqrt{\frac{\epsilon_p s}{D_e}} R - \frac{1}{R} \sinh \sqrt{\frac{\epsilon_p s}{D_e}} R}{\sqrt{\frac{\epsilon_p s}{D_e}} \cosh \sqrt{\frac{\epsilon_p s}{D_e}} R + \left(\frac{k_F}{D_e} - \frac{1}{R} \right) \sinh \sqrt{\frac{\epsilon_p s}{D_e}} R} \tag{11}$$

Inversion of this expression to obtain an analytical time domain solution is difficult, as it involves the evaluation of an oscillatory integrand which converges very slowly (Rasmuson, 1985b).

Moment analysis

The moment of the outlet peak, however, can be evaluated directly from Eq. 10 without inversion. Schneider and Smith (1968) have derived these expressions for the first absolute and second central moments of the exit peak for a chromatographic system similar to the one presented here. The moment for this gel filtration system are given by:

$$\mu_1 = \frac{\int_0^\infty C(L,t) t dt}{\int_0^\infty C(L,t) dt} = \frac{L}{V} \left(1 + \frac{\epsilon_p}{m} \right) + \frac{t_0}{2} \tag{12}$$

$$\mu_2 = \frac{\int_0^\infty C(L,t)(t-\mu_1)^2 dt}{\int_0^\infty C(L,t) dt} = \frac{2L}{V} \left[\frac{\epsilon_p^2 R^2}{15m} \left(\frac{1}{D_e} + \frac{5}{k_F R} \right) + \frac{D_L}{V^2} \left(1 + \frac{\epsilon_p}{m} \right)^2 \right] + \frac{t_0^2}{12} \tag{13}$$

Thus, by conducting pulse response experiments on a fixed bed of the matrix at different liquid velocities, the rate constants of the process may be estimated (Schneider and Smith, 1968). The moment method has been used before to estimate rate parameters from the chromatographic peaks obtained from gel filtration (Mehta et al., 1973; Suzuki, 1974; Nakanishi et al., 1977). These investigations, however, were concerned with axial dispersion and intraparticle diffusion of small solutes, rather than intraparticle protein diffusion. Application of second moment analysis usually involves the rearrangement of Eq. 13 to the following form:

$$\frac{\left(\mu_2 - \frac{t_0^2}{12} \right)}{\frac{2L}{V}} = \frac{\epsilon_p^2 R^2}{15m} \left(\frac{1}{D_e} + \frac{5}{k_F R} \right) + \frac{1}{V} \left[\frac{D_L}{V} \left(1 + \frac{\epsilon_p}{m} \right)^2 \right] \tag{14}$$

In the experimental conditions usually encountered in gel filtration, both the film transfer coefficient (k_F) and the quantity D_L/V are affected only slightly by the interstitial velocity in the column; therefore, the lefthand side of Eq. 14 will give a linear relation when plotted against inverse interstitial velocity ($1/V$) (Suzuki, 1974). The slope of this plot is related to the axial dispersion of the column and the intercept will depend on the intraparticle diffusivity and the external film diffusion. While this type of analysis is relatively simple and straightforward, it has the drawback of being very sensitive to peak tailing or baseline shifts that can occur experimentally (Anderssen and White, 1971).

HETP analysis

Arnold et al. (1985) found that the estimation of kinetic parameters by the HETP analysis of a chromatographic bed is affected only slightly by such experimental nonidealities. In this analytical method, the plate height, h, is given by:

$$h = \frac{\mu_2 L}{\mu_1^2} \tag{15}$$

Substitution of the expressions for the first and second moments (when the input pulse time, t_0, is negligible) given in Eqs. 12 and 13 yields the following expression for the theoretical plate height in terms of the system parameters.

$$h=\frac{2D_L}{V}+\frac{2\epsilon_p^2R^2}{15m(1+\epsilon_p/m)^2}\left(\frac{1}{D_e}+\frac{5}{k_FR}\right)V \tag{16}$$

This expression has the same form as the van Deemter equation in the case where the effects of molecular diffusion in the axial direction are negligible (Wankat, 1987). As mentioned previously, in gel filtration experiments the quantity D_L/V is essentially independent of flow rate; therefore, a plot of plate height vs. interstitial velocity should give a straight line. The slope of this line is related to the intraparticle diffusivity and the film transfer coefficient, and the intercept indicates the axial dispersion in the column.

In cases where the column is sufficiently long, the exit peaks will be Gaussian in shape. In these instances, the first moment will correspond to the retention time, which is the time at which the exit concentration is maximum ($\mu_1=t_m$), and the second moment will equal the square of the standard deviation of the outlet peak ($\mu_2=\sigma^2$). Hence, the plate height can be determined from the retention time and standard deviation of Gaussian outlet peaks according to the following relation:

$$h=\frac{\sigma^2L}{t_m^2} \tag{17}$$

Arnold et al. (1985) described a method by which the effects of film diffusion can be accounted for so that the intraparticle diffusion can be evaluated by this analysis. In this method, the contribution to HETP due to film transfer (h_F) is calculated using the correlated values of the film transfer coefficient as shown in Eq. 18. Finally, as shown in Eq. 19, the difference of the observed HETP and the film HETP will have a slope which is inversely proportional to the intraparticle diffusivity of the protein. This method is shown in Figure 1 for a typical set of data.

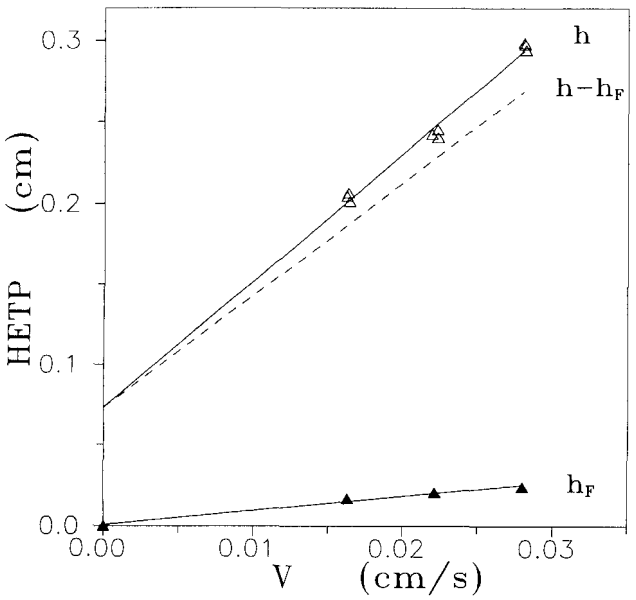

Figure 1. Procedure for evaluation of intraparticle diffusivity by HETP analysis.

$$h_F=\frac{2\epsilon_p^2RV}{3mk_F(1+\epsilon_p/m)^2} \tag{18}$$

$$h-h_F=\frac{2D_L}{V}+\frac{2\epsilon_p^2R^2V}{15mD_e(1+\epsilon_p/m)^2} \tag{19}$$

FFT analysis

Hsu and Dranoff (1987) introduced the use of the fast Fourier transform (FFT) to invert certain Laplace transforms that are of interest to chemical engineers. Chen and Hsu (1987) demonstrated that FFT inversion was an accurate and efficient method of obtaining breakthrough curves from a fixed-bed adsorber in the time domain. Hsu and Chen (1987) also extended the use of this elegant technique to the problem of zonal chromatography in their theoretical study of peak asymmetry. Recently, Ernst and Hsu (1991) have taken advantage of the fast computing speed of the FFT method for the estimation of rate parameters from chromatography peaks. This algorithm is based on a modified Rosenbrock multivariable optimization technique and can be used to estimate one or more rate parameters by fitting the time domain solution to experimental data. This algorithm was employed to evaluate axial dispersion coefficient, intraparticle diffusivity, and porosity for the separation of amino acids by fixed-bed cation exchange. In this work, the FFT technique will be used to obtain time domain solutions of the rate model (Eq. 10) using parameters that are determined by the moment or HETP analysis. These simulations will be compared to experimental chromatographic peaks to judge the validity of the parameters obtained from these other methods.

Experimental Methods

Materials

The intraparticle diffusivities of seven proteins were investigated: myoglobin (horse heart), lactoglobulin (milk), ovalbumin (chicken egg white), albumin (bovine serum), hexokinase

Table 1. Properties of Proteins Studied

Protein (Source)	M_w (Da)	D_0^{20} (cm^2/s)	r_s (nm)	D_0^4 (cm^2/s)
Myoglobin (Horse Heart)	16,890	11.3×10^{-7}	1.89	6.9×10^{-7}
β-Lactoglobulin (Milk)	35,400	7.8×10^{-7}	2.74	4.7×10^{-7}
Ovalbumin (Chicken Egg)	45,000	7.3×10^{-7}	2.93	4.4×10^{-7}
Albumin (Bovine Serum)	67,000	5.9×10^{-7}	3.59	3.6×10^{-7}
Hexokinase (Yeast)	102,000	5.9×10^{-7}	3.62	3.6×10^{-7}
Immunoglobin G (Rabbit Serum)	161,000	3.8×10^{-7}	5.62	2.3×10^{-7}
Catalase (Bovine Liver)	225,000	4.1×10^{-7}	5.21	2.59×10^{-7}

(yeast), immunoglobin G (rabbit serum), and catalase (bovine liver). All proteins, except immunoglobin G, were obtained from Sigma Chemical Co. and were used as supplied. Immunoglobin G was purified by Protein A affinity chromatography from rabbit serum. The physical properties of these proteins are listed in Table 1. The Stokes radius (r_s) was calculated for each protein by the Stokes-Einstein equation, using literature values of diffusivity at 20°C and the molecular weight (Tyn and Gusek, 1990).

$$D_0 = \frac{k_B T}{6\pi\mu r_s} \tag{20}$$

where D_0 is the diffusivity at temperature T (in K), k_B is the Boltzman constant, and μ is the viscosity of the solvent. The Stokes-Einstein equation was also used to estimate diffusivity at 4°C for the proteins from the diffusivity at 20°C. Fractionated blue dextran was prepared from Blue Dextran 2000 (Pharmacia-LKB Biotechnology) by gel filtration as we described earlier (Boyer and Hsu, 1992). The buffer used throughout this study was 50-Mm potassium phosphate, pH 7.0.

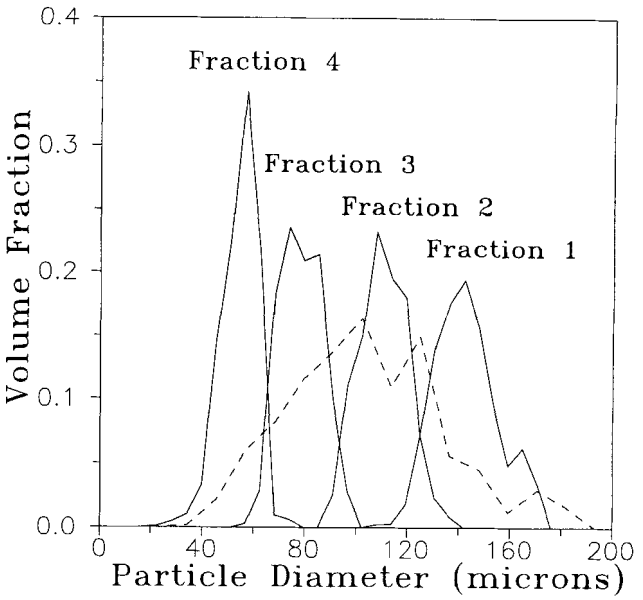

Figure 2. Particle size distributions of Sepharose CL-6B (dashed line) and fractions.

Fractionation of Sepharose CL-6B

The support matrix used in these studies was Sepharose CL-6B (Pharmacia-LKB Biotechnology), which is a 6% cross-linked agarose gel. Sepharose CL-6B has a particle size distribution of approximately 45–165 microns. The Sepharose particles were separated into four fractions of different size by the following two-step procedure. A primary fractionation was accomplished by fluidization of the particles in a long column. Particles are separated on the basis of settling velocity in this system, and fractions were collected by stepwise increases of flow rate. These fractions were further classified by extensive wet sieving through mesh screens with 125, 90 and 65 micron openings. The particle size distributions of the four fractions and the unfractionated Sepharose were determined by a photo-microscopic method. Photographs were taken of samples from each matrix at a magnification of 200 times, and the diameters of the particles in these photographs were measured. The particle diameter was then determined by comparing these photographic diameters with photographs of a stage micrometer taken at the same magnification.

Zonal chromatography experiments

All experiments were carried out at 4°C in columns having a diameter of 1.6 cm. The agarose columns were packed by passing buffer upward through the column at a high flow rate. Each experimental run consisted of the introduction of a pulse of protein at the inlet of the column and its subsequent elution. The input pulse was introduced by means of a sample injection valve (Rheodyne No. 5052) just before the column inlet. Liquid flow to the column was maintained at a constant rate during each run either by gravity or a peristaltic pump. The outlet peak was detected by monitoring the absorbance at 280 nm of the column effluent. Column bed voidage was measured by the analysis of the pulse response of fractionated Blue Dextran. Zonal chromatography of each protein was carried out, either in duplicate or triplicate, at three flow rates for each matrix.

The pulse responses of two proteins, albumin and myoglobin on columns of the Sepharose fractions were investigated. The fixed-bed volumes employed in these studies varied from 5 to 8 cm^3. Experimental runs were initiated by introduction of a 0.10-cm^3 pulse of a protein solution at 1 mg/cm^3. The flow rates studied in the experiments with fractionated Sepharose were nominally 50, 70, and 90 cm^3/h, which corresponded to superficial velocities in the column of approximately 0.007, 0.010 and 0.013 cm/s.

The zonal chromatography experiments on the unfractionated Sepharose were carried out in larger columns: the fixed-bed volumes were approximately 45 cm^3. The pulse responses for each of the seven proteins were studied with this matrix. The size of the input pulses in these experiments was 0.50 cm^3 of a 1-mg/cm^3 protein solution. The increased length of the column limited the usable flow rates to 70 cm^3/h or less, so the flow rates studied in these columns were nominally 40, 55 and 70 cm^3/h, which corresponded to superficial velocities of 0.006, 0.008 and 0.010 cm/s.

Results and Discussion

Zonal chromatography on Sepharose fractions

The particle size distributions of the four Sepharose fractions and the unfractionated Sepharose as determined by the photo-

Table 2. Results of Sepharose CL-6B Fractionation

Matrix	Volume Fraction	Average Radius (μm)	Standard Deviation (μm)
Fraction 1	0.20	70.9	11.7
Fraction 2	0.39	54.9	9.3
Fraction 3	0.28	38.8	8.0
Fraction 4	0.13	26.6	9.4
Sepharose CL-6B (unfractionated)	1.00	46.0	25.9

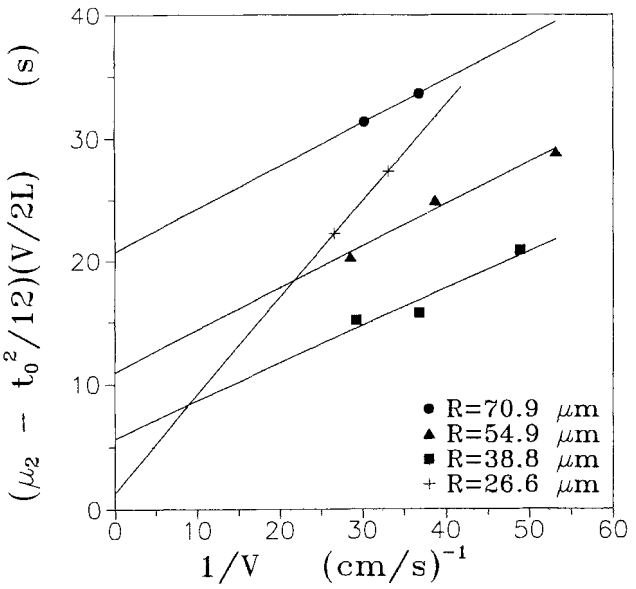

Figure 3. Moment analysis of myoglobin pulses on columns of fractionated Sepharose CL-6B.

microscopic method are shown in Figure 2. The particle diameter was found to vary from 40 to 175 μm, which agrees very well with the manufacturer's specifications. The four fractions show very little overlap and exhibit a small standard deviation (see Table 2). Rasmuson (1985a) suggested that the appropriate average radius should correspond to the equivalent surface area per unit particle volume. For nonuniform spherical particles, the average radius, R, can be calculated directly from the measured particle size distribution:

$$R = \frac{\sum_{i=1}^{N} R_i^3}{\sum_{i=1}^{N} R_i^2} \tag{21}$$

The average radii determined for the fractions as well as the unfractionated Sepharose are also listed in Table 2.

Since only small amounts of the fractionated gels were available, the columns used in chromatography experiments were relatively short. As a result, the pulse responses of proteins on these gels exhibited highly asymmetric peaks; therefore, the kinetic parameters were estimated by the moment analysis as described above. This analysis is shown for pulses of myoglobin on the four Sepharose fractions in Figure 3. As expected, linear relationships were obtained in this analysis.

As seen in Eq. 14, the slope of these plots can be related to the axial dispersion of the column. Axial dispersion arises from several sources in a real system: from liquid flowing through the packed bed and from external sources such as tubing and flow distributors. Chung and Wen (1968) have presented the following correlation for estimating the dispersion due to flow through a packed bed of particles.

$$\frac{D_L}{V} = \frac{2\epsilon R}{0.20 + 0.011 Re^{0.48}} \tag{22}$$

Table 3. Comparison of Axial Dispersion Determined by Experiment and Correlation for Fractionated Sepharose CL-6B

Matrix	Experimental D_L/V (cm)	Correlated* D_L/V (cm)
Fraction 1	0.067	0.025
Fraction 2	0.064	0.021
Fraction 3	0.063	0.013
Fraction 4	0.117	0.008

*Correlation of Chung and Wen (1968).

In this equation, Re is the particle Reynolds number ($=2R\epsilon V\rho/\mu$), where ρ is solvent density. This correlation predicts that the bed axial dispersion will increase almost proportionally with particle radius. Arnold et al. (1985) discussed an experimental method for estimating the dispersion due to external sources and found that the relative magnitude of the external dispersion is inversely proportional to the column length; therefore, shorter columns would be expected to exhibit greater degrees of external dispersion.

The overall axial dispersion (D_L/V) has been determined from the experimental data from each of the fractionated Sepharose columns. The void fraction of these columns was measured with fractionated Blue Dextran to obtain values for the parameter m. Values for intraparticle void fraction (ϵ_p) for myoglobin and albumin were determined by first moment analysis and were found to be 0.734 for myoglobin and 0.550 for albumin. Using these parameters, values of D_L/V were determined for each of the fractionated Sepharose columns and are listed in Table 3 along with the values for bed dispersion as estimated by the correlation. Comparison of these values shows that only 10% to 40% of the observed dispersion can be accounted for by dispersion due to liquid flowing through the packed bed; therefore, the remaining dispersion must be due to other external sources. Such a high degree of external dispersion is not unusual in columns as short as these. Axial dispersion was found to vary only slightly in columns of fractions 1, 2 and 3; however, a much higher value was observed for fraction 4. This was probably a result of the shorter column length that was used for the experiments on fraction 4. From these results it is clear that axial dispersion should be determined experimentally rather than by correlation, especially when short columns such as these are employed.

The intercepts of the moment analysis plots are related to both intraparticle and external protein diffusion. The external film transfer coefficient has been estimated by the correlation of Ohashi et al. (1981), which has been used by other researchers for protein adsorption (Liapis et al., 1989).

$$\frac{2k_F R}{D_0} = 2 + 0.51\left(\frac{E^{\frac{1}{3}}(2R)^{\frac{4}{3}}\rho}{\mu}\right)^{0.60} Sc^{\frac{1}{3}} \tag{23}$$

In this expression, Sc is the Schmidt number ($=\mu/\rho D_0$), and E is the energy dissipation rate defined by:

$$E = \frac{25(1-\epsilon)\epsilon^2 C_{D0} V^3}{R} \tag{24}$$

Table 4. Film Mass Transfer Coefficient and Intraparticle Diffusivity of Proteins in Fractionated Sepharose CL-6B

Matrix	Albumin k_F^* (cm/s)	Albumin D_e (cm²/s)	Myoglobin k_F^* (cm/s)	Myoglobin D_e (cm²/s)
Fraction 1	3.8×10^{-4}	6.3×10^{-8}	5.9×10^{-4}	20.3×10^{-8}
Fraction 2	4.2×10^{-4}	5.9×10^{-8}	6.8×10^{-4}	23.5×10^{-8}
Fraction 3	5.4×10^{-4}	5.6×10^{-8}	8.7×10^{-4}	23.7×10^{-8}
Fraction 4	7.4×10^{-4}	6.6×10^{-8}	11.8×10^{-4}	15.3×10^{-8}

*Determined by correlation of Ohashi et al. (1981).

where C_{D0} is the drag coefficient for a single particle. These experiments could all be characterized by creeping flow, since the Reynolds numbers were always less than 0.1; consequently, the drag coefficient is given by the Stoke's law: $C_{D0}=24/Re$ (Bird et al., 1960). The Ohashi correlation is valid in the following ranges (Liapis et al., 1989): $0.08<Re^f(=[E^{1/3}\times(2R)^{4/3}\rho/\mu])<4{,}600$; $505<Sc<70{,}600$. Re^f varied from 0.3 to 1.0 in these experiments and the Schmidt numbers were 22,880 for myoglobin and 43,540 for albumin; therefore, the correlation should be valid. Under the conditions employed in these experiments, the film transfer coefficient estimated by this correlation shows only a slight dependence on fluid velocity, therefore an average value can be used for the proteins on each gel. The average film transfer coefficients determined are listed in Table 4 for these experiments.

The values of intraparticle diffusivity can now be estimated from the intercepts of the second moment analysis plots by Eq. 14 and are also listed in Table 4 for the two proteins. Intraparticle diffusivity was found to vary little in the different Sepharose fractions. The values of myoglobin diffusivity ranged from 15.3 to 23.7×10^{-8} cm²/s, while that of albumin ranged from 5.7 to 6.7×10^{-8} cm²/s. These results suggest that a single average value of intraparticle diffusivity may characterize the diffusion of proteins in Sepharose particles regardless of size. Average values of the intraparticle diffusivity for these proteins

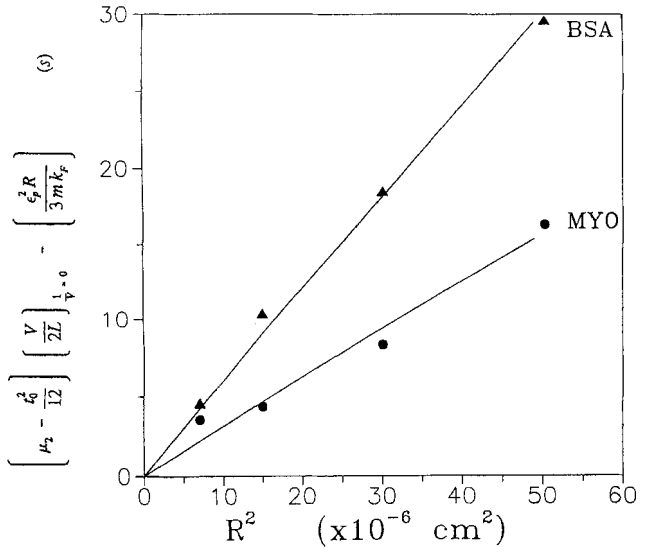

Figure 4. Variation of moment analysis intercepts (corrected for film transfer effects) with the particle radius squared.

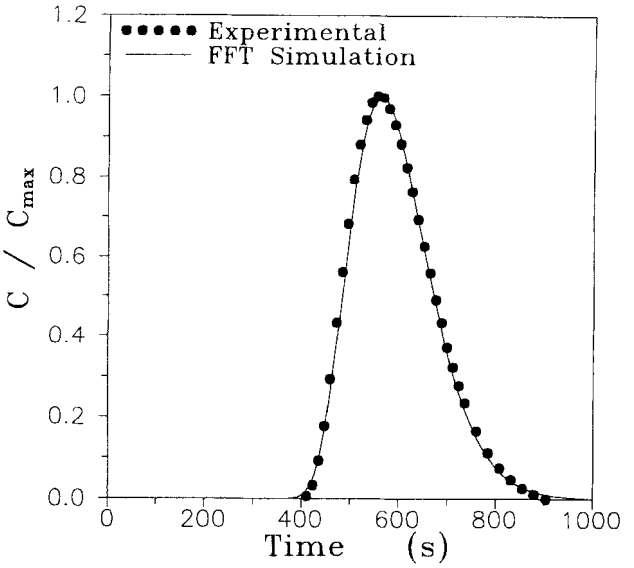

Figure 5. Comparison of experimental outlet peak for myoglobin on a column of Sepharose fraction 1 with that predicted by FFT simulation.

$L=3.8$ cm, $V=3.14\times10^{-2}$ cm/s, $R=7.09\times10^{-5}$ cm, $\epsilon=0.3789$, $\epsilon_p=0.734$, $D_e=21.7\times10^{-8}$ cm²/s, $D_L=2.1\times10^{-3}$ cm²/s, and $k_F=5.9\times10^{-4}$ cm/s.

were determined by plotting the intercepts of the moment analysis (corrected for the film transfer contribution) vs. the squared radius as shown in Figure 4. The slope of this plot is inversely proportional to the intraparticle diffusivity. By this analysis, the average intraparticle diffusivity was found to be 21.7×10^{-8} cm²/s for myoglobin and 6.3×10^{-8} cm²/s for albumin. Hence, the intraparticle diffusivity of myoglobin is 32% of its solution diffusivity of 68.5×10^{-8}, while that of albumin is only 17% of its solution diffusivity. These figures indicate that protein diffusion in the agarose matrix is restricted and that the degree of the restriction is greater for larger molecules. These phenomena will be investigated more fully in the second part of this study.

The parameters determined by moment analysis above have been used in the rate model for gel filtration to generate simulations of column behavior by the FFT technique for a number of experimental runs. A typical simulation is shown in Figure 5 along with the experimental data. The simulation corresponds very well with the observed peak. This agreement between model and experiment suggests that the parameters determined by moment analysis are accurate and valid. These results also demonstrate the utility of the FFT technique as a method of predicting the behavior of these gel filtration systems.

Since intraparticle diffusivity was found to be independent of the particle size, it is likely that the internal structure is similar in agarose particles of different size. These results suggest that a rigorous model, such as that of Chen and Hsu (1990) or Rasmuson (1985a) which can account for particles of different size with different properties, is not required for this case of protein gel filtration on agarose particles. Furthermore, it has been reported (Rasmuson, 1985a) that there should be very little difference in performance between fixed beds of monodisperse particles and fixed beds of particles that have a moderate Gaussian particle size distribution having the

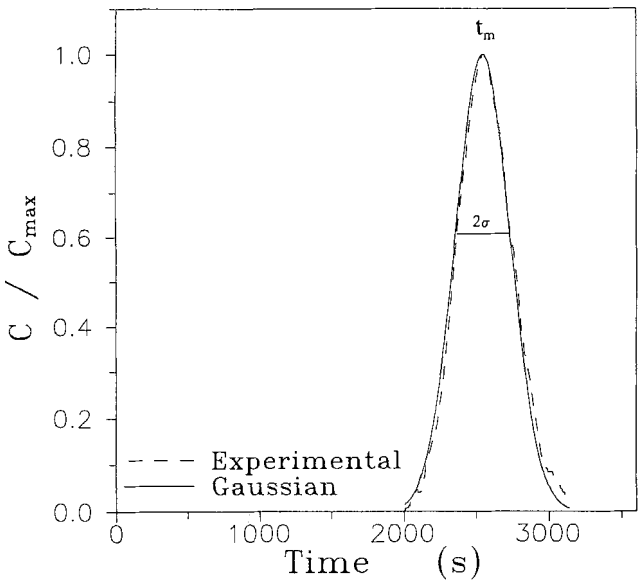

Figure 6. Comparison of experimental outlet peak with Gaussian curve: experimental determination of t_m and σ.

same average radius. Consequently, a simplified rate model, which assumes monodisperse particle size, should give reliable simulations for protein gel filtration on agarose particles.

Protein chromatography on unfractionated Sepharose CL-6B

Since particle size distribution is not an important factor in these gel filtration experiments as discussed above, the investigation of the effects of protein size on intraparticle diffusivity was carried out of fixed beds on unfractionated Sepharose CL-6B particles. Much longer columns were employed in these experiments to minimize the effects of external sources of dispersion and to reduce the resulting scatter in the experimental data. The exit peaks from protein pulses introduced to these longer columns were found to be essentially Gaussian in shape. As seen in Figure 6, a typical chromatogram exhibits only a small amount of tailing on both the leading and trailing edges of the peak. As a result, the HETP method was chosen for the analysis of these experimental data. The peak residence time (t_m) was taken to be the time that corresponded to the maximum outlet concentration (C_{max}), while the standard deviation (σ) was determined by measuring the peak width at a height of $C/C_{max} = e^{-1/2}$ ($\cong 0.607$), as shown in Figure 6.

Residence time analysis of these experiments is shown in Figure 7. This analysis is identical to first moment analysis with t_m replacing μ_1 in Eq. 12; thus, the intraparticle void fraction (ϵ_p) of each of the proteins can be determined from the slope of these plots. As in the experiments with fractionated Sepharose, the column void fraction was measured by zonal chromatography of fractionated Blue Dextran to evaluate the parameter m, which was used in these calculations. The values of intraparticle void fraction obtained by this analysis are listed in Table 5. It is observed that intraparticle void fraction generally decreases as the molecular weight of the protein is increased. Intraparticle void fraction usually yields a straight line when plotted vs. the logarithm of the protein molecular weight,

Table 5. Intraparticle Void Fraction of Proteins in Sepharose CL-6B

Protein	Intraparticle Void Fraction
Myoglobin	0.734
β-Lactoglobulin	0.625
Ovalbumin	0.610
Albumin	0.550
Hexokinase	0.549
Immunoglobulin G	0.457
Catalase	0.474

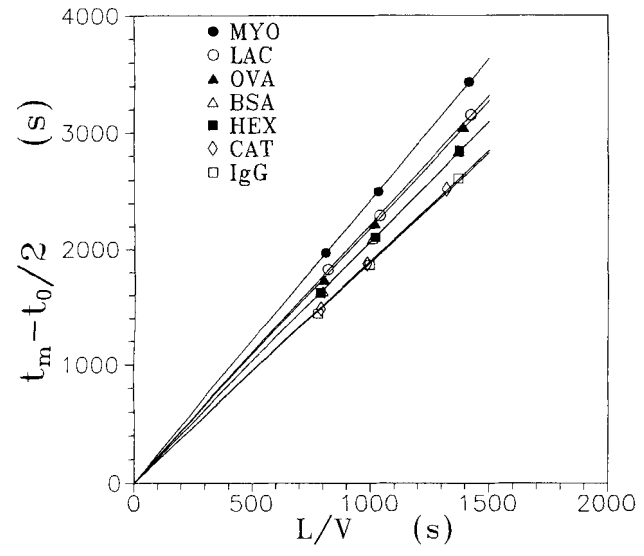

Figure 7. Retention time analysis of outlet peaks from protein pulses on columns of unfractionated Sepharose CL-6B.

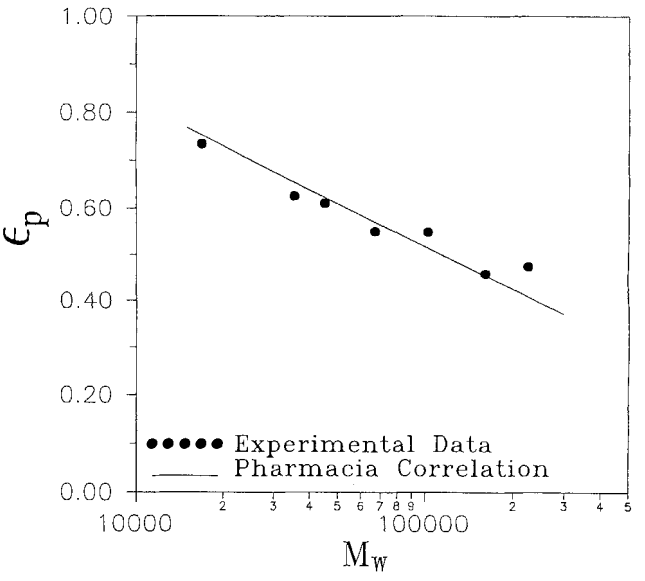

Figure 8. Correlation of intraparticle void fraction with protein molecular weight in unfractionated Sepharose CL-6B.

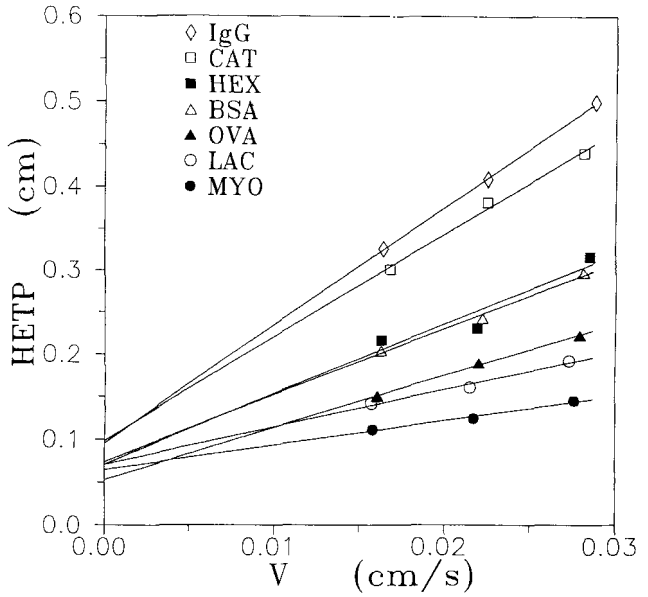

Figure 9. HETP analysis of protein pulses on columns of unfractionated Sepharose CL-6B.

as shown in Figure 8. The results obtained in this study were found to correspond very well to the published correlation provided by Pharmacia (1984) which is given by the solid line in Figure 8.

HETP analysis of these gel filtration experiments is shown in Figure 9. Linear relationships between plate height and interstitial velocity were observed for each of the proteins studied. As Eq. 16 shows, axial dispersion is directly related to the intercepts in Figure 9. The values of D_L/V were found to range from 0.027 to 0.049 cm, and there was no trend observed with protein size, indicating that the contribution of molecular diffusion to axial dispersion in these columns was small. These values of D_L/V correspond well with those measured by Suzuki (1974), who found values of D_L/V ranging from 0.028 to 0.080 cm in gel filtration columns. The correlation of Chung and Wen (1968) predicts a $D_L/V = 0.016$ cm for dispersion which arises due to liquid flow through the packed bed alone, which suggests that external dispersion is still present in the longer columns. The relative contribution of these external sources of dispersion, however, is much smaller than was observed in the short columns of fractionated Sepharose.

Before intraparticle diffusivity can be determined, film HETP (h_F) must be evaluated. As before, the Ohashi correlation (Eq. 23) was used for estimation of film mass transfer coefficients. Again, since the values for Re' for these experiments were in the range 0.4 to 0.6, and the Schmidt numbers varied from 20,000 to 65,000, this correlation should give valid estimates of the film transfer coefficients. Under the conditions employed in these experiments the effects of film transfer were small, generally accounting for less than 15% of the measured plate height.

Values of intraparticle diffusivity were determined from the slope (corrected for the effects of film transfer as given above) of the HETP plots in Figure 9. The results of this analysis are listed in Table 6. Intraparticle diffusivity was observed to vary from 23.5×10^{-8} cm^2/s for myoglobin, down to 2.5×10^{-8} cm^2/s for immunoglobin G. As expected, intraparticle diffusivity was found to decrease with increasing protein size. This behavior results from the combination of decreased solution diffusivity, as given by the Stokes-Einstein equation, and restricted diffusion in the agarose matrix. The extent to which diffusion is restricted by the matrix is indicated by the ratio of the intraparticle diffusivity to the solution diffusivity as shown in Table 6. These data indicated that the diffusional restrictions increased with the protein size and are in agreement with the results of Davies (1989), who found that the diffusion of fibrinogen ($M_w = 340{,}000$) was more severely restricted than that of γ-globulin ($M_w = 162{,}000$).

Table 6. Intraparticle Diffusivities and Diffusion Restriction of Proteins on Sepharose CL-6B

Protein	D_e (cm^2/s)	D_e/D_0
Myoglobin	23.5×10^{-8}	0.343
β-Lactoglobulin	12.5×10^{-8}	0.265
Ovalbumin	8.2×10^{-8}	0.184
Albumin	5.6×10^{-8}	0.155
Hexokinase	5.2×10^{-8}	0.146
Immunoglobin G	2.5×10^{-8}	0.102
Catalase	3.0×10^{-8}	0.119

The values of intraparticle diffusivity obtained for myoglobin and albumin by HETP analysis of pulses on the unfractionated Sepharose CL-6B compared very well to those determined by moment analysis from the fractionated Sepharose results: the values differ by less than 10%, which is very good considering the differences in particle size distribution, packing, and column geometry between these two sets of experiments. Furthermore, the validity of these values was

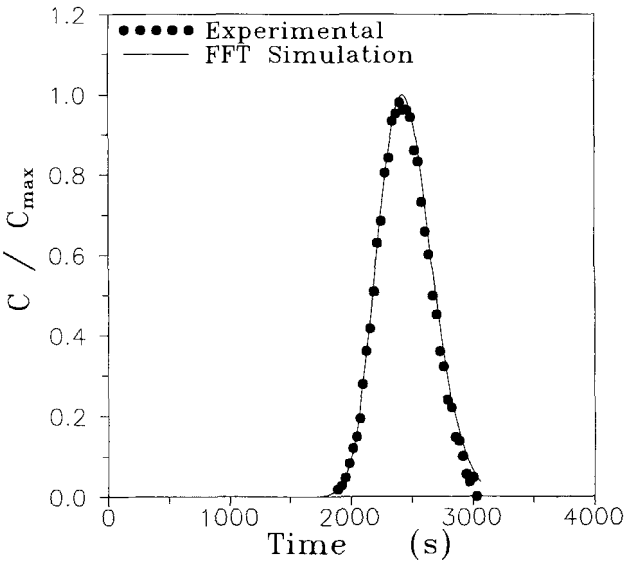

Figure 10. Comparison of experimental outlet peak for albumin on a column of unfractionated Sepharose CL-6B with that predicted by FFT simulation.

$L = 22.4$ cm, $V = 2.23 \times 10^{-2}$ cm/s, $R = 4.6 \times 10^{-5}$ cm, $\epsilon = 0.3403$, $\epsilon_p = 0.550$, $D_e = 5.6 \times 10^{-8}$ cm^2/s, $D_L = 8.2 \times 10^{-4}$ cm^2/s, and $k_F = 4.6 \times 10^{-4}$ cm/s.

confirmed by FFT simulation of the outlet peak, as shown in Figure 10.

Correlation of intraparticle diffusivity with protein size

Very early in the development of gel filtration as a method of protein purification, it was understood that proteins were separated not only by partial exclusion from the gel, but also by restricted diffusion of the proteins in the polymer matrix (Laurent and Killander, 1964). The observed decrease in diffusion rate was found to empirically obey Eq. 1, which indicated that the degree of diffusion retardation varies exponentially with the one-half power of polymer concentration. Ogston et al. (1973) were the first to develop a theoretical framework to explain this empirical equation. This theory is based on a stochastic model, in which the size of the random walk steps are reduced by the presence of the polymer fibers. In this model, the interaction radius is taken to be the sum of the fiber radius (r_f) and the Stokes radius (r_s) of the protein. From this model, which was largely geometrical, the following relationship for restricted diffusion in polymer gels was proposed.

$$\frac{D_e}{D_0} = \exp[-B(r_s + r_f)c_f^{1/2}] \tag{25}$$

More recently, Cukier (1984) has derived a model that described the diffusion of Brownian spheres in a semidilute polymer solution based on the concept of hydrodynamic screening. In this model, the polymer molecules were assumed to behave like long rods, which can be described as prolate ellipsoids with major and minor axes given by l and b, respectively. These rods were assumed to be fixed in space relative to the solvent and Brownian particles due to entanglement with other polymer rods. This physical situation was the characteristic of a Debeye-Bueche-Brinkman (DBB) fluid. The screening coefficient (κ_f), which indicated the resistance to fluid flow resulting from the fixed rod network, is given by:

$$\kappa_f^2 = \frac{3\pi n_f l}{\ln(l/b)} \tag{26}$$

The parameter n_f in this equation is the number density of the polymer rods. Restricted diffusion of the Brownian spheres in such a system is given by:

$$\frac{D_e}{D_0} = \exp(-\kappa_f r_0) \tag{27}$$

By substituting Eq. 26 for the screening coefficient, converting the number density of polymer to polymer (fiber) concentration (c_f), and inserting the relation for the interaction radius, the following expression can be obtained for particle diffusion in a semidilute polymer solution.

$$\frac{D_e}{D_0} = \exp\left[-\left(\frac{3\pi l N_A}{M_f \ln(l/b)}\right)^{1/2}(r_s + r_f)c_f^{1/2}\right] \tag{28}$$

In this expression M_f is the molecular weight of the polymer

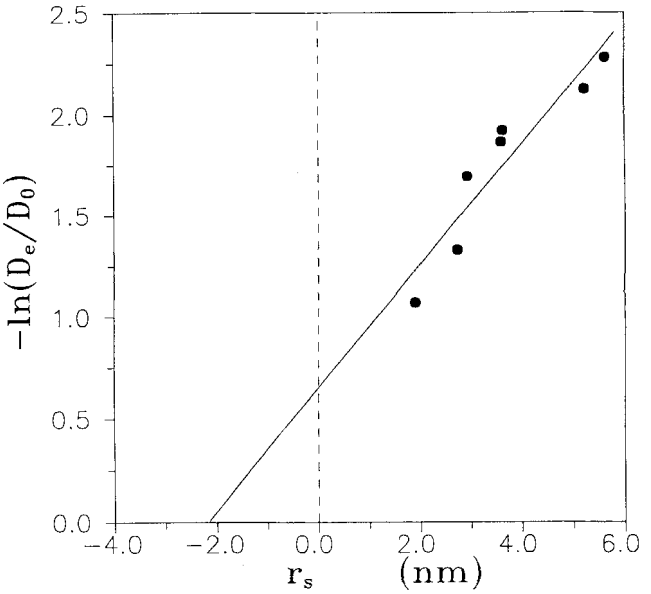

Figure 11. Ogston (1973) analysis of hindered diffusion of proteins in Sepharose CL-6B particles.

rod. Equation 28 has the same form as the model (Eq. 25) proposed by Ogston et al. (1973); only the coefficient in the exponent is different.

In this study, experimental data were empirically correlated according to the form of Eqs. 25 and 28; therefore, the difference between the theoretical values for the exponential coefficient predicted by these two models was not a critical issue. Based on these considerations, the coefficient will be expressed as the original empirical coefficient, B, from Eq. 1. Equations 25 and 28 can be linearized by taking logarithms of both sides of the equation.

$$\ln\left(\frac{D_e}{D_0}\right) = -B(r_s + r_f)c_f^{1/2} \tag{29}$$

The data for intraparticle diffusivity of the seven proteins have been plotted according to Eq. 29 in Figure 11. The data on this plot scattered along a line that intersects the abscissa at −2.3 nm, very close to the value of 2.5 nm obtained for the fiber radius (r_f) in agarose gels by both gel filtration (Laurent, 1967) and light scattering (Öbrink, 1968). Thus, there are both experimental and theoretical evidences which suggest that Eq. 29 will serve as a good correlation for protein diffusivity in agarose gels.

The degree of diffusion retardation in agarose gels was to the protein's Stokes radius as shown in Eq. 29. Although the stokes radius can be routinely determined by dynamic light scattering, it is not a widely used parameter as molecular weight. As a result, the application of this correlation based on the Stokes radius will be limited by either the availability of published values of Stokes radius or the ability to measure this parameter for the purified protein of interest. It is clear that a correlation, which is based on a more common parameter such as molecular weight, will be much more useful.

A very simple approach has been used to replace the Stokes radius in the correlation. The proteins have been assumed to

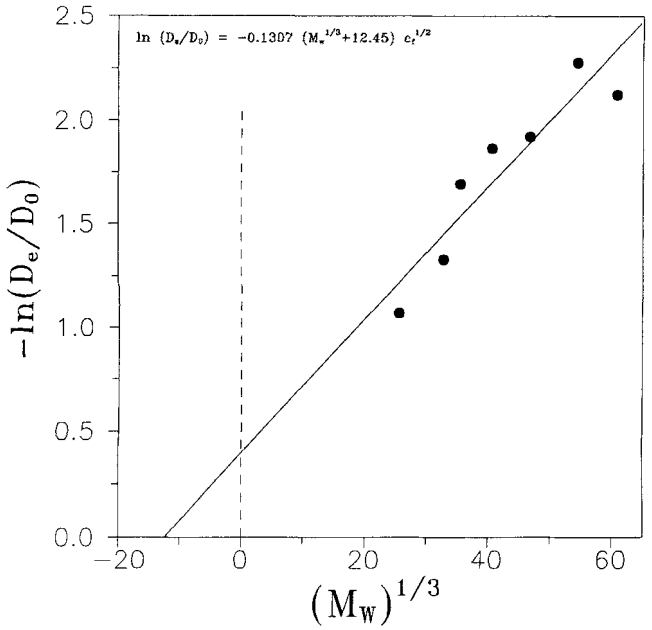

Figure 12. Correlation of restricted protein diffusion in Sepharose CL-6B particles based on protein molecular weight.

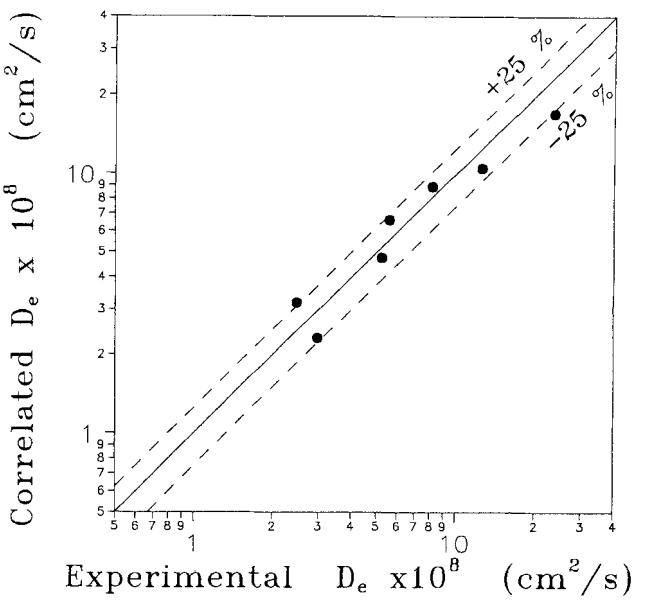

Figure 13. Comparison of intraparticle diffusivity predicted by Eq. 35 with experimentally determined values.

be spherical molecules with radius equal to the Stokes radius. In this case, the molar volume of the protein will be given by:

$$V_m = \frac{4}{3}\pi N_A r_s^3 \tag{30}$$

where V_m is the molar volume of the protein and N_A is Avagadro's number. If it is further assumed that a constant value of partial specific volume exists for proteins (Young et al., 1980), which allows the following relationship between molecular weight and Stokes radius to be obtained,

$$M_w = \frac{4}{3}\pi \frac{N_A}{\overline{V}} r_s^3 \tag{31}$$

where $\overline{V}$ is the protein partial specific volume. In this equation, it is observed that the Stokes radius is proportional to the molecular weight raised to the 1/3 power. Thus, Eq. 29 can be modified to eliminate the Stokes radius from the correlation as:

$$\ln\left(\frac{D_e}{D_0}\right) = -B_m(M_w^{1/3} + A_m)c_f^{1/2} \tag{32}$$

where A_m and B_m are constants.

The form of the proposed correlation for estimating the extent of diffusional restriction for a protein diffusing in agarose beads is shown in Eq. 32. Figure 12 shows the data for the seven proteins plotted according to this equation. The constants in the correlation, A_m and B_m, were obtained by the linear regression analysis of these data. The correlation (Eq. 33) thus obtained is represented by the solid line in Figure 12.

$$\ln\left(\frac{D_e}{D_0}\right) = -0.1307(M_w^{1/3} + 12.45)c_f^{1/2} \tag{33}$$

The units used for the polymer (fiber) concentration (c_f) in this correlation are g/cm^3 gel.

As shown in Eq. 33, the value of the solution diffusivity of a protein must be known to estimate its intraparticle diffusivity. Young et al. (1980) have developed the following correlation for prediction of protein diffusivity based on the protein's molecular weight:

$$D_0 = 8.34 \times 10^{-10}\left(\frac{T}{\mu M_w^{1/3}}\right) \tag{34}$$

This correlation can be introduced into the exponential form of Eq. 33 to give a correlation that allows the prediction of intraparticle diffusivity of proteins in agarose support matrices based solely on the protein's molecular weight. The proposed correlation is:

$$D_e = 8.34 \times 10^{-10}\left(\frac{T}{\mu M_w^{1/3}}\right)\exp[-0.1307(M_w^{1/3} + 12.45)c_f^{1/2}] \tag{35}$$

The intraparticle diffusivities for the seven proteins studied here have been estimated by Eq. 35. These results are plotted against the experimentally determined values in Figure 13. The agreement between the correlated and experimental values is fairly good, as most of the points fall inside the dashed lines, which indicate ±25%. The average error of all seven proteins was found to be 19%.

A few values of intraparticle diffusivity have been reported in the literature for proteins in Sepharose CL-4B matrices, which is a 4% cross-linked agarose gel (Arnold et al., 1985;

Table 7. Comparison of Literature Values of Intraparticle Diffusivity with Those Predicted by Eq. 35

Protein	Experimental D_e (cm^2/s)	Correlated D_e (cm^2/s)	Percentage Error (%)
Myoglobin*	39.0×10^{-8}	35.2×10^{-8}	9.7
Albumin*	9.6×10^{-8}	15.0×10^{-8}	56.3
γ-Globulin**	11.2×10^{-8}	7.8×10^{-8}	30.5
Fibrinogen**	3.6×10^{-8}	4.1×10^{-8}	13.3

*Data from Arnold et al. (1985).
**Data from Davies (1989).

Davies, 1989). Equation 35 was used to predict the intraparticle diffusivities of these proteins under the conditions studied for comparison. The results are shown in Table 7. Except for albumin, the values obtained by correlation compare very well to the experimental values. It is possible that the value for albumin contains some error, as the measured value observed is lower than that of γ-globulin, which has a molecular weight approximately twice that of albumin. Since Sepharose CL-4B is a cross-linked 4% agarose matrix, these results indicate that the correlation has the correct dependence on agarose concentration (c_f). These results are encouraging as it appears that the correlation may be adequate for the prediction of protein diffusivity in agarose gels of various concentrations.

It is realized that the proposed correlation is based on the intraparticle diffusivities of only seven proteins in a single agarose matrix and therefore may be subject to any irregularities that may occur in these cases. It is possible that as more values for intraparticle diffusivity are reported in the literature, the values of the coefficients used in the correlation may need modification. Such irregularities may include the effects of protein shape and flexibility, which were ignored in the development of the correlation. Proteins are known to have widely varying shapes, ranging from nearly spherical to long rod-like structures. The shape of the protein can be expected to have a significant effect on its intraparticle diffusivity. Since the proteins studied here were globular, it is likely that this correlation will slightly overpredict the diffusivity of proteins with rod-like structure, as the diffusion of these proteins will experience greater hindrance in the agarose matrix.

Another factor that may have important effects on intraparticle diffusivity is the flexibility of the protein's three-dimensional structure. Proteins that contain no disulfide cross-linkages often are more flexible than those that are cross-linked. A flexible protein would be expected to exhibit a higher diffusivity in a polymer matrix than a rigid protein of the same size, because it may be able to assume more favorable conformations for passage through the fibrous network. This factor may be responsible for the large discrepancies observed between correlated and measured values of diffusivity for myoglobin and immunoglobin G. Myoglobin contains no disulfide linkages and therefore has a very flexible three-dimensional structure. This flexibility may allow myoglobin to diffuse more rapidly in the agarose matrix than would be expected for a typical protein of that molecular weight. Immunoglobin G, on the other hand, contains many disulfide cross-linkages which give it a very rigid structure. This rigidity may account for the low value of intraparticle diffusivity that was determined for this protein.

The theoretical models of Ogsten (1973) and Cukier (1984) accounted for the variation of diffusivity with the polymer concentration; consequently, the empirical correlation proposed here also exhibits a concentration dependence. Although this study has investigated the protein diffusivity only in 6% cross-linked agarose particles, the results in Table 7 for 4% cross-linked agarose, as well as those from Laurent's (1967) study involving agarose particles ranging from 2 to 8% cross-linkage, suggested that this concentration dependence exhibited in the correlation may be valid. Taking into account these limitations, the proposed correlation should be valid for globular proteins having molecular weight in the range from 10,000 to at least 300,000 which are diffusing in agarose matrices containing from 2 to 8% agarose (these are the typical concentrations used for adsorbent particles). The values predicted can be expected to be accurate to within 25% which is adequate for many types of engineering calculations.

Conclusions

From the analysis of experiments with the fractionated Sepharose, it was found that protein diffusivity in agarose matrices was independent of the particle size. This result suggests that the interior structure of these particles is consistent and is not affected by differences in diameter. Other researchers have found that particle size distribution exerts very little effect on fixed-bed performance when the particle size distribution is Gaussian and when kinetic parameters may be assumed to be independent of particle size (Rasmuson, 1985a). These two findings, taken in conjunction, suggest that systems using agarose-based adsorbents should be adequately simulated by a simple fixed-bed adsorption model that assumes a monodisperse particle size.

In the second part of this study, it was found that protein diffusion in these agarose particles is severely restricted, in many cases the diffusivity was found to be less than 15% of the value for the protein in solution. The degree of the diffusion restriction was found to obey the theoretical models presented by Ogston et al. (1973) and Cukier (1984). These theoretical models served as the starting point for the development of an empirical correlation for the prediction of intraparticle diffusivity in agarose support matrices. The minimum parameters required in the correlation are the molecular weight of the protein and the agarose concentration of the matrix. This correlation should provide reasonably accurate predictions of a protein's intraparticle diffusivity for use in the modeling and scale-up of a chromatography process, without the completion of tedious and expensive experimental procedures.

The occurrence of restricted protein diffusion in polymer matrices, however, is not limited to the case of diffusion in adsorbent particles. It observed in many other important processes, such as membrane separation processes, immobilized cells and enzymes, constant-release drug devices, and a whole range of other biomedical devices that employ polymer matrices as supports. An understanding of the fundamental transport phenomena in these systems is critical for rational design and improvement of these systems. While the correlation proposed in this report is valid only for systems employing agarose matrices, the methodology presented in this work should provide a sound framework for the study of restricted diffusion in these other systems.

Acknowledgment

The authors would like to thank Lampire Biologicals (Coopersburg, PA) for their generous gifts of rabbit serum and Protein A-agarose.

Notation

A_m = constant in Eq. 32
b = minor axis length of polymer rod in Eqs. 26 and 28, cm
B = proportionality constant in Eqs. 1 and 29
B_m = proportionality constant in Eq. 32
c_f = polymer (fiber) concentration in particles, g/cm^3
$\underline{C}$ = solute concentration in mobile phase, mg/cm^3
C = Laplace domain solute concentration in mobile phase, mg/cm^3
C_{D0} = drag coefficient for a single particle in liquid flowing at ϵV
$C_{\max}$ = maximum concentration observed in a chromatographic peak, mg/cm^3
C_p = solute concentration in particle based on accessible particle volume, mg/cm^3
D_0 = solution diffusivity of solute, cm^2/s
D_e = effective intraparticle diffusivity of solute, cm^2/s
D_L = axial dispersion coefficient, cm^2/s
E = energy dissipation rate, given by Eq. 24, cm^2/s^3
h = height of an equivalent transfer plate, cm
h_F = height of a transfer plate due to film diffusion only, cm
k_B = Boltzman constant = 1.3805×10^{-16} g·cm^2/s^2·K
k_F = film mass transfer coefficient, cm/s
l = major axis of polymer rods in Eqs. 26 and 28
L = column length, cm
m = $\epsilon/(1-\epsilon)$
M_f = molecular weight of polymer rods in Eq. 28, g/mol
M_w = molecular weight, g/gmol
n_f = number density of polymer rods, 1/cm^3
N_A = Avagadro's number, 6.023×10^{23}/gmol
r = radial distance from center of particle, cm
r_f = fiber radius, cm
r_s = solute Stokes radius, cm
r_0 = interaction radius between polymer fiber and protein inside the particle, cm
R = average particle radius, cm
R_i = radius of the ith particle, cm
Re = Reynolds number, = $2R\epsilon V\rho/\mu$
Re^f = modified Reynolds number, = $E^{1/3}(2R)^{4/3}\rho/\mu$
s = Laplace transform variable, 1/s
Sc = Schmidt number, = $\mu/\rho D_0$
t = time, s
t_m = retention time of Gaussian peak, s
t_0 = input pulse time, s
T = temperature, K
$\underline{V}$ = interstitial velocity, cm/s
$\bar{V}$ = protein partial specific volume, cm^3/g
V_m = protein molar volume, cm^3/mol
z = axial distance from column inlet, cm

Greek letters

$\alpha(s)$ = function defined in Eq. 11
ϵ = column void fraction
ϵ_p = particle accessible void fraction
κ_f = screening coefficient for polymer rods, Eqs. 26-28
μ = solvent viscosity, g/cm·s
μ_1 = first absolute moment, Eq. 12, s
μ_2 = second central moment, Eq. 13, s^2
ρ = solvent density, g/cm^3
σ = standard deviation of Gaussian peak, s

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