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Surface and pore diffusion in macroporous and gel-filled gigaporous stationary phases for protein chromatography

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Abstract

A recently introduced method [Biotechnol. Prog. 13 (1997) 429] for determining intraparticle mass transfer parameters in high speed liquid chromatography is considered in the present study for the case where the eluite adsorbs onto the stationary phase. The validity of the method was verified theoretically using simulated elution profiles and then applied to experimental data obtained using columns packed with either a macroporous or a gel-filled gigaporous stationary phase. For this purpose, experimental measurements were made using α-lactalbumin and bovine serum albumin as eluites at several retention factors. Apparent intraparticle diffusivities measured for the gel-filled gigaporous stationary phase were seen to increase with the retention factor, which indicates that for this material surface diffusion is a significant mechanism of mass transfer under retained conditions. Data obtained on the macroporous stationary phase revealed that the intraparticle diffusivity was independent of the retention factor, which suggests that pore diffusion remains the principal mass transfer mechanism even under conditions where proteins are adsorbed on the column packing.

Keywords: Stationary phases, LC; Diffusivity; Proteins

1. Introduction

Chromatographic separations of proteins are important not only for analytical purposes, but are also widely used in the biotechnology industry for large scale preparative applications. A variety of stationary phases have been developed for industrial applications in recent years. The optimal use of these stationary phases requires detailed knowledge of their equilibrium and mass transport characteristics.

This work investigates the mass transfer characteristics of several novel stationary phases recently developed for large scale protein purification. Traditionally such studies have been carried out by frontal chromatography where the estimation of mass transfer resistances involves elaborate measurements, requires prior knowledge of the adsorption isotherm and is usually based on the numerical interpretation of breakthrough curves. However, due to the complex nature of chromatographic systems such methods are often subject to interpretation and prone to

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error. Alternatively, in elution chromatography the determination of mass transfer parameters is most commonly based on the evaluation of the moments of the eluite band as a function of eluent flow velocity under isocratic conditions [2–5]. However, this approach is difficult to apply to cases involving proteins and other slowly diffusing species especially at high flow velocities due to the non-Gaussian band shapes that result under these conditions.

It was recently shown by Farnan et al. [1] that instead of using the variation of the second moment with the flow velocity, the dependence of band asymmetry on the flow velocity can be used as a relatively simple method to estimate intraparticle mass transfer resistances. The present work extends the use of this method to the case where the eluites are retained by the column packing and consequently where surface diffusion and adsorption kinetics must be included in the analysis. The extended method is used to analyze mass transfer data in terms of the relative importance of surface diffusion, pore diffusion, adsorption kinetics, and intraparticle convection for two commercially available stationary phases intended for process scale protein chromatography.

2. Theory

2.1. Intraparticle mass transfer

Two independent material balances describe the movement of species through the chromatographic column—one for the interstitial space and the other for the intraparticle space in the column. When eluite transport within the particle space occurs purely by diffusion in the pores, the material balance for the intraparticle space can be written as:

$$\epsilon_i \frac{\partial \bar{c}}{\partial t} + (1-\epsilon_i)\frac{\partial \bar{q}}{\partial t} = D_{eff}\frac{1}{r_c}\frac{\partial}{\partial r_c}\left(r_c \frac{\partial \bar{c}}{\partial r_c}\right) \quad (1)$$

where

$$D_{eff} = \frac{\epsilon_i \lambda}{\theta} D_m \quad (2)$$

In this formalism, ϵ_i is the intraparticle void fraction and $\bar{c}$ and $\bar{q}$ are the concentrations (both in mass per unit volume) of the eluite in the particle pores and in the adsorbed phase, respectively, at the radial position r_c in the particle. Diffusion of the eluite within the pores of the particles is expressed by an effective diffusivity, D_{eff}, which is related to the free solution eluite diffusivity, D_m, through the intraparticle void fraction, ϵ_i, the hindrance parameter, λ, and the tortuosity of the intraparticle pore structure, θ, as described by Eq. (2).

Eqs. (1) and (2) are based on the premise that pore diffusion is the only mechanism for intraparticle mass transport within the particle. More generally, other mechanisms for intraparticle mass transport have also been identified including fluid convection within the pores [6–8] and diffusion of the eluite species in the adsorbed phase along the surface of the stationary phase [9–11]. When more than one mechanism for intraparticle mass transfer is evident, an overall or apparent diffusivity, D_{app}, is determined. This can be expressed as:

$$D_{app} = \left(\frac{\epsilon_i \lambda}{\theta}\right)_{app} D_m \quad (3)$$

Intraparticle convection and surface diffusion can be incorporated into Eq. (1) using the relation developed by Frey et al. [8] to yield:

$$\epsilon_i \frac{\partial \bar{c}}{\partial t} + (1-\epsilon_i)\frac{\partial \bar{q}}{\partial t} = D_{eff}\left(1 + \frac{2Pe'}{45}\right)\frac{1}{r_c}\frac{\partial}{\partial r_c} \times \left(r_c \frac{\partial \bar{c}}{\partial r_c}\right) + D_s \frac{1}{r_c}\frac{\partial}{\partial r_c}\left(r_c \frac{\partial \bar{q}}{\partial r_c}\right) \quad (4)$$

where D_s is the surface diffusivity and Pe' is the Peclet number for the fluid flow within the pores of the particle.

The concentration of the adsorbed species, $\bar{q}$, is related to the concentration of the species in the pore space, $\bar{c}$, through the adsorption isotherm. In the cases of interest here we assume that the amount of the protein present is sufficiently small that the adsorption isotherm is within the linear Henry's law domain, i.e. $K_{eq} = \bar{q}/\bar{c}$. In this regime of linear elution chromatography the amount adsorbed can be expressed by the retention factor, k', which is related to the equilibrium constant and the phase ratio, ϕ, by:

$$k' = K_{eq}\frac{(1-\epsilon_i)(1-\epsilon_e)}{\epsilon_e + \epsilon_i(1-\epsilon_e)} = K_{eq}\phi \quad (5)$$

Where ϵ_e represents the interstitial void fraction, the portion of the column volume between the particles of stationary phase. Using Eq. (5), the right-hand side of Eq. (4) can be rewritten as:

$$\left[D_{\text{eff}}\left(1 + \frac{2Pe'}{45}\right) + \frac{k'}{\phi} D_s \right] \left\{ \frac{1}{r_c} \frac{\partial}{\partial r_c}\left(r_c \frac{\partial \bar{c}}{\partial r_c}\right)\right\} \tag{6}$$

Eq. (6) indicates that when intraparticle convection occurs, the apparent intraparticle diffusivity depends on the velocity of the mobile phase in the column while if surface diffusion occurs the apparent intraparticle diffusivity depends on the retention factor. In general, intraparticle convection contributes significantly to intraparticle mass transport only when the nominal pore diameter is greater than 1/20 of the particle diameter and the reduced velocity is relatively high [8]. Neglecting the contribution of intraparticle convection to mass transport the apparent diffusivity, D_{app}, can be expressed in terms of D_s and D_{eff} as:

$$D_{\text{app}} = D_{\text{eff}} + K_{\text{eq}} D_s \tag{7}$$

Eq. (7) implies that the variation of D_{app} with K_{eq} can be used to determine both D_{eff} and D_s from experimental data. Finally for the case where adsorption kinetics contributes to the band spreading, Eq. (7) can be extended to yield the following result for D_{app}:

$$\frac{1}{D_{\text{app}}} = \frac{1}{D_{\text{eff}} + K_{\text{eq}} D_s} + \left(\frac{(1-\epsilon_e)K_{\text{eq}}}{\epsilon_e (1-\epsilon_e) K_{\text{eq}}} \right)^2 \frac{60}{k_{\text{ads}} d_p^2} \tag{8}$$

where k_{ads} is the adsorption rate constant and d_p is the nominal resin particle diameter.

2.2. Determination of the apparent diffusivity

For the case of proteins eluting from a chromatographic column under conditions of no retention and when the number of theoretical plates is small, i.e. within the entrance length of the column, it was shown [1] that the variation of peak asymmetry with the flow velocity offers a simple means to estimate intraparticle mass transfer resistances which avoids the difficulties inherent in determining higher statistical moments of asymetrical peak shapes. For this purpose it is convenient to define the peak asymmetry as:

$$\chi = \frac{V_A - V_I}{V_R - V_I} \tag{9}$$

where V_A and V_R are the apical elution volumes (measured at the peak apex) and the first moment of the eluted peak, respectively, and V_I is the interstitial volume of the packed column. Furthermore, an extended Peclet number, Pe_R, can be defined as:

$$Pe_R = Pe \frac{d_p}{4L} \frac{\theta}{\epsilon_i \lambda} \tag{10}$$

where $Pe = ud_p/D_m$

The reduced plate height, h, for the case where liquid mass transfer resistances can be neglected is given by [4]:

$$h = h_{AD} + \frac{\mu\theta}{\epsilon_i \lambda} Pe \tag{11}$$

where h_{AD} accounts for axial dispersion, flow maldistribution, and extracolumn band broadening and where, for spherical particles:

$$\mu = \frac{\epsilon_e (1-\epsilon_e)(\epsilon_i + (1-\epsilon_i)K_{\text{eq}})^2}{30(\epsilon_e + (1-\epsilon_e)(\epsilon_i + (1-\epsilon_i)K_{\text{eq}}))^2} \tag{12}$$

If h_{AD} can be neglected, i.e. when Pe is large, Eq. (11) becomes:

$$N = \frac{1}{4\mu Pe_R} \tag{13}$$

where N is the plate number. In order to develop a convenient means to relate χ to the physical properties of a chromatographic system, a number of simulations were performed using the analytical solution of Carta and co-workers [12,13]. Although χ in general is a function of a number of parameters, the simulation results indicate that to a good approximation χ can be considered as single-valued function of Pe_R. Thus the following relation:

$$\chi = \frac{1}{1 + (Pe_R/4.7)^{1.58}} \tag{14}$$

which was originally developed for an unadsorbed eluite can be used for the evaluation of χ. Fig. 1

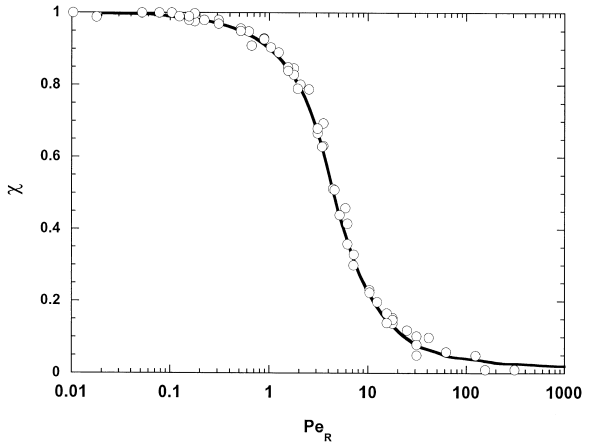

Fig. 1. Variation of χ with Pe_R calculated under retaining conditions (circles) compared with that predicted from Eqs. (10) and (14) (solid line). Column parameters used in the calculations were in the ranges: $0.05 < \epsilon_i < 0.6$, $0.3 < \epsilon_e < 0.6$, $0 < k' < 5$ and $500 < L/d_p < 3500$.

shows a comparison of Eq. (14) with calculations of χ for various values of Pe_R for the case where k' varies. These calculations were made using a revised form of the solution [1] originally published by Carta et al. [12,13]. As shown, Eq. (14) agrees well with the results of the rigorous calculations of peak shape. In contrast, Fig. 2 indicates that the use of N to form a single valued function of χ leads to a high level of scatter.

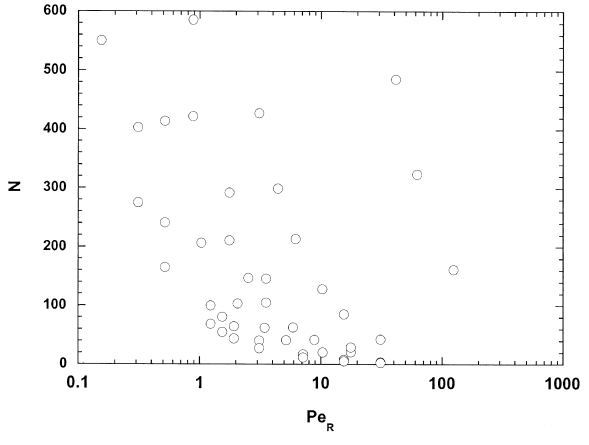

Fig. 2. Variation of the plate number, N, with Pe_R calculated using the column parameters given in Fig. 1.

3. Experimental

3.1. Materials

Bovine serum albumin (BSA) and α-lactalbumin were purchased from Sigma (St. Louis, MO). Polyvinyl pyrrolidone having a molecular mass of 1200 kDa and with the trade name Kol-90 was donated by BASF (Wayne, NJ). Reagent grade sodium chloride and concentrated hydrochloric acid was supplied by Baxter (McGaw Park, IL). Ultrapure Tris was obtained from USB (Cleveland, OH). Deionized water was prepared using a Barnsted Nanopure water filtration system (Dubuque, IA).

Buffer solutions were prepared by dissolving the appropriate amount of Tris and NaCl to give the required concentration. The solution was adjusted to the appropriate pH using 1 *M* HCl and a Radiometer Model PMH 82 pH meter (Radiometer America, Westlake, OH), calibrated between pH 7.00 and 10.00 immediately prior to each titration.

3.2. Columns

All columns were No. 316 stainless steel, 75×4.6 mm. The macroporous Macro-Prep High Q strong anion-exchanger particles, having a diameter of 50 μm and a pore size of 1000 Å, were donated by Bio-Rad Laboratories (Hercules, CA). Q HyperD F gel-filled anion-exchanger particles having a nominal diameter of 35 μm were donated by Biosepra (Marlborough, MA).

The Q Hyper D particles were screened before use, and the 325–400 mesh (38–44 μm) fraction was used to pack the column. These particles were packed at 2000 p.s.i. using a 60% (v/v) slurry in 50 m*M* Tris–HCl pH 8.5 containing 1.0 *M* NaCl. Macro-Prep High Q particles were first introduced into the column in the dry state. The packing liquid, Tris–HCl at pH 8.5 containing 0.5 *M* NaCl, was then pumped through the column at elevated flow velocities for 1 h in order to stabilize the column. The process was repeated until no further reduction in bed height was observed.

3.3. Instrumentation

All experiments were performed on a Hewlett-

Packard Model 1090 (Palo Alto, CA) liquid chromatograph equipped with a DRV5 high-performance pumping system and an auto injector. A Perkin-Elmer (Norwalk, CT) LC-95 variable wavelength UV–Vis detector, fitted with a 1.4 μl flow cell, was placed in close proximity to the left hand side of the chromatograph at the same level as the body of the valve of the injection system in order to reduce the dead volume of the connecting tubing. Connecting tubing was 125 μm I.D. PEEK with finger tight fittings (Upchurch Scientific, Oak Harbor, WA).

Data was collected using Hewlett-Packard Chemstation software, which was installed on a Hewlett-Packard Vectra 486DX33 IBM compatible computer running the Microsoft Windows 3.1 operating system. The LC-95 detector was connected to the computer through a Hewlett-Packard Model 35900 D/A interface.

3.4. Procedures

3.4.1. Determination of column domains

Several experiments were performed to measure the volumes explored in the packed column by two markers of widely different molecular masses, NaCl and PVP-Kol 90, as well as by two proteins. The mobile phase was 50 m*M* Tris–HCl at pH 8.5 containing 0.5 *M* NaCl. The flow-rate was 0.2 ml min^{-1}, which was low enough to obtain symmetrical peaks. The retention times were sufficiently long that it was unnecessary to correct for the delay time between the sample introduction and the beginning of data acquisition, which was less than 1 s. Each measurement was repeated five times, and the error in the elution times was less than 1% for each eluite at any given set of conditions.

3.4.2. Peak shape analysis

α-Lactalbumin and bovine serum albumin were chromatographed over a wide range of Peclet numbers at several retention factors for each of the stationary phase and eluite combinations. Analyte retention was controlled by adjusting the sodium chloride concentration in the eluents. The retention factor was calculated from the numerically determined first moment of the analyte peak under retaining and non-retaining conditions. Special care was taken to ensure the fidelity and accuracy of data handling and analysis. For this purpose, the detector was operated at the lowest possible time constant, 20 ms, with computer-based data acquisition occurring at the complementary rate of 50 Hz. Hewlett-Packard Chemstation software was used to determine the zeroth and first central moments for the peaks with manually identified integration limits. The volumetric first moment and the apical elution volume were corrected for the time interval between the physical act of injection and the commencement of data acquisition. The true elution volume was determined from the variation of the observed elution volume with the eluent flow-rate, as the reciprocal slope of the regression obtained by plotting the reciprocal flow-rate against the observed elution time.

4. Results and discussion

4.1. Determination of column domains

Due to the large molecular mass of PVP-Kol 90 it was assumed that this molecule is excluded from the particle pore space and therefore had an elution volume equal to the column interstitial void volume. Interstitial void fractions for the columns packed with the macroporous and gel-filled gigaporous phases were 0.4 and 0.43, respectively. The difference between the elution volume of the proteins and that of PVP was used to determine the intraparticle void fraction explored by the proteins, and the results are summarized in Table 1. Values of the intraparticle void fraction calculated in the same way for the dipeptide Leu-Val are also shown as they are considered to be estimates for the total volume of liquid within the column and representative of the total porosity of the particle.

4.2. Determination of apparent diffusivity

4.2.1. Macroporous particles

Figs. 3 and 4 show the dependence of apical elution volume on *Pe* for the Macro-Prep High Q stationary phase with α-lactalbumin and BSA as eluites at several different retention factors. Also shown are the best fitting curves calculated using Eq. (13) with the fitted $(\epsilon_i \lambda/\theta)_{app}$ parameters listed in Table 2. As shown by the plots there is good

Table 1
Intraparticle void fractions for the macroporous and the gel-filled gigaporous column packings. The mobile phase was 50 m*M* Tris–HCl, pH 8.5, containing 0.5 *M* NaCl and the flow-rate was 0.2 ml min^{-1}

Tracer	M.W. (kDa)	p*I*	ϵ_i	
			Macroporous	Gel-filled gigaporous
Leu-Val	0.23	–	0.53	0.51
α-Lactalbumin	14.2	4.8	0.49	0.10
BSA	64	5.0	0.44	0.02

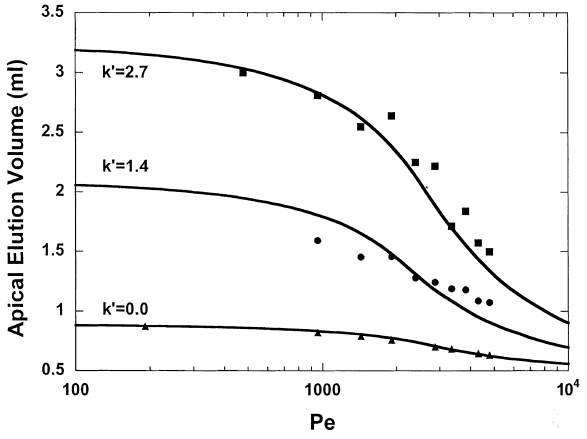

Fig. 3. Observed and calculated variation (according to Eq. (13)) of the apical elution volume with *Pe* using α-lactalbumin as an eluite on Macro Prep High Q column at several different retention factors. Eluent: 50 m*M* Tris–HCl, pH 8.5, with NaCl added to achieve the stated retention factor.

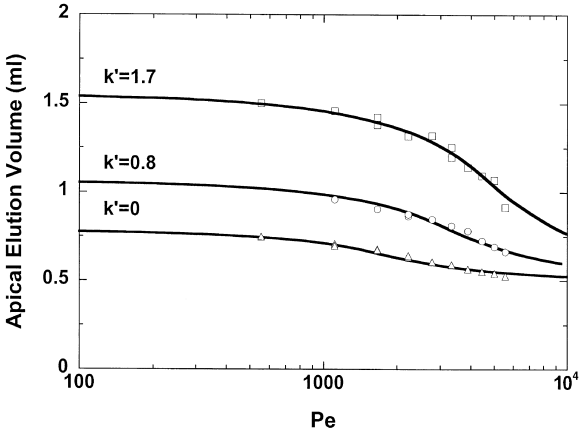

Fig. 4. Observed and calculated variation of the apical elution volume with *Pe* using BSA as the eluite on the Macro Prep High Q column at several retention factors. Eluent: 50 m*M* Tris–HCl, pH 8.5, with NaCl added to achieve the stated retention factor.

agreement between the experimental data and the theoretical curves.

As anticipated due to it's relatively large molecular size, BSA encounters a higher mass transfer resistance than the smaller α-lactalbumin. This is reflected in the respective values of the term $(\epsilon_i \lambda/\theta)_{app}$, which are 0.072 and 0.107 for BSA and α-lactalbumin. From the column domain experiments summarized in Table 1 it is seen that the difference in ϵ_i shown (i.e. about 10%) does not account for the entire difference in the observed values of $(\epsilon_i \lambda/\theta)_{app}$ for the two proteins. Instead, λ/θ is approximately 30% less for BSA than for α-lactalbumin. Furthermore, it is seen in view of Eq. (7) that in the case of both albumin and α-lactalbumin a single value of D_{app} permitted the fitting of the apical elution data obtained at different k'. This important finding shows that pore diffusion is the dominant mechanism for mass transfer, as opposed to surface diffusion. The results also indicate that the adsorption of the proteins by the stationary phase surface does not significantly alter the accessibility of the particle interior, as has been suggested to occur in particles of smaller pore size [14].

4.2.2. Gel-filled gigaporous particles

Figs. 5 and 6 illustrate the dependence of the apical elution volume on *Pe* obtained when α-lactalbumin and BSA were eluted from a column packed with gel-filled gigaporous particles at several values for k'. It is seen that there is a good agreement between the experimentally determined variation of V_A with *Pe* and the corresponding curves calculated using the relations in Section 2.2 and the fitting parameters given in Table 2.

In previous work [1] the partioning and mass transfer behavior inside similar gel-filled gigaporous particles as used in the present study were examined

Table 2
Compilation of mass transfer parameters obtained from data measured on the macroporous and gel-filled gigaporous strong anion-exchanger columns with three eluites at several retention factors in the range 0–6.7

Stationary phase	Eluite	Retention factor, k'	$(\epsilon_i \lambda/\theta)_{app}$	Biot No.
Macro Prep	α-Lactalbumin	0	0.11	35
Macro Prep	α-Lactalbumin	1.4	0.11	35
Macro Prep	α-Lactalbumin	2.7	0.11	35
Macro Prep	BSA	0	0.07	52
Macro Prep	BSA	1.3	0.07	52
Macro Prep	BSA	4.7	0.07	52
Q HyperD F	α-Lactalbumin	0	0.03	93
Q HyperD F	α-Lactalbumin	0.9	0.17	19
Q HyperD F	α-Lactalbumin	2.4	0.28	11
Q HyperD F	BSA	0	0.01	1790
Q HyperD F	BSA	2.2	0.20	32
Q HyperD F	BSA	6.7	0.40	16

The calculated Biot numbers are also listed.

under non-retaining conditions. The results were consistent with models in which the eluites diffuse through a medium composed of liquid surrounding solid cylinders which represent the polymer chains in the gel. According to the models, the polymer chains have a nominal diameter of 10 Å, a length equal to the pore diameter and occupy 5% of the pore volume. Previously [1] two models had been used. The first by Ogston [15] described the dependence of ϵ_i on the protein diameter. Variation of θ/λ with protein diameter was described using the second model, developed by Cukier [16]. Combining the formalisms of both models we can derive an equation to describe the dependence of $\epsilon_i \lambda/\theta$ on the eluite diameter, this equation can be written thus:

$$\frac{\epsilon_i \lambda}{\theta} = \frac{1}{\phi_{sol}} \exp\left[\frac{3}{\sqrt{2}}\left(1+\frac{d_{elu}}{d_{pol}}\right)^4 \times \left(\frac{\phi_{pol}^3}{\ln(l_{pol}/d_{pol})}\right)^{1/2}\right] \quad (15)$$

Fig. 7 illustrates calculations using Eq. (15) for the case of the gel-filled gigaporous particles with the

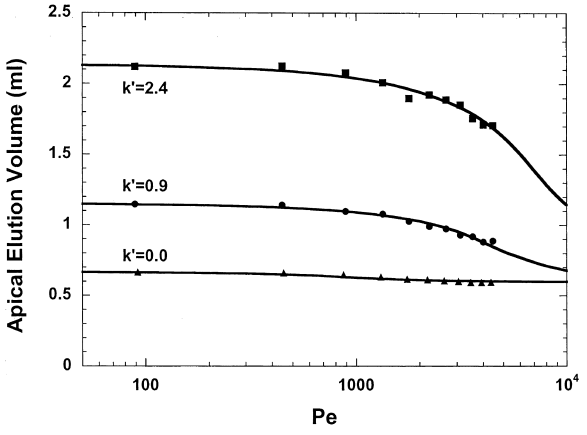

Fig. 5. Observed and calculated variation of apical elution volume with *Pe* using α-lactalbumin as the eluite at several retention factors for the gel-filled gigaporous stationary phase.

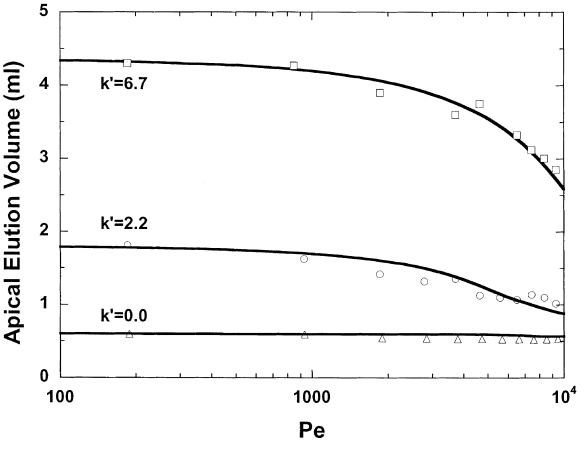

Fig. 6. Observed and calculated variation of apical elution volume with *Pe* using BSA as a probe at several retention factors for the gel-filled gigaporous stationary phase.

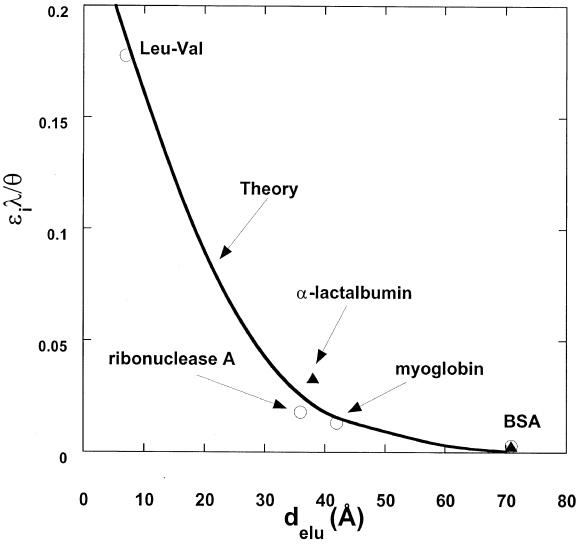

Fig. 7. Comparison of the experimentally observed dependence of the mass transfer parameter ($\epsilon_i\lambda/\theta$), on the diameter of the eluite molecule with that predicted using Eq. (14) for un-retained eluites. Data points shown by open circles were taken from Ref. [1] while the solid triangular data points were measured in the present study.

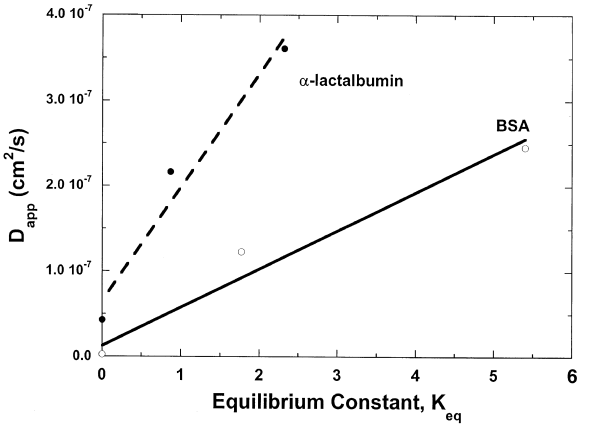

Fig. 8. Apparent diffusivity as a function of K_{eq} observed for α-lactalbumin and BSA on a gel filled gigaporous stationary phase. D_{app} values were obtained by fitting the data shown in Figs. 5 and 6.

parameters determined as mentioned above. For comparison, the experimentally determined values of $(\epsilon_i\lambda/\theta)_{app}$ in Table 2 and those published previously [1] are also shown to illustrate the good agreement between the data and the predictions of Eq. (15).

Examination of the data tabulated for the gel-filled gigaporous and the macroporous stationary phase shown in Table 2 reveals that for the macroporous stationary phase $(\epsilon_i\lambda/\theta)_{app}$ is independent of the retention factor, whereas for the gel-filled gigaporous stationary phase a distinct increase in $(\epsilon_i\lambda/\theta)_{app}$ is seen to occur with increasing retention.

According to Eqs. (7) and (8), an increase in D_{app} with K_{eq} suggests that surface diffusion contributes to mass transfer (assuming that D_s is constant), while a decrease in D_{app} with K_{eq} suggests that adsorption kinetics contributes to mass transfer (assuming k_{ads} is a constant). The fact that the former situation is seen to apply to gel filled gigaporous stationary phases indicates that surface diffusion is a significant contributor to the rate of mass transfer for the material. More specifically, according to Eq. (6) to estimate the surface diffusivity, D_s, it is necessary to know the apparent diffusivity as a function of K_{eq}, with the slope of the functional relation yielding the surface diffusivity. Fig. 8 illustrates the variation of the apparent diffusivity, D_{app}, with K_{eq} observed using the gel-filled gigaporous stationary phase. For both α-lactalbumin and BSA, a linear variation of D_{app} with the k' is seen, with the data indicating that the surface diffusivities for α-lactalbumin and BSA in the gel-filled gigaporous support are 1.33×10^{-7} cm^2 s^{-1} and 4.5×10^{-8} cm^2 s^{-1}, respectively. These values are approximately 5–10% of the free solution diffusivites [17] and somewhat larger than the pore diffusivities for this column packing.

4.3. Film mass transfer

In the theoretical framework for interpreting the experimental data it was assumed that the liquid film mass transfer resistance was negligible in comparison to the intraparticle mass transfer. This assumption can be verified by calculating the Biot number for each eluite at each retention factor on both stationary phases. The correlation of Carberry [18] for predicting film mass transfer coefficient, at the lowest flow velocity, was used in conjunction with the apparent intraparticle diffusivities to estimate the Biot number defined as follows [19]:

$$Bi = \frac{k_f d_p}{2D_{app}} \tag{16}$$

Since the intraparticle mass transfer resistance does not vary with K_{eq} for the macroporous stationary phase, the Biot number is seen to be invariant with the retention factor, although it is a different value for the two eluites employed. Nevertheless, for both eluites in this case, the values of *Bi* were at least an order of magnitude higher than unity, which indicates that the film mass transfer is still a negligable contribution to the overall mass transfer resistance in the flow velocity range studied here.

The Biot number calculated for the gel-filled gigaporous stationary phase reveal a contrasting situation in that *Bi* decreases rapidly with increasing k'. However, over the range of k' studied here, $0 < k' < 6.7$, *Bi* was greater than 10, which indicates that the liquid film resistance can still be neglected. However, for higher k' value, which is a situation frequently encountered when frontal chromatography is used to investigate mass transfer behavior, it is likely that the major source of mass transfer resistance would be in the liquid film surrounding the column packing particles. Such a conclusion was recently reached by Carta and co-workers for this same column packing [20–22].

5. Conclusions

The measurement and theoretical interpretation of the dependence of peak asymmetry for retained eluites on the flow velocity is demonstrated here to yield a convenient method for the evaluation of intraparticle mass transfer resistances by linear elution chromatography. The method is particularly convenient for investigating mass transfer behavior at high flow-rates where eluite peaks are not Gaussian in shape, and methods involving the evaluation of statistical moments are difficult to apply.

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