Prediction of Adsorption and Desorption of Protein on Dextran Based Ion-Exchange Resin

The equilibrium and kinetic adsorption and desorption of bovine serum albumin (BSA), fraction V, on Sephadex A-50 has been studied. The equilibrium data have been presented by a Langmuir isotherm for both adsorption and desorption conditions. The kinetics of adsorption in a batch system may be represented by a simple diffusional model. The kinetics of desorption of BSA in a batch system show a very fast initial rate due to the rapid shrinkage of the resin with changes in salt concentration. This has been modeled by including a protein exclusion term in the kinetics for desorption. The above information has been used to predict the breakthrough of BSA in a column operation. Under the pressure exerted in a column flow system the Sephadex A-50 is severely compressed. The effect this has on the breakthrough rate of BSA has been demonstrated.

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SCOPE

There is a definite need for more published systemic studies dealing with protein separations. At present much of the published work in this area deals with very specific and applied systems in which it is not clear whether what is presented is the best or optimized system. In this work a simple two-phase resistance model has been applied to predict both batch kinetics and column breakthrough for bovine serum albumin (BSA) on a dextran-based resin.

The equilibrium isotherms are represented by Langmuir isotherms for both adsorption and desorption. The importance the equilibrium distribution has in establishing the rate controlling step for the kinetic model is demonstrated. The rate of adsorption for batch operation or the breakthrough curve can be predicted once the equilibrium isotherm of the proteins is known.

During protein desorption caused by an increase in the salt concentration the resin undergoes rapid shrinkage, which greatly enhances the initial rate. This can be modeled by including a protein exclusion term in the two-phase resistance model.

For a resin with soft, gellike structures like that used in this study there is the possibility of severe bed compression during column operation. This has been shown either to severely reduce the maximum flow rate that can be used, or under high flow rates to significantly reduce the capacity of the resin for protein. It is believed the approach and general model presented here would be applicable to other resins and multicomponent protein separations.

CONCLUSIONS AND SIGNIFICANCE

For Sephadex A-50 the equilibrium isotherm for BSA in a 0.005M phosphate buffer has been determined under adsorption and desorption conditions at a pH of 6.9 and 6.5, respectively. Under adsorption conditions (no NaCl) the maximum capacity of Sephadex A-50 for BSA is approximately 8,000 mg protein/g dry resin. With 1% NaCl present (desorption conditions) the maximum capacity drops to approximately 400 mg/g. A Langmuir isotherm was used to fit the data.

Batch kinetics for both adsorption and desorption have been studied. For adsorption the simple two-phase diffusion model predicts the observed kinetics very well. For desorption the severe shrinkage of the resin enhances the rate of mass transfer significantly. This has been successfully modeled by including a term for excluded protein during shrinkage.

Finally, the results have been applied to predict the breakthrough rate of BSA for a column packed with Sephadex A-50. Due to the "soft" structure of Sephadex A-50 the bed is severely compressed during operation, resulting in a lower maximum capacity for the resins (~7,000 mg/g). The breakthrough curve can be predicted assuming film diffusion controlling and a Langmuir isotherm with the maximum capacity reduced to account for bed compression.

The general model used here should be applicable to other resins and proteins and should allow the prediction and optimization of protein separations in either batch or column operation.

TABLE 1. PROPERTIES OF DEAE SEPHADEX A-50 ION EXCHANGER

Description	Weakly basic anion exchanger
Functional group	Diethylaminoethyl
Counterion	Chloride
Size* (average radius determined by calibrated microscope)	0.116 mm (0% NaCl, before adsorption) 0.116 mm (0% NaCl, after adsorption) 0.083 mm (1% NaCl, after desorption)
Density* g (dry)/cm^3 packed bed	0.0155 (0% NaCl)
Voidage of packed bed* (extraparticle voidage)	0.61
Effective pH range	2–9
pK of DEAE group	9.5
Total capacity**	3.5 ± 0.5 mg/g
Available capacity†	
α-lactalbumin (MW 14,000)	741 mg/g
Albumin (MW 67,000)	7,566 mg/g
Hemoglobin (MW 69,000)	5,000 mg/g
Ferritin (MW 440,000)	74 mg/g

* All values were obtained in 0.005 M phosphate buffer, pH 6.9 for 0% NaCl, pH 6.5 for 1% NaCl.
** Total capacity was estimated in Tris-HCl buffer, pH 6.9, ionic 0.1 (Pharmacia, 1980). This approximate value will vary with the starting conditions chosen.
† Value was determined in 0.01 M Tris-HCl buffer, pH 9.0 (Pharmacia, 1980).

INTRODUCTION

There exists a large number of studies of the separation of proteins using ion-exchange chromatrography, both on the laboratory scale and on the commercial scale (Curling, 1980; Heide, 1977; Janson, 1982; Sober, 1965; Wang, 1982). In all these studies rules of thumb and time-consuming column experimentation have been used to obtain the separation. As these types of separations become more common (Curling, 1983) and higher-quality purification is demanded, a method to optimize the separations with the minimum amount of experimentation is needed. At present no such method of predicting protein separations exists.

It is the purpose of this work to extend the simple diffusion model presented by Graham and Fook (1982) to explain the rate of adsorption and desorption of bovine serum albumin (BSA) on Sephadex A-50 for both batch operation and column operation.

EXPERIMENTAL

The ion-exchange resin DEAE-Sephadex A-50 was used for the equilibrium and kinetic studies. The properties of this resin as measured in this lab and obtained from the manufacturer's specifications are summarized in Table 1. The protein used was bovine serum albumin, fraction V.

Equilibrium Studies

The equilibrium data were obtained by using a shallow-bed reactor that gave efficient contact between the solution and the ion-exchange resin. Typically, the bed height was 10–20 mm, representing 1–2 mL of resin (0.02 to 0.04 g resin on a dry basis). Flow rates ranged from 0.02–0.08 mL/s. Samples were collected continuously and analyzed for protein using U.V. absorption at 280 nm. A 0.005M phosphate buffer at a pH of 6.9 was used in all studies. For desorption conditions the same procedure was used but 1% NaCl was added to the protein solution. The temperature was maintained constant at 20 ± 1°C by room temperature control.

Since the pI of BSA is approximately 4.9 under all the conditions studied, the BSA molecules would be highly charged. However, with no salt present, even with over 8,000 mg of protein per gram of resin adsorbed the resin radius was essentially independent of protein concentration. However, with 1% NaCl present the radius of the resin is significantly reduced from 0.116 to 0.083 mm (see Table 1). The importance of this is discussed under the results for the desorption of BSA.

Rate Studies

For batch kinetic studies 0.2 to 1.0 g resin (dry basis) was agitated in 500 mL of protein solution at 30 rps. Four-milliliter samples were withdrawn periodically for protein analysis. The temperature was maintained constant at 20 ± 0.5°C by a constant-temperature bath. The kinetics of adsorption were measured with and without 1% NaCl. In both cases the pH was 6.9 and a 0.005 M phosphate buffer was used. For desorption studies the same procedures were used except that the resin injected initially contained 3,200 mg/g protein and the solution was 500 mL of 0.005 M phosphate buffer with 1% salt.

For the column studies a column diameter of 8.7 mm was used. Ten milliliters of preconditioned resin (equivalent to 0.155 g of dry resin) was placed in the column. It was found necessary to pass buffer through the column at a flow rate of approximately 6×10^{-8} m^3/s (220 mL/h) for 1–2 h. During this time the resin compacted and the measured resin height decreased. For the experiment reported here a protein concentration of 1 mg/g was used and a flow rate of 1×10^{-9} m^3/s (36 mL/h) ± 10% was maintained using a peristaltic pump. Without the initial conditioning of the bed at a high flow rate the desired flow rate of 1×10^{-9} m^3/s could not be maintained constant with the pumping system used. The outlet from the column was continuously monitored with U.V. at 280 nm.

THEORY

Rate of Adsorption for Batch Operations

The two-phase diffusion model given by Graham and Fook (1982) was used as the basis for adsorption. The mass flux is given by

$$N = k(C - C_i) = \bar{k}(\bar{C}_i - \bar{C}) = K(C - C^*) \tag{1}$$

where the overall mass transfer coefficient is defined by

$$\frac{1}{K} = \frac{1}{k} + \frac{1}{\bar{k}m'} \tag{2}$$

The final result for this model (Graham and Fook, 1982; Tsou, 1983 is

$$\ln[1 - F(t)] = -\frac{K\bar{A}\theta}{\bar{W}}\left(\frac{\bar{W}}{W} + \frac{1}{m''}\right) \tag{3}$$

All the symbols are defined in the Notation. Equation 3 is a general relation giving fractional attainment of equilibrium $F(t)$ as a function of total elapsed time θ. In this development the rate constant K and equilibrium parameters m' and m'' were assumed constant, which is generally a reasonable assumption over a moderate protein concentration range.

In the limit where film diffusion controls (m' very large) Graham and Fook approximate

$$K = k = \frac{D\rho_s}{\delta} \tag{4}$$

and Eq. 3 reduces to

$$\ln[1 - F(t)] = -\frac{D\bar{A}\rho_s\theta}{\delta\bar{W}}\left(\frac{\bar{W}}{W} + \frac{1}{m''}\right) \tag{5}$$

In the limit where particle diffusion controls the mass transfer coefficient is approximated (Tsou, 1983) by

$$K = \bar{k} = \frac{15\bar{D}}{r^2}\frac{\bar{W}}{\bar{A}} \tag{6}$$

and Eq. 3 reduces to

$$\ln[1 - F(t)] = -\frac{15\bar{D}m'}{r^2}\theta\left(\frac{\bar{W}}{W} + \frac{1}{m''}\right) \tag{7}$$

Rate of Desorption for Batch Operations

In this study, desorption was achieved by increasing the salt (NaCl) concentration. The salt contains the counterion, Cl^-,

which can facilitate equilibration. However, the swelling property of the ion exchanger used in this experimental work, DEAE-Sephadex A 50, is very sensitive to the ionic strength. In the experimental region studied, an increase in salt concentration from 0 to 1% caused the swollen exchanger to shrink by as much as 72% of its original volume. This significant change in volume greatly enhanced the rate of desorption, which cannot be explained by the two-film theory alone. However, by assuming an additive process of protein exclusion during shrinkage that occurs independently of the mass transfer by diffusion, a simple rate expression for desorption can be derived. Although this assumption is not physically justifiable, the rate equation thus derived can satisfactorily explain the rate data and provides a reasonable value for the effective diffusivity inside the particle.

The kinetics of swelling and shrinkage of ion exchangers is a complicated problem, one which has as yet received very little attention. Swelling and shrinking are accompanied by profound changes in the medium in which the processes take place. As swelling progresses, the matrix expands and the solute molecules thus become more mobile. However, it is unlikely that swelling and shrinking can be adequately described in terms of a solute diffusion coefficient which depends on the solvent concentration only. Rather, one must expect that this coefficient is also affected by the mechanical strain of the network (Crank, 1953; Downes, 1959), and possibly by time-dependent relaxation phenomena of the network (Parks, 1953).

Pratt and Cooney (1973) studied the kinetics of swelling of dextran gel, Sephadex G-25 and G-50, due to changes in salt concentration and found the kinetics were of first order.

For desorption the flux is given by

$$N = \bar{K}(\bar{C}^* - \bar{C}) \tag{8}$$

where $\bar{K}$ is an overall mass transfer coefficient defined by

$$\frac{1}{\bar{K}} = \frac{1}{\bar{k}} + \frac{\bar{m}'}{k} \tag{9}$$

The final general result for desorption (Graham and Fook, 1982; Tsou, 1983) is

$$\ln [1 - F(t)] = \int_0^\theta -\frac{\bar{A}}{\bar{W}} \bar{K} \left(1 + \bar{m}'' \frac{\bar{W}}{W}\right) dt \tag{10}$$

For the common case where $\bar{m}''(\bar{W}/W \ll 1$ Eq. 10 becomes

$$\ln [1 - F(t)] = \int_0^\theta -\frac{\bar{A}}{\bar{W}} \bar{K} dt \tag{11}$$

For desorption the equilibrium is unfavorable and one may often assume particle diffusion controls so the concentration at the particle surface is in equilibrium with the solution. Using Eq. 6 the time rate of change of $\bar{C}$ due to diffusion is then

$$\left(\frac{dc}{dt}\right)_D = \frac{\bar{W}}{\bar{A}} \frac{15D}{r^2} (\bar{C}^* - \bar{C}) \tag{12}$$

Mass transfer for desorption is considered to depend on two independent mechanisms. One is the mass transfer due to diffusion. The other is mass transfer due to exclusion caused by the shrinking. As for the mass transfer due to shrinking, it is assumed that mass is left behind with the excluded volume. So if the internal concentration of the particle is $\bar{C}$, the concentration of protein being excluded will be $(\bar{C}/\bar{V})d\bar{V}$. However, since equilibrium conditions exist at the particle surface, the true concentration of protein being excluded should be $[(\bar{C}/\bar{V}) - (\bar{C}^*/\bar{V})]\bar{V}$ and

$$\left(\frac{d\bar{C}}{dt}\right)_E = \frac{\bar{C} - \bar{C}^*}{\bar{V}} \frac{d\bar{V}}{dt} \tag{13}$$

By assuming the rate of shrinkage is of first order and independent of diffusion, Eq. 13 becomes

$$\left(\frac{d\bar{C}}{dt}\right)_E = \frac{\bar{C} - \bar{C}^*}{\bar{V}} K_v(\bar{V}_f - \bar{V}) \tag{14}$$

where

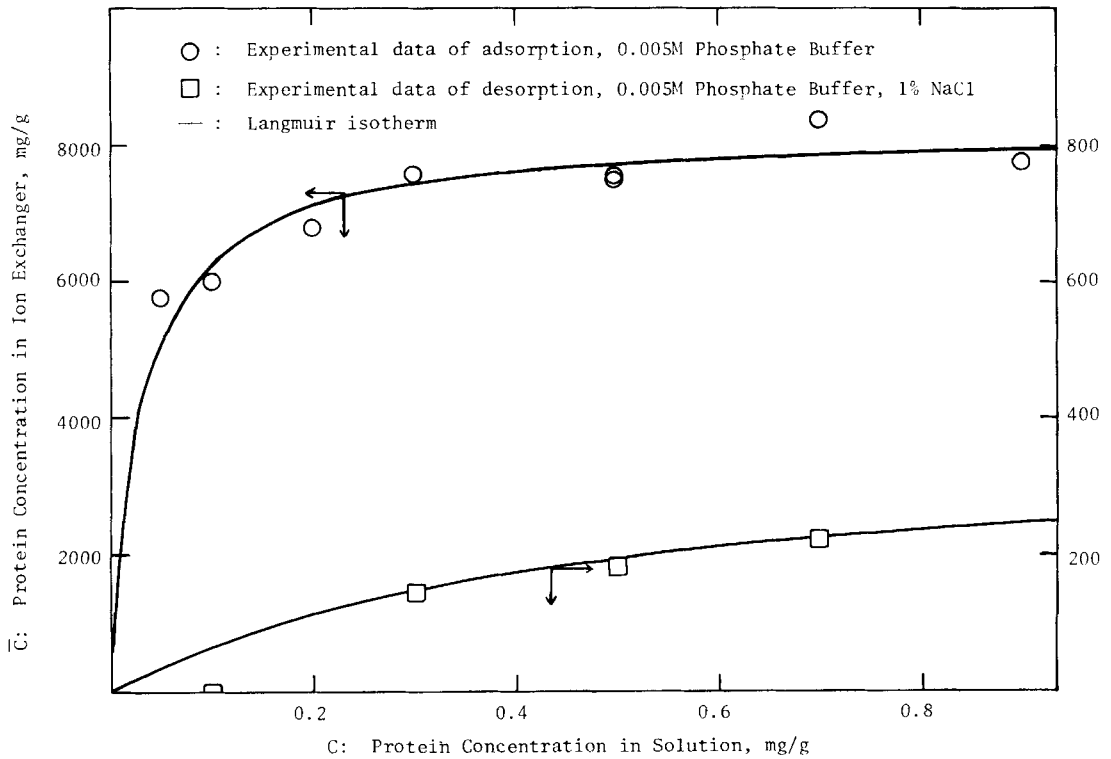

Figure 1. Equilibrium curves for adsorption and desorption on DEAE-Sephadex A-50 ion-exchange resin.

TABLE 2. EXPERIMENTAL CONDITIONS FOR EQUILIBRIUM STUDIES

	Adsorption	Desorption
Buffer	0.005 M Phosphate	0.005 M Phosphate
NaCl content	0%	1%
pH	6.9	6.5
C_m	8224 mg/g	364 mg/g
K_e	32.7 g/mg	2.26 g/mg
Langmuir isotherm	$8{,}224\frac{32.7C}{1+32.7C}$	$364\frac{2.26C}{1+2.26C}$

K_v: rate constant for shrinkage, s^{-1}.

Combining Eq. 14 with Eq. 12, the overall rate of change of $\bar{C}$ can be obtained.

$$\frac{d\bar{C}}{dt} = \left(\frac{d\bar{C}}{dt}\right)_D + \left(\frac{d\bar{C}}{dt}\right)_E \tag{15}$$

$$\frac{d\bar{C}}{dt} = \left(\frac{15\bar{D}}{r^2} - \frac{1}{V}\frac{dV}{dt}\right)(\bar{C}^* - \bar{C}) \tag{16}$$

Comparing Eq. 16 with Eq. 8, it can be shown that

$$\bar{K} = \left(\frac{15\bar{D}}{r_2} - \frac{d(\ln \bar{V})}{dt}\right)\frac{\bar{W}}{\bar{A}} \tag{17}$$

Substituting Eq. 17 into Eq. 11 and integrating from $t = 0$ to $t = \phi$, the following rate expression can be derived.

$$\ln[1 - F(t)] = \frac{-45\bar{D}}{r_f^2 K_v}\left(\frac{1}{6}\ln\frac{z^2+z+1}{(1-z)^2} + \frac{1}{3^{1/2}}\tan^{-1}\frac{2z+1}{3^{1/2}}\right) + \frac{45\bar{D}}{r_f^2 K_v}\left(\frac{1}{6}\ln\frac{y^2+y+1}{(1-y)^2} + \frac{1}{3^{1/2}}\tan^{-1}\frac{2y+1}{3^{1/2}}\right) + \ln\left(\frac{1+(R-1)\exp(-K_v\theta)}{R}\right) \tag{18}$$

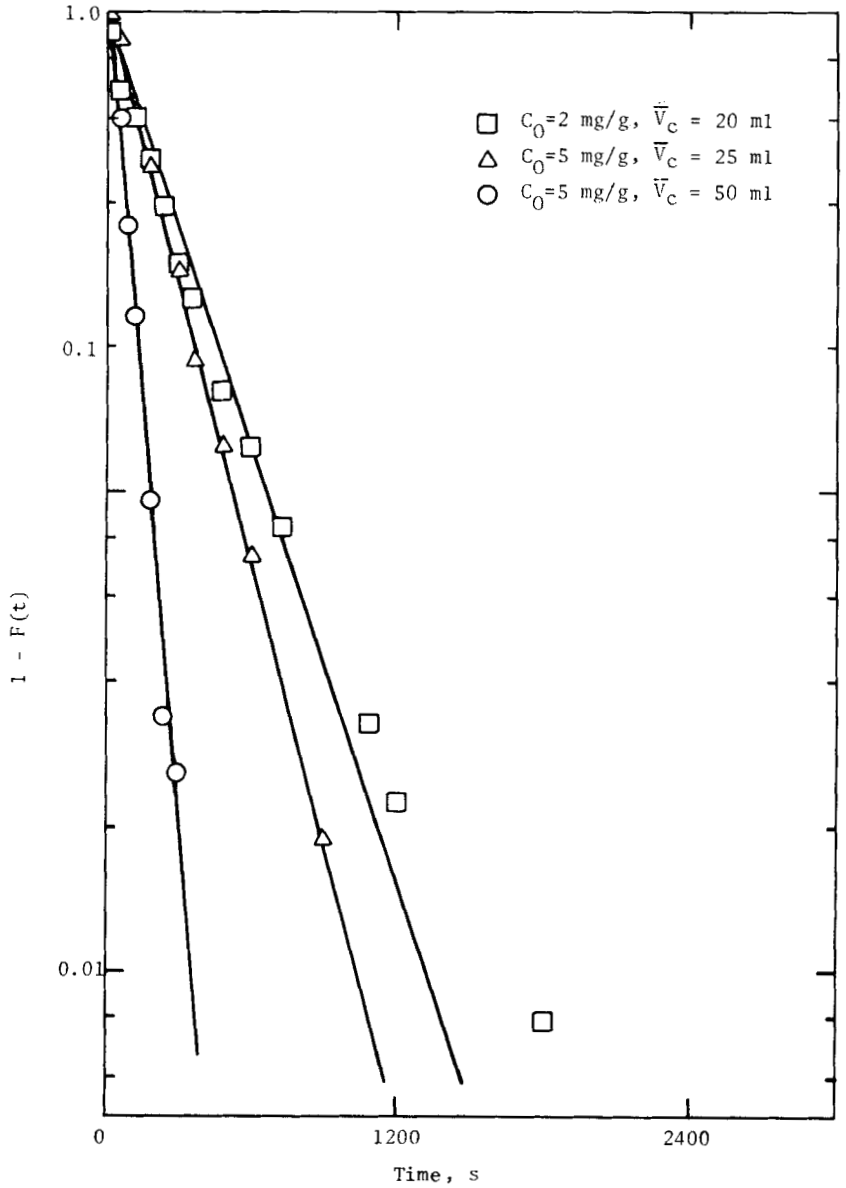

Figure 2. Plot of $\ln[1 - F(t)]$ vs. time for adsorption under film diffusion control.

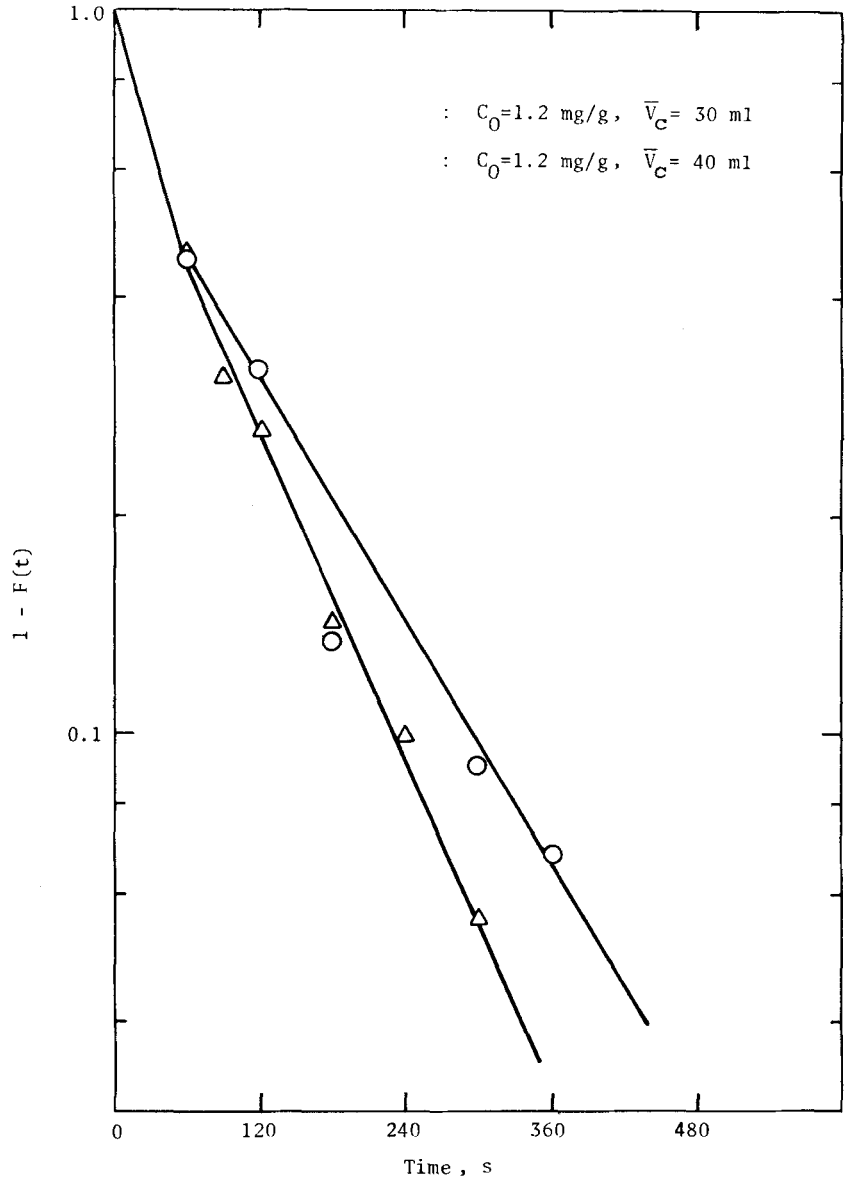

Figure 3. Plot of ln[1 − $F(t)$] vs. time for adsorption under particle diffusion control.

where

$$z = [1 + (R - 1)\exp(-K_v\theta)]^{1/3}$$

$$y = R^{1/3}$$

$$R = \bar{V}_0/\bar{V}_f$$

where

$\bar{V}_0$ = initial volume before desorption

r_f = final effective radius of particle

The above equation involves two adjustable parameters, $\bar{D}$ and K_v, which can be determined by fitting the equation to experimental data. It is clear from Eq. 18 that mass transfer by exclusion becomes less important and the curve of ln[1 − $F(t)$] vs. θ approaches a straight line as the time increases. Therefore, the effective diffusivity inside the particle $\bar{D}$ can be obtained by the slope of the asymptote, and subsequently the rate constant K_v can be determined by fitting the data for the initial period.

RESULTS

Equilibrium

The equilibrium results are plotted in Figure 1 and tabulated in Table 2. It can be seen from Figure 1 that the uptake of protein by Sephadex A-50 can be characterized satisfactorily by an adsorption isotherm of the Langmuir type. The parameters in the Langmuir isotherm, K_e and $\bar{C}_m$, were determined by linear regression of the experimental data and are given in Table 2. Considering the large amount of protein being adsorbed by the ion-exchange resin at 0% NaCl (8,200 mg/g) and the significant drop in adsorption caused by adding 1% NaCl (360 mg/g) it is clear that DEAE-Sephadex A 50 ion-exchange resin has great potential for concentrating and purifying BSA. These studies are currently being extended to multicomponent systems. A generalized form of the Langmuir isotherm as used by Liapis and Rippin (1977) may be necessary when more than one solute is present.

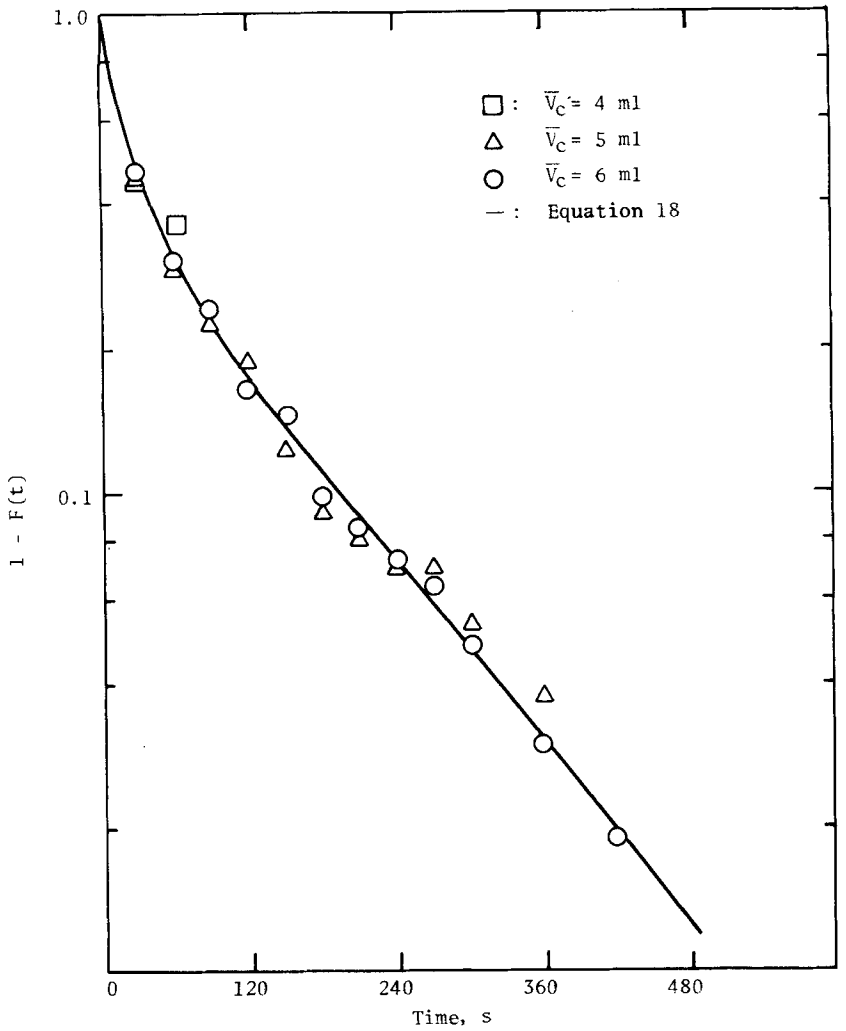

Figure 4. Plot of ln[1 − $F(t)$] vs. time for desorption.

Batch Kinetics—Adsorption

Considering the high adsorption of BSA shown by the equilibrium curve under adsorbing condition, values of m' and m'' will for most conditions be very large. For large values of m' the kinetics will be film-diffusion-controlled, Eq. 5. For large values of m'' the kinetics will be film-diffusion-controlled, Eq. 5. simplifies to

$$\ln [1 - F(t)] = \frac{D\bar{A}\rho_s}{\delta \bar{W}} \theta \left(\frac{\bar{W}}{W}\right) \tag{19}$$

which for spherical particles becomes:

$$\ln [1 - F(t)] = -\frac{3(1-\epsilon_p)}{r}\frac{\rho_s}{\rho}\left(\frac{\bar{W}}{W}\right)\frac{D}{\delta}\theta \tag{20}$$

$$= -\frac{3(1-\epsilon_p)}{r}\frac{\bar{V}}{V}\frac{D}{\delta}\theta \tag{21}$$

Figure 2 presents the experimental results obtained for adsorption under conditions for which film diffusion controlled and thus Eq. 21 applies. In all cases the volume of the solution was 500 mL and the stirring speed was 30 rps. In a separate experiment using Dowex 50W-X8 ion-exchange resin and a Na^+-H^+ exchange, the film thickness was determined for these conditions to be 5.2×10^{-3} mm. This is a case of ion exchange accompanied by a neutralization reaction for which the interdiffusion coefficient can be calculated from ionic mobilities. The mass transfer coefficient defined by Eq. 4 will be a function of diffusivity, but the film thickness is assumed a constant and only a function of stirring speed for dimensionally similar systems. The average resin radius was 0.116 mm, the solution density was 1.0 g/mL, and the density of the resin in a packed column was 0.0155 g/mL. The voidage of the packed bed was 0.61. Various initial protein concentrations (from 2 to 5 mg/g) and volumes of resin reported as packed bed volume (from 20 to 50 mL) were used. In each case a straight line was obtained from which the value of diffusivity D could be calculated. The values obtained ranged from 4.7 to 6.9×10^{-11} m^2/s. These values are in good agreement with the values of 5.8×10^{-11} m^2/s report by Wagner and Scheraga (1956), 5.9×10^{-11} m^2/s reported by Akeley and Gosting (1953), and 6.1×10^{-11} m^2/s reported by Creeth (1952). Differences in these values could be due to differences in pH, buffer type, and concentration (Keller, 1971; Fair et al., 1978. Equation 21 therefore represents a convenient and accurate approximation for the kinetics of protein adsorption when film diffusion is controlling.

It is practically impossible to get pure particle diffusion controlling under conditions favoring adsorption. Rate data for particle diffusion controlling therefore were obtained by adsorbing BSA in a solution with 1% NaCl. Figure 3 shows these rate data plotted according to Eq. 7. The initial rate period, represented by a single line, is in the region of combined film and particle

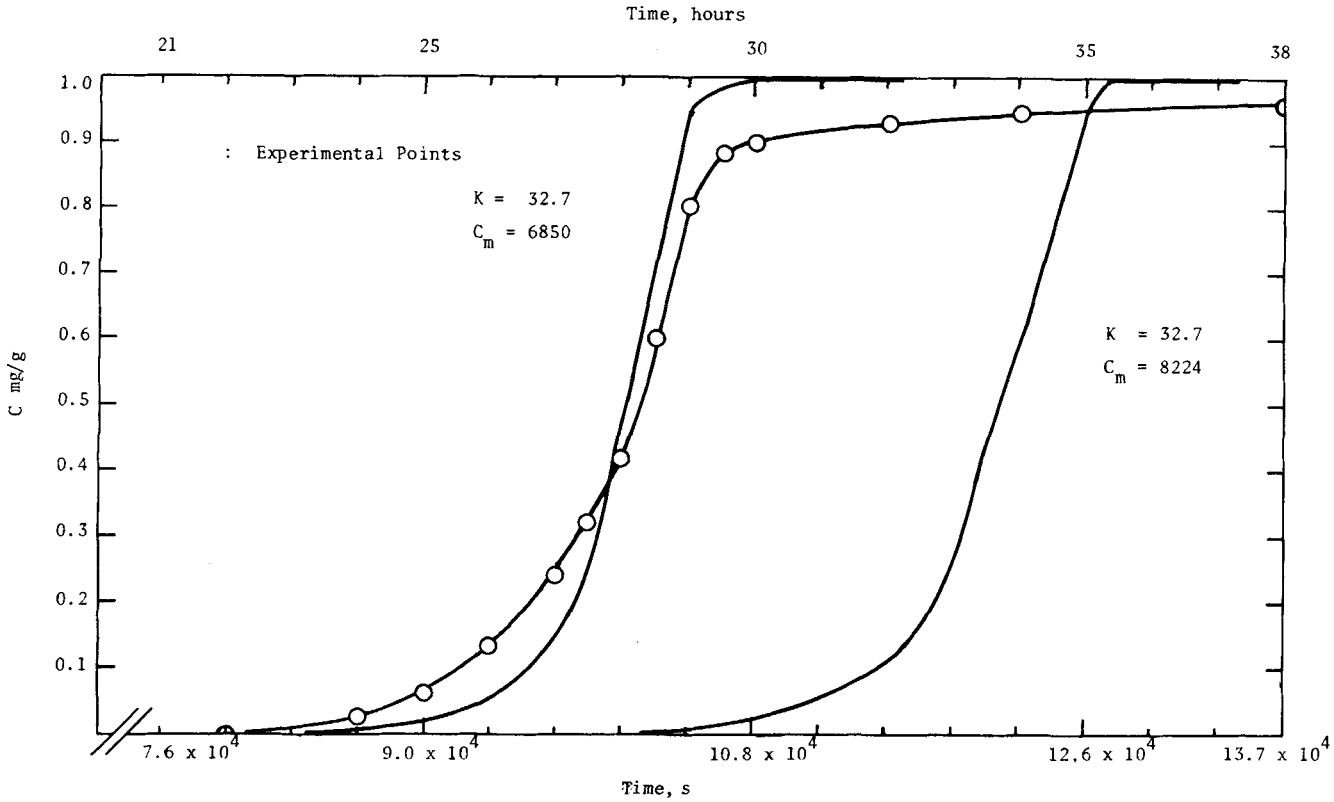

Figure 5. Breakthrough curve for BSA on Sephadex A-50. C_o = 1 mg/g; pH = 6.9; column dia. = 0.01m; flow rate = 1.0×10^{-9} m^3/s.

diffusion control. In these experiments the same solution volume (500 mL) and stirring rate (30 rps) were used. The initial protein concentration was 1.2 mg/g, the salt concentration was 1%. The average resin radius was 0.083 mm and the density of the resin in a packed column was 0.043 g/mL. Average values of $m' = 150$ and $m'' = 6.7 \times 10^{-3}$ were used in the calculation. Using the two different resin volumes the effective particle diffusivity was calculated. The values obtained were 2.1 and 2.5×10^{-8} cm^2/s, representing values about 1/20 of those for free diffusion. These values are similar to values reported by Colton et al. (1975) and reflect the much more open pore structure of Sephadex A-50 as compared to the protein resin used in studies of Graham and Fook (1982).

Batch Kinetics—Desorption

The initial protein concentration in the ion-exchange resin for the desorption studies was 3,215 mg/m. Small quantities (4 to 6 mL) of the adsorbed ion exchanger were used to satisfy the condition $\bar{m}''(\bar{W}/W) \ll 1$.

The value of $R = V_o/V_F$ was 2.8 and the final effective radius was 0.083 mm. The two adjustable parameters $\bar{D}$ (effective particle diffusion coefficient) and K_v (the shrinkage rate constant) were determined by fitting Eq. 18 to the experimental data, as shown in Figure 4. The diffusion obtained by fitting the shape of the final asymptote in Figure 4 (when the resin shrinking process was negligible) and K_v was then obtained by fitting the initial curvature of Figure 4 (when the resin shrinking process was dominant). The values obtained were $K_v = 0.04\ s^{-1}$ and $\bar{D} = 3.3 \times 10^{-12}\ m^2/s$. It can be seen from Figure 4 that Eq. 18 correlates the experimental data well. The value $\bar{D}$ is in reasonable agreement with the values of $2\text{–}2.5 \times 10^{-12}\ m^2/s$ obtained from the adsorption studies.

This work is now being extended to multicomponent protein separation. The details of the work presented here could easily be incorporated into the general mathematical model presented by Liapis and Rippin (1977).

Kinetics of Column Operation

The breakthrough curve obtained as outlined in the Experimental section is shown in Figure 5 along with the two computer predictions of the breakthrough rate. The computer program incorporated a two-film model with the assumption of a nonlinear equilibrium (the Langmuir isotherm) at the interface. The solution of the governing equations was approximated numerically using Vanier's third-order convergence method of characteristics. The inclusion of particle diffusion in the computer solution had very little effect on the predicted breakthrough curve. The breakthrough curve was essentially determined by film diffusional effects and the equilibrium. A value of $D = 5.8 \times 10^{-11}\ m^2/s$ was used for the diffusion coefficient of BSA and the film thickness was determined by the empirical correlation of Glueckauf (1955) for packed ion-exchange columns. Details of the computer program are given by Tsou (1983) and Holland and Liapis (1983).

The first theoretical prediction of the breakthrough was obtained using the Langmuir isotherm determined under batch conditions with $\bar{C}_m$ = 8,224 and K_e = 32.7 g/mg. It can be seen that this predicts a breakthrough rate that is much too slow compared to the experimental curve. However, if the total capacity $\bar{C}_m$ is determined from the breakthrough curve a value of 6,850 mg/g is obtained. This reduced capacity could easily be explained by the fact that under column operations the bed has been highly compressed, resulting in a restriction and loss of some of the exchange site. Using this value for the total capacity ($\bar{C}_m$ = 6,850 mg/g) and the same K_e value as before (K_e = 32.7 g/mg) a much better prediction of the experimental breakthrough curve is obtained (see Figure 5).

Preliminary work with DEAE-Sepharose Fast Flow resin shows that while the new fast flow resin has less than one-fifth the capacity of Sephadex A-50 for BSA, it has much better flow characteristics. It does not exhibit the large changes in resin volume with salt concentration that would make regeneration or recovery of the protein bound on Sephadex A-50 very difficult in a column operation. It also appears that pressure compaction due to flow can be eliminated.

For multicomponent systems the mathematical models presented by Hsieh et al. (1977) and Balzi et al. (1978) look very promising for incorporating the work presented here for multicomponent protein separations.

NOTATION

$\bar{A}$ = overall surface area for mass transfer, m^2 or cm^2; for spheres = $\frac{3}{r}\bar{V}(1-\epsilon_p)$

C = protein concentration in bulk of well-stirred solution at any time t, mg/kg or mg/g

$\bar{C}$ = average protein concentration in resin at any time t, mg/kg or mg/g

$C(o)$ = initial solution protein concentration, mg/kg or mg/g

$\bar{C}(o)$ = initial resin protein concentration, mg/kg or mg/g

C_∞ = final or equilibrium solution protein concentration, mg/kg or mg/g

$\bar{C}_\infty$ = final or equlibrium resin protein concentration, mg/kg or mg/g

C_i = protein concentration in solution at resin-solution interface, mg/kg or mg/g

$\bar{C}_i$ = protein concentration in resin at resin-solution interface, mg/kg or mg/g

C^* = protein concentration in the solution that would be in equilibrium with the average particle concentration $\bar{C}$ at any time, mg/kg or mg/g

$\bar{C}^*$ = protein concentration in resin that would be in equilibrium with bulk solution concentration, mg/kg or mg/g

$\bar{C}_m$ = maximum adsorption of protein, Langmuir constant, mg/kg or mg/g

D = diffusion coefficient of protein in solution, m^2/s or cm^2s

$\bar{D}$ = diffusion coefficient of protein in resin, m^2/s or cm^2/s

$F(t)$ = fractional conversion defined by $(\bar{C}(o) - \bar{C})[\bar{C}(o)-\bar{C}(\infty)]$

k = solution side mass transfer coefficient, $kg/m^2{\cdot}s$ or $g/cm^2{\cdot}s$

$\bar{k}$ = resin side mass transfer coefficient, $kg/m^2{\cdot}s$ or $g/cm^2{\cdot}s$

K = overall mass transfer coefficient defined by Eq. 2, $kg/m^2{\cdot}s$ or $g/cm^2{\cdot}s$

$\bar{K}$ = overall mass transfer coefficient defined by Eq. 9, $kg/m^2{\cdot}s$ or $g/cm^2{\cdot}s$

K_e = Langmuir constant, kg/mg or g/mg

K_v = rate constant for shrinking process, s^{-1}

m' = slope of a chord between a point defined by$(C^*,\bar{C})$ and a point at the intersection of a line drawn from any operating point $(C,\bar{C})$ with a slope equal to $-k/\bar{k}$

m'' = $(\bar{C}_\infty - \bar{C})/(C_\infty - C^*)$

$\bar{m}''$ = $(\bar{C}_\infty - \bar{C}^*)/(C_\infty - C)$

N = flux of protein, $mg/m^2{\cdot}s$ or $mg/cm^2{\cdot}s$

r = effective resin particle radius, m or mm

r_f = final radius of resin particles after desorption, m or mm

t = time, s

V_c = volume of packed column of resin, m^3 or mL

$\bar{V}$ = volume of resin particles, m^3 or mL

$\bar{V}_o$ = initial volume of resin particles before desorption, m^3 or mL

$\bar{V}_F$ = final or equilibrium volume of resin particles after desorption, m^3 or mL

W = amount of solvent, kg or g

$\bar{W}$ = amount of ion exchange resin, kg or g

Greek Letters

ϵ_p = voidage of packed bed

δ = effective solution film thickness, m or cm

ρ_s = density of solvent, kg/m^3 or g/mL

$\bar{\rho}$ = density of packed bed, kg/m^3 or g/mL

θ = total elapsed time, s

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