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# MODELLING SINGLE-COMPONENT PROTEIN ADSORPTION TO THE CATION EXCHANGER S SEPHAROSE® FF

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## SUMMARY

The equilibrium and kinetic characteristics of the adsorption of bovine serum albumin (BSA) and lysozyme to the strong cation exchanger S Sepharose FF have been determined. The rates of protein adsorption have been compared to two different models, the first being based on a single lumped kinetic parameter, whilst the second model considers the individual transport processes occurring prior to the adsorption reaction, that is taking into account diffusion across the liquid film surrounding individual particles and also the diffusion within the ion-exchanger particle itself. It was found that the adsorption of lysozyme to S Sepharose FF, in both batch, agitated tanks and in packed beds was consistent with both models. In the case of BSA however, the agitated tank adsorption profile was consistent only with the pore diffusion model and neither model correctly predicted the latter part of the breakthrough profile observed in packed-bed experiments.

## INTRODUCTION

Ion-exchange adsorbents have found widespread use in the purification of proteins, both in the laboratory and in the production plant, since the introduction in 1956 of the first ion exchanger spcifically designed for proteins[1]. This has recently been highlighted in a study by Bonnerjea *et al.*[2] which showed that ion exchangers were used in 75% of all the published purification protocols that they examined. This widespread use of ion exchangers is due to their versatility, relative cheapness and their acceptance by the regulatory authorities in the production of pharmaceutical proteins. This is in contrast to affinity adsorbents which, although they result in good resolution, are limited to one protein or group of proteins, are expensive and their use is currently questioned by regulatory bodies.

We have been studying the adsorption of proteins to ion exchangers to investigate how different properties of the ion exchangers affect their adsorption performance, and with the aim of producing simple models of the purification process to assist in process design. Previous studies examined how the properties of different anion exchangers, such as functional groups and the particle matrix, affected

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performance[3]. In this paper we have obtained experimental data for the binding of bovine serum albumin (BSA) and lysozyme to the strong cation exchanger S Sepharose FF. These results have been compared to the results calculated from two different models of the adsorption process. The characteristics of the adsorption of each protein to the ion exchanger were determined by several different types of experiment. The equilibrium capacity of S Sepharose FF for each of the proteins was established by determining adsorption isotherms. However, the adsorption of protein by an ion exchanger is not an instantaneous event and mass transfer effects must also be considered. The dynamic approach to equilibrium was therefore examined by studying the rate of uptake of protein in a shaken vessel. Finally the adsorption of protein to packed beds of S Sepharose FF was studied by determining breakthrough profiles. The completion of these studies provided a characterised system which was then used as the basis for studies of the simultaneous adsorption of BSA and lysozyme to S Sepharose FF from a solution containing both proteins. These studies will be reported in a following paper[4].

## THEORY

### *An equilibrium model*

When discussing protein adsorption to ion exchangers, the adsorbent is frequently considered as consisting of functional groups the charge of which is balanced by associated counter-ions, whilst the protein molecule is considered to exist in an ionized state in solution. On adsorption to an ion exchanger, a protein molecule displaces the counter-ions which were previously associated with the charged groups of the ion exchanger. For an ion exchanger equilibrated with monovalent counter-ions, this process can be represented by an equilibrium of the form:

$$\mathrm{A}\cdot n\mathrm{I} + \mathrm{P}^n \rightleftharpoons \mathrm{A}\cdot\mathrm{P} + n\mathrm{I} \tag{1}$$

where A represents the adsorption site on the ion exchanger, I represents the counter-ions, P the protein molecule and $n$ the number of charges involved in the interaction per adsorbed protein molecule. If the change in bulk-fluid concentration of the counter-ion I is small as a result of protein adsorbing to the ion exchanger, as is the case when buffered solutions containing I are used, then equilibrium 1 may be simplified to:

$$\mathrm{A} + \mathrm{P} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{A}\cdot\mathrm{P} \tag{2}$$

and hence the rate of change of the adsorbed protein concentration is given by:

$$\frac{\mathrm{d}q}{\mathrm{d}t} = k_1 c(q_\mathrm{m} - q) - k_{-1}q \tag{3}$$

where $c$ is the soluble protein concentration, $k_1$ and $k_{-1}$ are the adsorption and desorption rate constants respectively, $q$ is the adsorbed protein concentration, $q_\mathrm{m}$ the maximum protein capacity of the ion exchanger and $t$ represents time.

At equilibrium eqn. 3 equates to zero and hence:

$$q^* = \frac{c^* q_m}{c^* + \frac{k_{-1}}{k_1}} \tag{4}$$

where the superscript * denotes values when equilibrium has been established between solid and liquid phases. Substituting $K_d = k_{-1}/k_1$ into eqn. 4 gives:

$$q^* = \frac{c^* q_m}{c^* + K_d} \tag{5}$$

where $K_d$ is the dissociation constant of the protein–ion-exchanger complex. Eqn. 5 is the form of the Langmuir adsorption isotherm that we have previously used to describe the adsorption of BSA to anion exchangers[3]. The Langmuir isotherm is used frequently to describe the adsorption of proteins to various adsorbents, including ion exchangers, for example Leaver[5], Chase[6] and Annesini and Lavecchia[7]. Rearranging eqn. 5 gives the linear form of:

$$\frac{c^*}{q^*} = \frac{K_d}{q_m} + \frac{c^*}{q_m} \tag{6}$$

from which the dissociation constant, $K_d$ and the maximum protein capacity of the ion exchanger, $q_m$, can be calculated by least squares linear regression analysis.

*The approach to equilibrium—models of the uptake of protein*

*Kinetic rate constant model.* We have previously used a kinetic rate constant model, based on a single "lumped" adsorption rate constant, to describe the adsorption of proteins to affinity adsorbents[8]. The model takes an empirical approach to the adsorption process and assumes that all the rate limiting processes can be represented by kinetic rate constants. In such an approach, the rate of mass transfer of protein to the adsorbent is assumed to be described by eqn. 3 above. For batch adsorption in a stirred tank, the protein concentration in solution at time $t$ is given by the analytical solution of eqn. 3, namely:

$$c = c_0 - \frac{v}{V}\left[\frac{(b+a)\left(1 - \exp\left\{-\frac{2av}{V}k_1 \cdot t\right\}\right)}{\left(\frac{b+a}{b-a}\right) - \exp\left\{-\frac{2av}{V}k_1 \cdot t\right\}}\right] \tag{7}$$

where

$$a^2 = b^2 - \left(\frac{c_0 \cdot V}{v}\right)q_m \tag{8}$$

$$b = \frac{1}{2}\left(\frac{c_0 \cdot V}{v} + q_m + \frac{K_d V}{v}\right) \tag{9}$$

and $c_0$ is the initial liquid phase protein concentration, $v$ is the volume of ion exchanger present and $V$ the volume of liquid external to the ion exchanger.

If it is assumed that the effects of axial dispersion are negligible then eqn. 3 can also be solved analytically to describe adsorption in packed columns, first performed by Thomas[9] for classical ion exchange processes and adapted by Chase[8] for the adsorption of proteins to affinity adsorbents.

*Film and pore diffusion model.* A more rigorous approach to modelling the adsorption process is to consider the different steps that occur during protein uptake. These are commonly defined as transport through the liquid film surrounding the adsorbent particles, diffusion within the pores of the adsorbent and finally the adsorption reaction itself. We have recently shown that the affinity adsorption of immunoglobulin G to protein A immobilised on agarose matrices is described well by a combination of surface-film resistance and porous-diffusion resistance[10]. Such a model might also be expected to account for the rates of protein adsorption to ion exchangers. The following assumptions are used as the basis for the construction of the model:

(1) The adsorbent is made of a porous material, into which the solute must diffuse, in a manner described by an effective pore diffusivity, $D$. $D$ is assumed to be independent of concentration and is based on the porosity of the particle towards small molecules, rather than the actual extent to which molecules of a particular protein can penetrate the particle.

(2) Mass transfer to the surface of the adsorbent is governed by a film model characterised by a mass transfer coefficient, $k_f$.

(3) Surface reaction between the adsorbate and an adsorption site is described by a reversible second order reaction. Adsorption is isothermal, and its equilibrium behaviour can be represented by the Langmuir equation. Surface diffusion in which adsorbate moves directly between adsorption sites without interim desorption into the liquid phase is assumed to occur at a negligible rate and hence a term to describe this process is not thought appropriate.

(4) The adsorbent particles are spherical, with uniform size and density, and the functional groups of the ion exchanger are distributed evenly throughout the interior of the particle.

(5) Axial dispersion, $D_x$, is negligible in packed bed simulations.

For diffusion of protein in the liquid within the ion exchanger particle, the point concentration of protein, $c_i$, is given by:

$$\varepsilon\frac{\partial c_i}{\partial t} = \varepsilon D\left(\frac{\partial^2 c_i}{\partial r^2} + \frac{2\partial c_i}{r\partial r}\right) - (1-\varepsilon)\frac{\partial q_i}{\partial t} \tag{10}$$

where $\varepsilon$ is the particle porosity, $q_i$ the point concentration of adsorbed protein and $r$ the radial coordinate within the ion-exchanger particle. The particle porosity was determined from a knowledge of the solids content of the adsorbent. S Sepharose FF is formulated from 6% agarose and hence the particle porosity was taken as being 0.94.

The rate of mass transfer through the external film relates the bulk liquid concentration, $c$, to the concentration in the pore liquid at the surface of the particle. The expression is:

$$\left.\frac{\partial c_i}{\partial r}\right|_{r=R} = \left.\frac{k_f}{D\varepsilon}(c - c_i)\right|_{r=R} \tag{11}$$

At the centre of the particle

$$r = 0 \qquad \frac{\partial c_i}{\partial r} = 0 \tag{12}$$

If a second order rate of surface reaction is assumed then the rate of change of adsorbed protein concentration is given by eqn. 3 above. At equilibrium this gives a form of the Langmuir equation with maximum capacity $q_m$ and dissociation constant $K_d = k_{-1}/k_1$, eqn. 5 above.

For adsorption and desorption in a stirred tank, the rate of change of bulk concentration of protein, $c$, is given by:

$$\frac{dc}{dt} = -\left(\frac{3vk_f}{RV}\right)(c - c_i)\Big|_{r=R} \tag{13}$$

The correlation used to estimate the liquid film mass transfer coefficient, $k_f$, of protein to the adsorbent particles in stirred tank experiments was that given by Geankoplis[11].

$$k_f = \frac{2D_{AB}}{d} + 0.31\left(\frac{\mu}{\rho D_{AB}}\right)^{-\frac{2}{3}}\left(\frac{\Delta\rho\mu g}{\rho^2}\right)^{\frac{1}{3}} \tag{14}$$

where $\rho$ is the particle density, $\Delta\rho$ is the density difference between the adsorbent particle and the liquid, $\mu$ is the liquid viscosity and $g$ is the gravitational constant. The molecular diffusivity of lysozyme and BSA in free aqueous solution, $D_{AB}$, was estimated using the semi-empirical equation of Polson[12]:

$$D_{AB} = 9.4 \cdot 10^{-15}\frac{T}{\mu(M_A)^{\frac{1}{3}}} \tag{15}$$

where $M_A$ is the relative molecular mass of A and $T$ is the absolute temperature. Taking 14 500 and 66 300 as the relative molecular masses of lysozyme and BSA respectively, gives molecular diffusion coefficients in acetate buffer, $D_{AB}$, of $1.2 \cdot 10^{-10}$ m$^2$/s and $7.3 \cdot 10^{-11}$ m$^2$/s respectively.

For adsorption in a packed bed, the equation of continuity in the mobile phase is given by:

$$D_x\frac{\partial^2 c}{\partial x^2} - l\frac{\partial c}{\partial x} - R_i = \frac{\partial c}{\partial t} \tag{16}$$

where $x$ is the axial coordinate in the bed, $l$ the interstitial velocity of liquid in the bed and $R_i$ is rate of interface mass transfer. The rate expression is:

$$R_i = \frac{3}{R}\frac{(l - \varepsilon_b)}{\varepsilon_b} D\frac{\partial c_i}{\partial r}\Big|_{r=R} \tag{17}$$

where $\varepsilon_b$ is the porosity of the packed bed. Using data from molecular-exclusion experiments performed with very high-molecular-weight dextran, the porosity of packed beds of S Sepharose FF was taken to be 0.35.

At each point in the column, the concentration outside the fluid film is related to the liquid phase concentration at the surface of the particle by:

$$k_f(c - c_i)\Big|_{r=R} = \varepsilon D \frac{\partial c_i}{\partial r}\Big|_{r=R} \tag{18}$$

To estimate $k_f$ in a packed bed, the following correlation of Foo and Rice[13] was used:

$$Sh = 2 + 1.45 Re^{\frac{1}{2}} Sc^{\frac{1}{3}} \tag{19}$$

where $Sh = k_f d/D_{AB}$, $Re = u\rho d/\mu$, $Sc = \mu/(\rho D_{AB})$, $u$ is the superficial velocity of liquid flow through the column and $d$ is the mean particle diameter.

## EXPERIMENTAL

### *Materials*

BSA and lysozyme (EC 3.2.1.17) were obtained from Sigma (Poole, U.K.), catalogue numbers A-3912 and L-6876, respectively. BSA has a relative molecular mass of 66 300 daltons[14] and an isoelectric point (p*I*) of pH 4.7 (ref. 15), whilst lysozyme has a relative molecular mass of 14 500 daltons[16] and a p*I* of pH 11.1 (ref. 17). The choice of these two proteins was largely determined by cost considerations as the capacity of ion exchangers (commonly in the range 50–100 mg protein per ml of ion exchanger) meant that gram quantities of pure proteins were required for these studies.

All solutions were buffered with 0.1 *M* sodium acetate–acetic acid, pH 5. Sodium acetate, acetic acid and sodium chloride were all laboratory grade reagents. S Sepharose FF was a gift from Pharmacia LKB (Uppsala, Sweden). Known volumes of ion exchanger were obtained by allowing a suspension of the ion exchanger to settle in a measuring cylinder overnight and then adjusting the liquid volume to equal that of the settled ion exchanger. Aliquots of a known volume of a 50:50 (v/v) suspension were then obtained by the use of a Gilson Pippetman automatic pipette.

### *Adsorption isotherms*

Isotherms for the adsorption of each protein to S Sepharose FF were determined in batch experiments. A known volume of a 50:50 (v/v) suspension of ion exchanger in buffer was added to each of a series of flasks containing known volumes of buffered protein solution at different concentrations. The flasks were incubated overnight in a shaking water bath at 25°C to allow equilibrium to be established. The ion exchanger was then allowed to settle under gravity for approximately 30 min and the resulting supernatant was filtered before determining the equilibrium concentration of protein in the soluble phase by UV spectrophotometry. The amount of protein adsorbed to the S Sepharose FF was then calculated by mass balance.

*Kinetics of batch adsorption*

The rate of adsorption of protein to a suspension of S Sepharose FF was determined in experiments in which the soluble phase protein concentration of a batch system was continuously monitored. This was achieved by recycling the liquid phase; the liquid was first passed through a 2-$\mu$m HPLC pump inlet filter to prevent removal of suspended S Sepharose FF from the reaction vessel and then through a continuous flow UV spectrophotometer before return to the experimental vessel. The reaction vessel was incubated and agitated in a shaking water bath maintained at 25°C. A typical experiment consisted of 25 ml of buffer containing protein at a concentration of 2 mg/ml. In order to achieve a rapid response time the volume of the recycle was kept as small as possible (approximately 1 ml) and the solution was pumped at a flow-rate of 7 ml/min. Experiments were commenced by the addition of 0.5 ml of a 50:50 (v/v) suspension of S Sepharose FF. The output from the UV spectrophotometer was connected to a chart recorder, and the protein concentration in the liquid phase at selected times was determined from the chart recorder trace and reference to calibration data. The protein concentrations were normalised by dividing the concentration $c$, at time $t$, by the protein concentration at time zero, $c_0$.

*Frontal analysis*

Breakthrough curves were determined in order to evaluate packed bed performance. All column experiments were performed with 2 ml (settled volume) of ion exchanger packed in a chromatography column, 1 cm diameter (0.785 $cm^2$ cross-sectional area), mounted vertically. It was found that the volume of ion exchanger used gave a bed height of 2.2–2.3 cm, equivalent to a packed volume of approximately 1.75 ml. All experiments were performed at a volumetric flow-rate of 1 ml/min (superficial velocity 1.27 cm/min) and flow was always in an upward direction. Protein was applied to the beds at a concentration of 1 mg/ml ($c_0$) and the optical density at 280 nm of the outlet stream was recorded. For the determination of breakthrough curves, the beds were loaded until the protein concentration in the outlet stream equalled, or was approaching, that of the inlet stream, $c_0$. At the end of the adsorption phase protein was eluted from S Sepharose FF with 1 *M* sodium chloride in 0.1 *M* acetate buffer. Data were plotted in the form of normalised concentration, $c/c_0$, of the outlet stream against the amount of protein applied. Time zero was taken as the point at which the adsorbate solution first entered the bed.

*Computer simulations*

The equations of the kinetic rate constant model were solved using programs written in BASIC running on a BBC microcomputer[8]. The governing differential equations of the film and pore diffusion model were solved using a finite difference method using the University of Cambridge IBM 3084 mainframe computer as described previously[10].

## RESULTS

*Adsorption isotherms*

The isotherms for the adsorption of BSA and lysozyme to S Sepharose FF in 0.1 *M* sodium acetate buffer, pH 5 are shown in Fig. 1. The experimental data for both

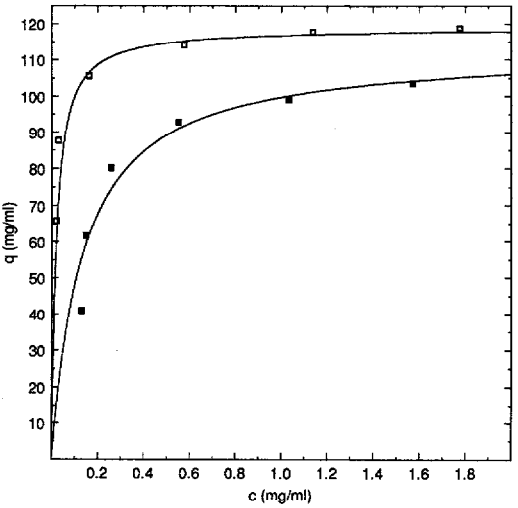


Fig. 1. Adsorption isotherms for the binding of lysozyme (□) and BSA (■) to S Sepharose FF in 0.1 *M* acetate buffer, pH 5 at 25°C. The data are plotted as mg protein adsorbed per ml S Sepharose FF against mg/ml protein in solution. The Langmuir constants were determined by linear regression and are presented in Table I.

proteins fitted well to a Langmuir isotherm and the characteristic parameters $K_d$ and $q_m$ are shown in Table I. The adsorption capacities of the adsorbents are based on the volume that the adsorbent would occupy when packed in a bed. The maximum capacities for the two proteins are similar when compared on a mass basis but the dissociation constants are only similar when compared on a molar basis.

*Kinetics of adsorption in a stirred tank*

The experimental data for the rates of protein adsorption in a stirred tank were compared with the two models described above; namely the kinetic rate constant model and the film and pore diffusion model. In each model there was only a single unknown parameter describing the rate of protein adsorption and the value of this parameter could be determined by finding the best fit to the experimental data. With the kinetic rate constant model, a simulation of the rate of protein adsorption was made using eqn. 7. The only unknown parameter was the apparent rate constant, $k_1$ as values of the isotherm parameters $q_m$ and $K_d$ were taken to be those determined in the batch isotherm experiments described above and the other parameters were known from the conditions of the experiment. $k_{-1}$ is given simply by $K_d \cdot k_1$ since the ratio of the reverse to the forward rate constant is the dissociation constant. The simulation was run with a variety of values of the unknown parameter $k_1$ and the value of this parameter was thus determined as the value that gave the best fit to the experimental curve. Similarly for the pore and diffusion model, simulations of the rate of protein adsorption were made by solving eqns. 11–14 for a variety of values of the only unknown parameter $D$. Again the value of $D$ was taken to be that value which gave the best fit to the experimental curve.

The agreement between the simulations and the experimental uptake curve is

TABLE I

VALUES OF $K_d$, $q_m$, $k_1$, $k_f$ AND $D$ FOR THE ADSORPTION OF BSA AND LYSOZYME TO S SEPHAROSE FF

| | $K_d$ (mg ml$^{-1}$) | $K_d$ (M) | $q_m$ (mg ml$^{-1}$) | $q_m$ (mol l$^{-1}$) | $k_1$ (ml mg$^{-1}$ s$^{-1}$) | $k_1$ (l mol$^{-1}$ s$^{-1}$) | $k_f$[a] (m s$^{-1}$) | $k_f$[b] (m s$^{-1}$) | $D$ (m$^2$ s$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Lysozyme | 0.019 | $1.3 \cdot 10^{-6}$ | 120 | $8.4 \cdot 10^{-3}$ | $1.7 \cdot 10^{-3}$ | 23.88 | $8.0 \cdot 10^{-6}$ | $8.1 \cdot 10^{-6}$ | $5.0 \cdot 10^{-11}$ |
| BSA | 0.133 | $2.0 \cdot 10^{-6}$ | 113 | $1.7 \cdot 10^{-3}$ | $5.0 \cdot 10^{-4}$ | 33.15 | $5.0 \cdot 10^{-6}$ | $5.6 \cdot 10^{-6}$ | $8.5 \cdot 10^{-12}$ |

[a] Values obtained from the correlation for stirred tanks (eqn. 14).
[b] Values obtained from the correlation for packed beds (eqn. 19).

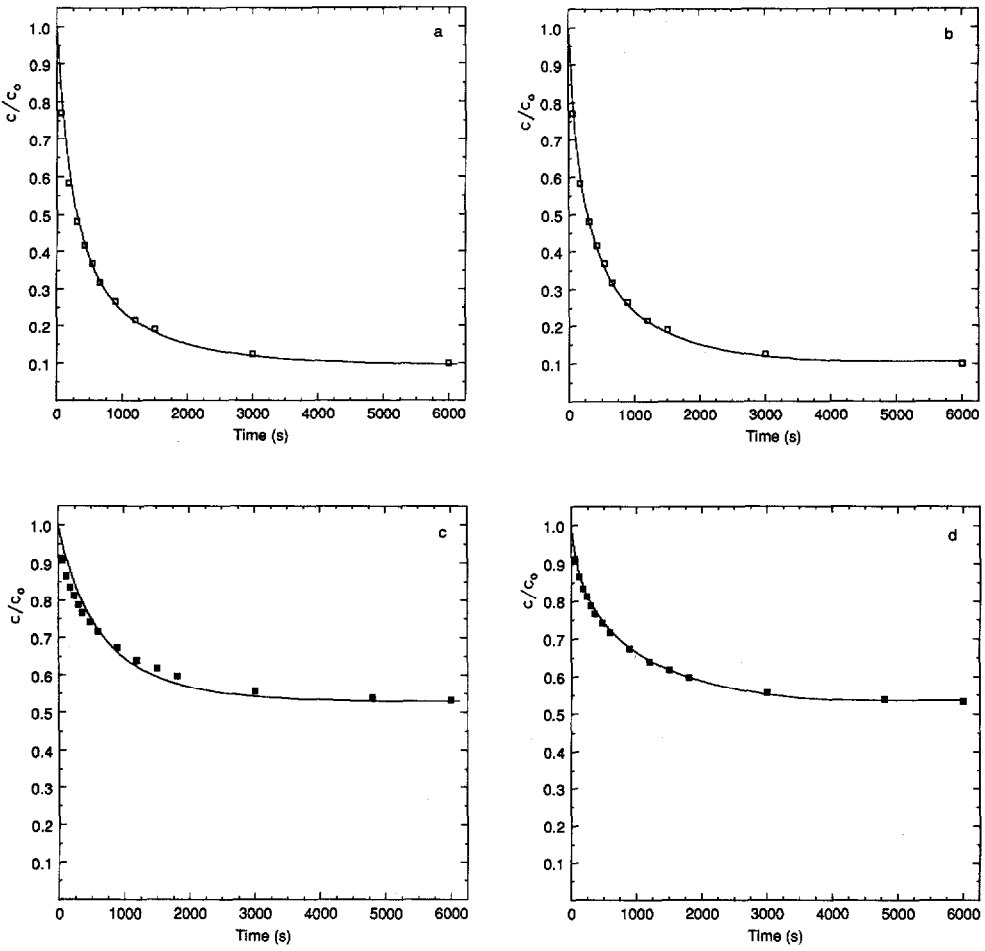


Fig. 2. Batch adsorption profiles for the uptake of lysozyme (□) and BSA (■) by S Sepharose FF in suspension in agitated vessels. The solid lines were calculated by solution of the appropriate equations describing the adsorption profiles for the two models discussed in the text. (a) The adsorption of lysozyme and the adsorption profile predicted by the kinetic rate constant model. (b) The adsorption of lysozyme and the adsorption profile predicted by the film and pore diffusion model. (c) The adsorption of BSA and the adsorption profile predicted by the kinetic rate constant model. (d) The adsorption of BSA and the adsorption profile predicted by the film and pore diffusion model. The parameters used in the calculations are listed in Table I.

shown in Fig. 2. Table I shows the best fit values for the rate constant, $k_1$, for the kinetic rate constant model and the effective diffusivity, $D$, for the film and pore diffusion model. It was possible to obtain equally good fits to both models for the adsorption of lysozyme (Fig. 2a and b) but the uptake profile for the adsorption of BSA fitted better to the pore and film diffusion model (Fig. 2d). When the latter system was compared with the kinetic rate constant model (Fig. 2c), it was not possible to find a value of the unknown parameter, $k_1$, that was appropriate throughout the entire time course of adsorption.

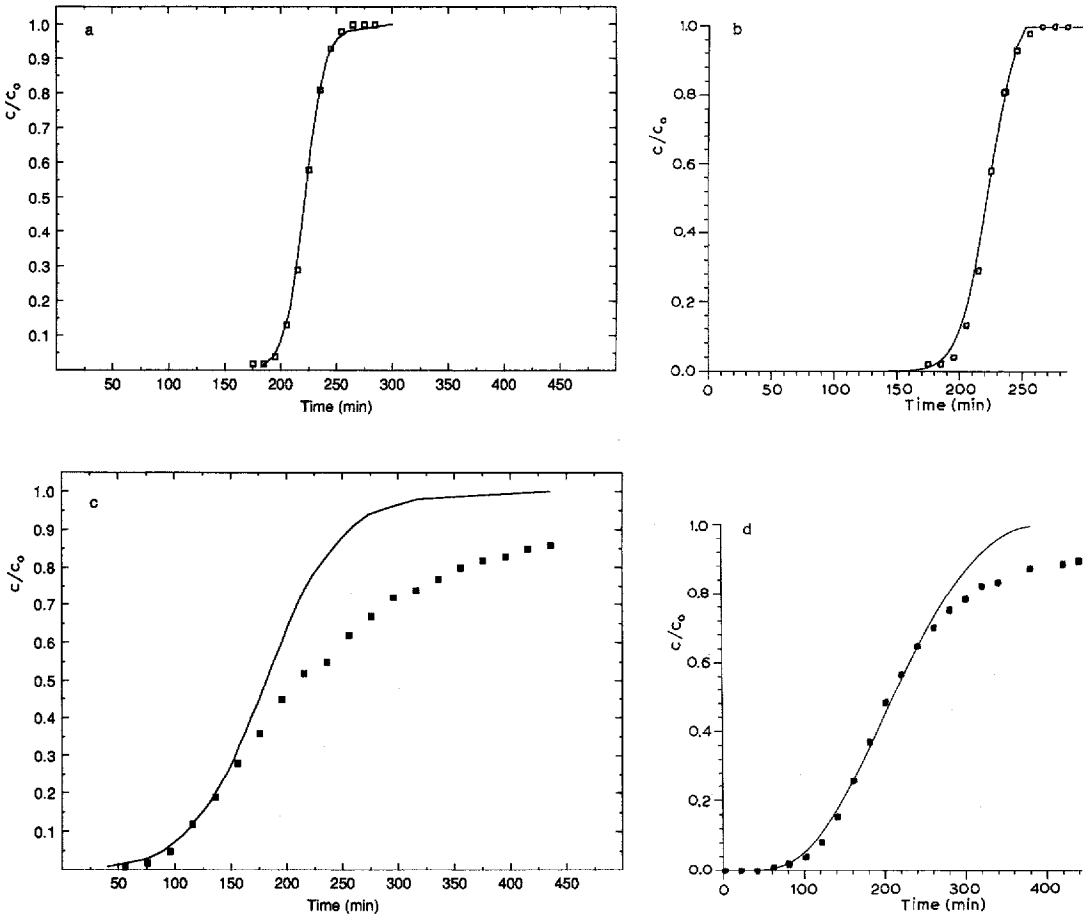


Fig. 3. Breakthrough profiles for the adsorption of lysozyme (□) and BSA (■) to S Sepharose FF in packed beds. The beds used were 1 cm diameter and 2.3 cm high. All flow-rates were 1 ml/min in an upward direction and protein solutions at a concentration of 1 mg/ml. The solid lines were calculated by solution of the appropriate equations describing the adsorption profiles in packed beds for the two models discussed in the text. (a) The adsorption of lysozyme and the adsorption profile predicted by the kinetic rate constant model. (b) The adsorption of lysozyme and the adsorption profile predicted by the film and pore diffusion model. (c) The adsorption of BSA and the adsorption profile predicted by the kinetic rate constant model. (d) The adsorption of BSA and the adsorption profile predicted by the film and pore diffusion model. The parameters used in the calculations are listed in Table I with the exception of (d) in which the value of $q_m$ used was 130 mg/ml.

*Frontal analysis*

Frontal analysis experiments were performed to see whether the two models of protein adsorption that had been used to model uptake in a stirred tank could also describe adsorption in a packed bed. The experimental breakthrough curves are shown in Fig. 3. The figure also shows the curves predicted by the two models using the values of the equilibrium and rate parameters determined in the isotherm and batch uptake experiments. However, the values of $k_f$ used in the film and pore diffusion model were

those appropriate for adsorption in a packed bed and were estimated from the correlation given above (eqn. 19).

For lysozyme, it was found that both models gave a good fit to the experimental breakthrough curve using the parameters derived from batch experiments (Fig. 3a and b). However, neither model gave a precise fit to the breakthrough curve obtained with BSA (Fig. 3c and d). The experimental curve for the latter system was very asymmetric and approached the inlet concentration very slowly. Indeed, it appeared that the equilibrium capacity of the bed for BSA was greater than that predicted from the adsorption isotherm experiments. A value for the apparent maximum capacity in fixed bed uptake was not able to be determined by integrating the area above the breakthrough curve as the outlet concentration had not risen completely to the inlet value by the end of those experiments. Both models failed to predict the later stages of breakthrough correctly although the film and pore diffusion model did predict some degree of asymmetry to the shape of the curve and a good fit to the earlier stages of adsorption was obtained. The best fit with this model was obtained if the value of $q_m$ used in the simulations was increased from the value found in batch uptake experiments (113 mg/ml) to 130 mg/ml. Attempts were also made to obtain better fits to the experimental data by varying the values of the rate parameters from those obtained from the batch uptake experiments. Hence, with the kinetic rate constant model, simulations were carried out with other values of $k_1$ and other values of $D$ were used in the film and pore diffusion model. However, in both cases, it was not possible to obtain better fits to the top end of the curve without resulting in a lack of fit to the earlier part of the experimental curve. This approach to obtain a better fit was thus abandoned as it was considered better to retain an accurate description of the early part of the breakthrough curve. Possible reasons for the poor fit to the latter stages of the breakthrough curve for BSA adsorption are discussed in detail below.

## DISCUSSION

The Langmuir isotherm has been successfully used to describe the adsorption of both BSA and lysozyme to the cation exchanger S Sepharose FF in single component experiments. This is consistent with our studies of the adsorption of BSA to anion exchangers in which a Langmuir isotherm was also found to be appropriate[3]. The values of the dissociation constant, $K_d$, for the adsorption of the two proteins to S Sepharose FF are similar in molar terms, a somewhat surprising observation as the pH used in this study, pH 5, was very close to the isoelectric point of BSA and the molecule would have been carrying a net negative charge of $-2$ (ref. 18). As such it may be expected that the protein would only bind weakly to an anion exchanger and not at all to a cation exchanger such as S Sepharose FF. In fact there was a considerable degree of BSA adsorption to S Sepharose FF, indicating that although the net charge of the molecule may be negative, there must be an asymmetric distribution of charges on the molecule such that a considerable degree of positive charge is present in one region[18] which may interact with the negatively charged sulphonic groups present in S Sepharose FF. Kopaciewicz *et al.*[19] have reported similar studies in which a number of proteins, including BSA were found to bind to ion exchangers at pH values at which the net charge carried by the proteins was the same as that carried by the functional group on the ion exchanger. In molar terms, the maximum capacity of S Sepharose FF

for lysozyme is several times greater than for BSA. A possible reason for this is the smaller size of the lysozyme molecule which could have two effects. Firstly more molecules of lysozyme than BSA may be able to fit onto a given surface area and secondly the smaller lysozyme molecule will be able to penetrate regions of the ion-exchanger particles which are too restricted for the larger BSA molecule to enter, this being the working principle behind molecular exclusion separation methods.

The use of the Langmuir isotherm to describe protein adsorption has recently been criticised by Velayudhan and Horváth[20]. They point out that a consideration of the nature of adsorption of proteins to many adsorbents, including ion exchangers, indicates that proteins do not bind at individual, independent sites, one of the assumptions of the theoretical derivation of the Langmuir isotherm, but that multivalent attachment involving several functional groups on both the protein and the adsorbent occurs. Such multivalent adsorption has been demonstrated by several authors in different systems, including ion exchangers. Gosling[21] for example, has demonstrated the expulsion of 10–15 moles of chloride for every mole of BSA that was adsorbed to a diethylaminoethyl (DEAE) derivatised ion-exchange material. Velayudhan and Horváth[20] also point out that the Langmuir isotherm can not account for changes in protein retention with changes in salt concentration. However, many authors have found that experimental protein-adsorption data from a variety of different systems can be described by a Langmuir type isotherm and constants derived from it can be used in models of the dynamic processes of protein adsorption, as demonstrated in this paper. Whilst accepting its limitations, the Langmuir isotherm remains therefore, a simple and useful tool which can be used to help in the study of protein adsorption and to compare and contrast different ion exchangers.

Two different models, both utilising equilibrium constants derived from the Langmuir isotherm, have been used to describe protein-adsorption profiles in stirred tanks. The shapes of the predicted curves were compared to experimental data for each of the proteins being studied. In the case of lysozyme the kinetic rate constant model gave a curve which followed closely the experimental adsorption profile throughout the whole period of the experiment. A similar fit could not be obtained to the adsorption profile of BSA. A possible reason for the relatively poor fit of the BSA adsorption data to the kinetic rate constant model is that diffusion of BSA within the S Sepharose FF particles is severely hindered and hence adsorption of BSA may occur initially in the outer regions of particles. As the diffusion paths are short, adsorption appears to take place rapidly whilst later phases of adsorption, which must take place deeper in the particle as the outer regions are filled, are thus much slower than the initial rate. The lysozyme molecule, being smaller than BSA, is able to penetrate the particles more easily and adsorption could be occurring at a more even rate throughout the whole adsorption period. The resulting lysozyme adsorption profile is a shape that can be successfully described by the kinetic rate constant model. This hypothesis could be further investigated by performing a series of experiments in which the adsorption process is stopped at different time points, followed by visualising the location of the adsorbed protein within the adsorbent particle.

The fits of the film and pore diffusion parameter model are very close to the experimental data for both proteins. In the case of lysozyme the experimental data fitted this model slighter better than the kinetic rate constant model. As expected, for both proteins, the values of the effective pore diffusion coefficient, $D$, were found to be

lower than the calculated molecular diffusion coefficients in free solution, $D_{AB}$. The ratios of the molecular diffusivity in free solution, $D_{AB}$, to the effective diffusivity in the adsorbent particle, $D$ are 2.4 for lysozyme and 8.6 for BSA, which provides strong evidence that the diffusion of BSA within the particle is more restricted than for the smaller lysozyme molecule. The ability to get excellent agreement between theoretical and experimental profiles for both proteins suggests that the film and pore diffusion model can be used successfully to predict the rates of adsorption of proteins to ion exchangers in mixed tank situations.

Both the kinetic rate constant model and the film and pore diffusion model predict breakthrough curves very close to the experimental points obtained for lysozyme. Both the sharpness of the curve and its symmetry is mirrored in each of the calculated curves. The reasons why BSA behaved anomalously in the fixed bed experiments are not known. The ion exchanger had a greater capacity for BSA when loaded in a packed bed mode than had been predicted from isotherms that were the result of stirred tank experiments. This additional capacity appeared to be characterised by slow adsorption kinetics as evidenced by the slow rise in the value of $c/c_0$ during the later part of the breakthrough curve. The time–concentration profile seen by the adsorbent is much different in packed bed and stirred tank experiments and this may partially explain the difference observed. It is well established that BSA can form dimers in solution[22] and it is possible that the high local concentrations of BSA that are present when this protein is adsorbed to the ion exchanger, and the constant flow of fresh BSA solution in packed bed experiments, may promote the formation of dimers. If this were the case, the apparent additional adsorption capacity may be the result of multi-layer binding of BSA molecules to molecules that are already adsorbed. Indeed when BSA eluted from S Sepharose FF was analysed by molecular exclusion chromatography two peaks were observed. The retention volume of the peaks suggested the presence of BSA monomer and dimer forms, indicating that dimer formation was occurring in the packed bed. Although there was evidence for some dimers in the fresh, unadsorbed solutions, the concentration of the dimer in the eluted protein was much enhanced.

The results presented here illustrate the use of two different models to describe the adsorption of proteins to the strong cation exchanger S Sepharose FF. In the case of lysozyme both models predicted accurately the observed adsorption profiles in mixed tanks and in packed beds. The predictions from both models of BSA adsorption were not as accurate as those of lysozyme adsorption, however, the apparent agreement may be sufficient for most design purposes. Although it is recognised that the kinetic rate constant model is a gross simplification of the actual adsorption process, it may be a useful method for the prediction of the adsorption of proteins to ion exchangers, given its simplicity and the small amount of computing power required in contrast to the more rigorous film and pore diffusion model. Similar studies with an affinity adsorption system involving the adsorption of immunoglobulin G to agarose based affinity adsorbents showed that only the film and pore diffusion model described the experimental data accurately[10].

SYMBOLS

A adsorption site on the ion exchanger
$c$ soluble protein concentration
$c_i$ point concentration of protein
$c_0$ initial, or inlet liquid phase protein concentration
$d$ mean particle diameter
$D$ effective pore diffusivity
$D_{AB}$ molecular diffusivity in free solution
$D_x$ axial dispersion coefficient
$g$ gravitational constant
I counter-ion
$k_1$ adsorption rate constant
$k_{-1}$ desorption rate constant
$k_f$ liquid film mass transfer coefficient
$K_d$ dissociation constant for the protein–ion-exchanger complex
$l$ interstitial velocity of liquid in the bed
$M_A$ relative molecular mass of A
$n$ the number of charges involved in the interaction between an adsorption site and a single protein molecule
P protein molecule
$q$ concentration of protein adsorbed to the ion exchanger
$q_i$ point concentration of adsorbed protein
$q_m$ maximum protein capacity of the ion exchanger
$r$ radial coordinate of ion exchanger particle
$R$ radius of ion exchanger particle
$R_i$ rate of interface mass transfer
$t$ time
$T$ absolute temperature
$u$ superficial velocity of liquid flow through the column
$v$ volume of ion exchanger
$V$ volume of liquid external to the ion exchanger
$x$ axial coordinate of packed bed
$\varepsilon$ porosity of ion exchanger particle
$\varepsilon_b$ porosity of packed bed
$\mu$ liquid viscosity
$\rho$ particle density

*Superscript*
* value when system is at equilibrium

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