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PREDICTION OF THE PERFORMANCE OF PREPARATIVE AFFINITY CHROMATOGRAPHY

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SUMMARY

This paper describes a theoretical approach to the prediction of the performance of preparative affinity separations for biological macromolecules in packed columns. The approach, which is applicable to conventional low-pressure packed column methods as well as high-performance liquid chromatography techniques, requires knowledge of certain parameters that describe the interactions between adsorbent and adsorbate during the affinity separation procedure. We have measured the parameters appropriate to the adsorption stages of affinity systems involving immobilised Cibacron Blue and immobilised monoclonal antibodies against β-galactosidase. The theoretical predictions appear to agree well with the experimental performance of batch and packed column affinity systems. The influence of the factors that govern the performance of the adsorption stage of the separation procedure is explained in detail, and the possible advantages of using HPLC techniques in macropreparative affinity chromatography are discussed.

INTRODUCTION

Affinity chromatography is a powerful technique that has been used increasingly frequently over the last 30 years for the purification of biological macromolecules[1–3]. Its main advantages as a bioseparation technique are that a very high degree of purification of the desired species can be obtained in a single step process and that packed column techniques can readily be automated. There is considerable current interest in the adoption of affinity chromatography as a chemical engineering unit operation for the isolation of biological compounds on an industrial scale[4,5]. However, a prerequisite of such a step is that the process of affinity chromatography is well understood so that the performance of such systems can be predicted and the influence of different procedures, such as the adoption of HPLC techniques, can be compared. Packed column affinity chromatography techniques fall mainly into two major categories; pulse methods and frontal methods[6].

Pulse methods

A small sample of crude material is applied to the column, which is subse-

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quently eluted by techniques which lower the affinity of the adsorbate for the immobilised phase. The process is often referred to as elution development. The technique is frequently used for analytical procedures in order to measure the amount of adsorbate present in the applied sample. The method is also used in so-called "quantitative" affinity chromatography, which enables the parameters of the interactions between biomacromolecules and soluble or immobilised ligands to be measured[7,8]. Pulse methods of operation can be used for separating mixtures of compounds which have different affinities for the immobilised phase if the conditions of operation result in chromatographic separation of the compounds along the length of the column. However, as only small samples of material are applied in each cycle of operation, pulse methods are not ideally suited for macropreparative applications where the maximum usage of the column for the isolation of adsorbate is desirable.

Frontal methods

In this mode of packed column operation, sample is fed continuously into the column until the available capacity of the column is exhausted and adsorbate begins to appear in the column outlet. The variation of the concentration of adsorbate in the column outlet as a function of time is known as a breakthrough curve, and the measurement of breakthrough curves for such systems is referred to as frontal analysis[1]. This method of operation is particularly suitable for preparative affinity separations where the adsorbent has a specific affinity for only one species of molecule in the input stream. Under these circumstances, a substantial proportion of the available capacity of the adsorbent is utilised for binding adsorbate, an important consideration when the adsorbent itself is of high value, as is the case when immobilised antibodies are used for isolation purposes[9].

Further discussion in this paper will be restricted to affinity separation systems based on frontal methods rather than elution development, as the former mode of operation is more suited for use on a macro-preparative scale. General theories describing the performance of affinity chromatography systems are not well developed; in a recent review of column adsorption theories and their possible application for describing affinity chromatography, Yang and Tsao[6] have pointed out that the assumptions of linear adsorption isotherm or linear adsorption–desorption kinetics, which have been used frequently to obtain analytical solutions describing the performance of conventional liquid or gas chromatography, are not applicable to affinity chromatography procedures, particular when these are operated under conditions of frontal analysis. The unique property of adsorption specificity that occurs in affinity systems results in non-linear adsorption isotherms and the difficulties encountered in the mathematical treatment of such systems are the main reason for the lack of theoretical analysis.

The main aims of the work reported here are to present a simple model which successfully describes affinity chromatography and to use this model for developing methods to predict the performance of packed column and batch affinity separations. The experimental performances of two different types of affinity separation system are compared with the performances predicted by the theoretical approach following the measurement of certain of the parameters that describe the adsorption process. The apparent success of the theoretical model in accounting for the experimental observations has enabled prediction of the influence of many of the operating param-

eters on the performance of an affinity separation. Hence, affinity processes can be designed and optimised and the possible advantages of the application of HPLC techniques to macro-preparative affinity chromatography determined.

THEORY

Construction of models to describe affinity chromatography processes

Affinity separations exploit the interactions that can occur in biological systems when two different molecules can interact reversibly to form a strong non-covalent complex. Such interactions include those that occur between an enzyme and its substrate or inhibitor, an antibody and its antigen, hormones and their receptors, and those between complementary sequences present in nucleic acids. By immobilizing one of the components involved in the interaction, an absorbent with an affinity for the other component can be created. If a highly specific interaction is exploited, an adsorbent with an affinity for only one compound can be synthesized. For simplicity, we have assumed that the immobilised binding sites (B) on the affinity adsorbent have an affinity for only one species of adsorbate (A) and that the interaction can be described by an equilibrium relationship of the form:

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AB \tag{1}$$

where k_1 and k_2 are the rate constants governing the forward and backward directions of the reaction. The ratio of the constants (k_2/k_1) is equal to the dissociation constant (K_d) that describes the equilibrium relationship. The rate constants k_1 and k_2 do not simply represent the rates of adsorption and desorption of the adsorbate onto the immobilised binding site on the surface of the adsorbent but will also include significant contributions from resistances to mass transfer from the bulk of the mobile phase to the adsorption site that also occur in such systems. These mass transfer resistances also include the resistance in the fluid outside the particles (film diffusion resistance), that within the particles due to diffusion into the pores (pore diffusion or particle diffusion resistance) and the resistance on the wall surface of the pore (surface reaction resistance)[10]. Rather than attempting to derive models based on rigorous considerations of the nature of the resistances, we have decided to adopt an empirical approach and to assume that the adsorption and desorption processes can each be described by an experimentally determinable rate constant and to see how well experimental results correlate with the theoretical models.

The rate of mass transfer to the immobilised phase in an interaction described by eqn. 1 is given by:

$$\frac{dq}{dt} = k_1 \cdot c \cdot (q_m - q) - k_2 \cdot q \tag{2}$$

where c is the concentration of adsorbate in solution, q is the solid-phase concentration of the adsorbed material per unit volume of settled adsorbent, and q_m is the maximum adsorption capacity of the adsorbent.

The equilibrium position of such a system will be:

$$\frac{dq^*}{dt} = k_1 \cdot c^* \cdot (q_m - q^*) - k_2 \cdot q^* = 0 \tag{3}$$

where the superscript * represents the value at equilibrium. From eqn. 3, it can be shown that at equilibrium, the solid phase concentration of the adsorbed material will vary with the concentration of the adsorbate in a manner described by:

$$q^* = \frac{q_m \cdot c^*}{(K_d + c^*)} \tag{4}$$

where $K_d = k_2/k_1$, *i.e.* the dissociation constant of the equilibrium reaction. Eqn. 4 predicts that the adsorption isotherm should be non-linear and of the type first described by Langmuir[11] often referred to as a "favourable" isotherm.

Batch adsorption

For a batch adsorption system of total volume V, with an initial adsorbate concentration (c_0) and containing a settled volume v of adsorbent, the concentration of adsorbate in the soluble phase at equilibrium will be given by:

$$c^* = \left(c_0 - \frac{v \cdot q^*}{V}\right) \tag{5}$$

Hence from eqn. 3:

$$\left(c_0 - \frac{v \cdot q^*}{V}\right)(q_m - q^*) - K_d \cdot q^* = 0 \tag{6}$$

and solving for q^* gives

$$q^* = \frac{V}{2v}\left(b - \sqrt{b^2 - \frac{4q_m \cdot c_0 \cdot v}{V}}\right) \tag{7}$$

where $b = (K_d + c_0 + q_m \cdot v/V)$. Substituting eqn. 7 back into eqn. 5 gives

$$c^* = c_0 - \frac{1}{2}\left(b - \sqrt{b^2 - \frac{4q_m \cdot c_0 \cdot v}{V}}\right) \tag{8}$$

Hence, at equilibrium, the amount of adsorbate that will remain in the soluble phase and the amount that will be bound to the adsorbent can be readily calculated, once values for K_d and q_m are known.

More complicated expressions, which take into account the intraparticular voidage of the adsorbent, can also be derived by the above procedure, although the error involved in ignoring this voidage is very often negligible.

Packed column adsorption

We have decided to adapt rate theories of chromatography rather than plate theories in order to model the performance of packed column affinity chromatography systems. Rate theories consist of sets of material balance equations together with appropriate boundary and initial conditions. The equation of continuity of the mobile phase is frequently formulated as being[6]

$$D_x \cdot \frac{\partial^2 c}{\partial x^2} - l \cdot \frac{\partial c}{\partial x} - R = \frac{\partial c}{\partial t} \tag{9}$$

where D_x is the axial dispersion coefficient, l is the linear velocity, R is the rate of interface mass transfer, x is the distance along the bed and t is the time. The literature contains a number of models of chromatographic processes based on rate theories because of the variety of expressions for R that can be used in eqn. 9. Many of these expressions require knowledge of parameters associated with the physical nature of the adsorbent, and these parameters are often not readily measured. Many of the solutions obtained for eqn. 9 are only applicable to chromatography systems that can be described by a linear isotherm[6]. We assumed that the rate of interface mass transfer in packed column systems can be described in an identical manner to that we have adopted to describe batch operations. Hence, using eqn. 2,

$$R = k_1 \cdot c \cdot (q_m - q) - k_2 \cdot q \tag{10}$$

If the assumptions are made that the effect of axial dispersion is negligible (*i.e.* $D_x = 0$), and that the boundary conditions are that the column is initially devoid of bound adsorbate and that the concentration of the adsorbate in the inlet stream (c_0) is constant, then it is possible to obtain an analytical solution to the differential eqns. 9 and 10. The solution obtained is essentially identical in form to that obtained by Thomas[12] for similar differential equations describing fixed-bed ion-exchange and fixed-bed adsorption of gases. Thomas' solution has to be modified slightly in order to be able to use the nomenclature that has been adopted here to describe affinity separations, but the original form of the solution is unchanged. The variation of c/c_0 measured at the outlet of a column at a time t after a non-adsorbed component would have emerged from the column is given by:

$$\frac{c}{c_0} = \frac{J(n/r, nT)}{J(n/r, nT) + |1 - J(n, nT/r)| \cdot \exp|(1 - r^{-1})(n - nT)|} \tag{11}$$

where

$$r = 1 + c_0/K_d$$
$$n = q_m \cdot k_1 \cdot h \cdot A_c/f$$
$$T = f \cdot t \cdot (K_d + c_0)/A_c \cdot q_m \cdot h$$

The packed column is of height (h) and cross-section area (A_c) and f is the volumetric flow-rate through the column. The function $J(d, e)$ of the two variables d and e is defined by Hiester and Vermeulen[13], and is given by:

$$J(\alpha, \beta) = 1 - e^{-\beta} \int_0^{\alpha} e^{-\xi} I_0 (2\sqrt{\beta\xi})\, d\xi$$

According to Thomas[12], values of J can be calculated more easily from an asymptotic series when α and β are large. The first two terms of the series are

$$J(\alpha, \beta) \approx \frac{1}{2}[1 - \text{erf}(\sqrt{\alpha} - \sqrt{\beta})] + \frac{\exp[1 - (\sqrt{\alpha} - \sqrt{\beta})^2]}{2\pi^{\frac{1}{2}}[(\alpha\beta)^{\frac{1}{4}} + \beta^{\frac{1}{4}}]}$$

and the error is less than 1% when $\alpha\beta > 36$. Using this approximation, we have been able to compute values of c/c_0 as a function of time (t) for a wide range of fixed bed adsorption procedures. The values of K_d, k_1 and q_m that have been used in these predictions are the values determined from small-scale batch experiments. The use of eqn. 11 allows the shape of a breakthrough curve for packed column adsorption to be predicted by using values of parameters that are readily measured in batch experiments.

EXPERIMENTAL

Affinity systems

In order to check the validity of the theoretical approach to predicting the performance of affinity systems, experiments have been carried out with two types of affinity chromatography systems. The experimental data have allowed determination of the parameters describing the affinity interactions which can be used in theoretical predictions of adsorption performance. Further experiments have subsequently been carried out to test the validity of these theoretical predictions. The affinity systems investigated were:

(i) Cibacron Blue Sepharose CL-6B. The binding of the proteins, bovine serum albumin, and hen egg white lysozyme to Cibacron Blue Sepharose CL-6B (Pharmacia Fine Chemicals) was studied. To simplify the investigation, only the adsorption of pure proteins from 0.05 *M* Tris–HCl buffer, pH 7.2, was investigated. Elution of the adsorbed proteins was achieved with 1 *M* KCl in the above Tris buffer.

(ii) Immobilised monoclonal antibodies. The adsorption of *E. coli* β-galactosidase on an immobilised monoclonal antibody against β-galactosidase was chosen as a suitable model immunoadsorbent system, as the concentration of antigen could easily be followed by measuring β-galactosidase activity. The β-galactosidase system does have some limitations as a model immunoadsorbent system. The molecular weight of the β-galactosidase molecule (*ca.* 464,000) is substantially larger than the molecular weight of antigens commonly purified by immunoaffinity techniques, and the large molecule may have difficulties penetrating the pores present in the immunoadsorbents. As the β-galactosidase molecule consists of four identical polypeptide chains, there is the possibility that it could be bound to immobilised monoclonal antibodies by multiple bonds, thus complicating the characterisation of the binding reaction[14]. Monoclonal anti-β-galactosidase, prepared from the ascites fluid of a rat, was the kind gift of Dr. F. Shand, Department of Immunotechnology, Wellcome

Research Laboratories, Beckenham, U.K. The antibody was immobilised on two different support materials by the following techniques:

(a) Immobilisation on CNBr-activated Sepharose 4B. 50 mg of antibody in 10 ml of 10 m*M* sodium phosphate buffer, 0.65% sodium chloride, pH 7.5 (PBS buffer), was added to 10 ml (settled volume) of swollen CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals) in 40 ml of 0.1 *M* $NaHCO_3$ buffer, 0.5 *M* sodium chloride, pH 8.3, and the suspension was gently agitated for 2 h at 20°C. Any remaining reactive groups on the support were blocked by suspending the adsorbent in 0.5 *M* Tris–HCl buffer, pH 8.0 for a further 2 h. Excess non-coupled protein was removed by alternate washing with 0.1 *M* sodium acetate buffer, pH 4, and $NaHCO_3$ buffer, pH 8.3, both containing 0.5 *M* sodium chloride. Five such washing cycles were carried out, and the adsorbent was resuspended and stored in 0.05 *M* Tris–HCl buffer, pH 7.2 in the presence of 0.01% thimerosal prior to use.

(b) Immobilisation on a silica-based adsorbent. Antibodies were also immobilised on a porous silica affinity support matrix; "Affinity Adsorbent", Boehringer (Mannheim, F.R.G.). The adsorbent is supplied in a form preactivated with glutardialdehyde. The size of the silica particles is about 100–200 μm, the size of the pores is about 300 nm and the specific surface is about 10 m^2/g. 2 g of affinity adsorbent was added to 50 ml of PBS buffer, containing 100 mg of antibody, and the flask was shaken for 4 h at 20°C in a water bath. Unreacted protein was removed by extensive washing in a sintered funnel with 1.5% (w/v) sodium chloride solution, and any remaining unreacted aldehyde groups were blocked by suspension of the adsorbent in 0.3 *M* ethanolamine–HCl buffer, pH 7.5, for 1 h at 20°C. The gel was washed extensively with 0.5 *M* propionic acid containing 0.9% sodium chloride, followed by 0.9% sodium chloride. The adsorbent was stored in PBS buffer in the presence of 0.01% (w/v) thimerosal.

Elution of adsorbed β-galactosidase from the adsorbents was achieved with 6 *M* urea in 20 m*M* Tris–HCl, 10 m*M* ethylene diamine tetraacetic acid, 10 m*M* sodium chloride, 0.1 *M* 2-mercaptoethanol, pH 7.2. Urea was removed by exhaustive dialysis against the above buffer and, typically, more than 80% of β-galactosidase activity was recovered under these conditions.

Batch isotherms

The appropriate affinity adsorbent was added to flasks containing various amounts of protein in 50 m*M* Tris–HCl buffer, pH 7.2, in a final volume of 10 ml. The flasks were shaken in a water bath for at least 6 h in order for the system to come to equilibrium. Samples (1 ml) were removed and centrifuged (5000 *g*, 2 min) and the supernatant was either assayed for enzyme activity (for studies with immobilised anti-β-galactosidase) or the amount of protein in solution was determined from measurements of the optical adsorption at 280 nm (for studies with immobilised Cibacron Blue). The amount of enzyme bound to the adsorbent (q^*) was calculated as the total amount of enzyme present at the beginning of the experiment less the amount still in the soluble phase at equilibrium. Controls were carried out to ensure that there was no loss of enzyme activity under these conditions.

Rearrangement of eqn. 4 yields:

$$c^*/q^* = c^*/q_m + K_d/q_m \tag{12}$$

Values of K_d and q_m were determined from straight-line plots of c^*/q^* against c^*. The intercept of such plots on the c^* axis is at $-K_d$ and the gradient of the line is $1/q_m$.

Kinetics of batch adsorption

In order to follow the kinetics of adsorption in batch systems, two procedures were used:

(i) Immobilised Cibacron Blue system. The soluble phase of the batch system was monitored continuously for protein concentration using the apparatus described in Fig. 1a. Soluble phase was continuously removed from the batch reactor by passage through a 20-μm porosity net filter and pumped through a continuous flow UV spectrophotometer to measure protein concentration. The output of the spectrophotometer was connected to a chart recorder so that a permanent record could be kept of the time course of reduction of protein concentration in the soluble phase. The liquid stream was continuously returned to the reactor so that the overall volume of the system remained constant. The volume of the external fluid circuit was kept as small as possible (approx. 1 ml) and a fast pump flow-rate was used to achieve a rapid response time. In a typical experiment, adsorbent (1 ml of a 1:1 suspension in buffer) was added to 20 ml of protein solution (50 mM Tris–HCl, pH 7.2) at 20°C and recycling was continued until equilibrium was achieved, as evidenced by the lack of further reduction in the concentration of protein in the soluble phase.

(ii) Immobilised antibody systems. One of the experimental systems used to investigate the kinetics of the adsorption of β-galactosidase on the immobilised an-

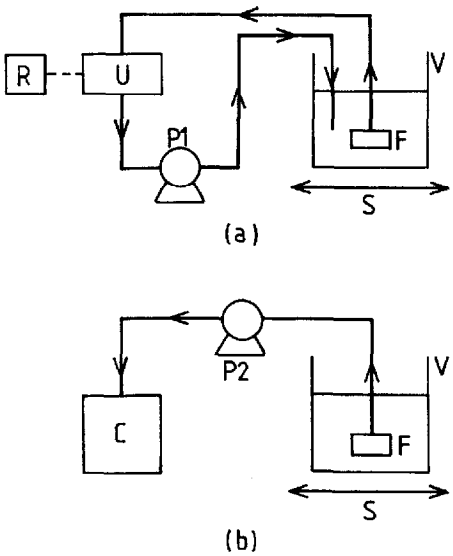

Fig. 1. Apparatus for measuring the kinetics of batch affinity adsorption. (a) System used for Cibacron Blue–Sepharose CL-6B system. (b) System used for immunoadsorbent systems. C = fraction collector; F = 20-μm porosity net filter (Amicon adjuster assembly and net filter for a 16 mm bore chromatography column); P = peristaltic pump (flow-rate P1 = 10 ml min^{-1}, P2 = 0.5 ml min^{-1}); R = chart recorder; S = reciprocating shaker system in thermostatted water bath; U = continuous-flow UV spectrophotometer (280 nm); V = batch adsorption vessel.

tibodies is shown in Fig. 1b. The soluble phase was again removed continuously by passage through a filter but at a slower rate (0.5 ml min^{-1}) and was collected in a series of 0.5 ml fractions. Each fraction was thus a sample of the soluble phase at each minute of the reaction. A large volume of solution in the batch system was used so that the extent of the loss of soluble phase was negligible. The collected fractions were subsequently assayed for β-galactosidase activity in order that the time course of the loss of enzyme from the soluble phase could be determined. In experiments where adsorption was expected to continue over a period of a number of hours, an alternative procedure was used. Samples (0.5 ml) were removed from the batch reactor at intervals and the soluble and solid phases separated by centrifugation. Samples of the soluble phase were subsequently assayed for β-galactosidase activity.

The two procedures described above are suitable for following batch adsorption kinetics in systems which approach equilibrium over periods of at least a few minutes. The systems would not be suitable for measuring faster associations and it may not be possible to measure k_1 values for conventional high-performance liquid chromatography (HPLC) adsorbents by this type of procedure. The time taken for the system to come to equilibrium depends not only on the value of the association rate constant k_1 but also on the concentrations and amounts of adsorbent and adsorbate present in the batch system. Hence, it is possible to observe systems with a high value of k_1 by using low concentrations of adsorbent and adsorbate.

Determination of adsorption rate constants k_1 and k_2

In order to determine the value of the adsorption rate constant k_1 that was applicable to the kinetic profile of batch adsorption, the experimental data was compared to a series of predicted results that were constructed by computer methods. The predicted results were calculated by assuming that the rate of mass transfer of adsorbate to the adsorbent could be accurately described by eqn. 2 and the values of K_d and q_m needed in this equation were the values determined from previous isotherm experiments. The experimental kinetic results were compared with the predicted results for a range of values of k_1. The value of k_1 applicable to the system investigated was taken to be that value which resulted in a predicted time course that best fitted the experimental results. An example of this approach is described in Results and shown in Fig. 4. Eqn. 1 shows that k_2, the rate constant of the dissociation reaction, can be calculated as being equal to $k_1 \cdot K_d$ and hence does not have to be measured independently as the value of K_d has previously been measured in isotherm experiments.

Measurement of breakthrough curves

Breakthrough curves where measured in experiments where solutions of pure protein in 0.05 *M* Tris–HCl were pumped through packed columns of the appropriate affinity adsorbent. The outlet of the column was monitored for the level of adsorbate, either by continuous measurement of the optical adsorption at 280 nm (for the immobilised Cibacron Blue systems) or by measuring the β-galactosidase activity of collected fractions (for the immobilised antibody system). The lay-out of the micro-computer controlled experimental equipment used for operating packed bed procedures is shown in Fig. 2. The micro-computer is able to control the position of the solenoid valves which determine which fluid stream is applied to the bed and also

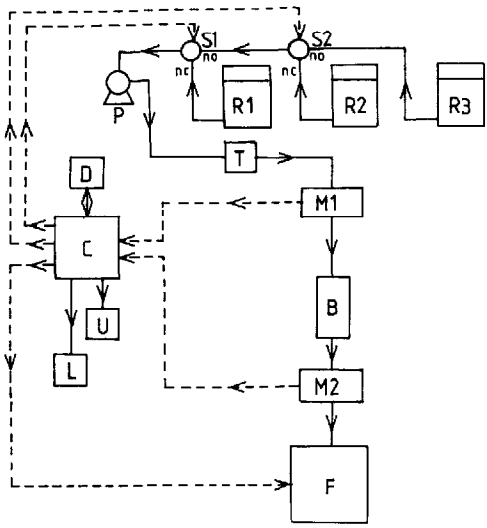

Fig. 2. Micro-computer-based system for the automatic control of packed bed operation used in the measurement of breakthrough curves. B = packed bed of affinity adsorbent; C = micro-computer; D = data storage unit (disk drive or cassette tape); F = fraction collector; L = line printer; M = flow spectrophotometer; P = peristaltic pump; R = reservoir (1 = adsorbate solution, 2 = elution buffer, 3 = re-equilibration buffer); S = solenoid valve (no = port normally open, nc = port normally closed); U = visual display unit. —— = a path of fluid flow; --- = an electrical connection.

logs the experimental data obtained. The system will be described more fully elsewhere[15].

Assay of β-galactosidase activity

β-Galactosidase was assayed by measuring colourimetrically the release of *o*-nitrophenol from the substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG). 50 μl of the solution to be assayed (*ca.* 1–2 units ml^{-1}) was added to 1.5 ml of an assay cocktail containing 0.1 *M* sodium phosphate buffer, pH 7.3, 0.11 *M* 2-mercaptoethanol, 1 m*M* magnesium chloride at 37°C. The enzyme was activated by incubation at 37°C for 3 min and the assay was then started by addition of 50 μl of a 0.068 *M* solution of ONPG and the incubation continued at 37°C. When the optical density at 410 nm had risen to approximately 0.4, the time that had elapsed after addition of substrate was noted and 1 ml of 1 *M* sodium carbonate was added to terminate the reaction and to develop the full colour of the released *o*-nitrophenol. The optical density of the samples was measured at 410 nm in a Pye Unicam SP6500 UV–visible spectrophotometer. One unit of β-galactosidase activity is defined as the amount of enzyme that can hydrolyse 1 μmole of ONPG to *o*-nitrophenol and galactose per min at 37°C and pH 7.3.

Assay of concentration of bovine serum albumin and lysozyme

The concentrations of bovine serum albumin and lysozyme in solutions of the pure proteins were determined by measuring the optical density of the solution at 280 nm. The assays were calibrated for each protein by measuring the optical density of protein solutions of known concentration.

Computer methods

Computer programs were written to calculate the values of the J functions used in eqn. 11, to simulate the adsorption process for batch systems, and to generate theoretical predictions of the influence of various parameters on the shape and position of adsorption breakthrough curves. All computer programs were written in BASIC to run on a Grundy Business Systems Newbrain Model AD micro-computer. Copies of the listings of the programs are available from the author.

RESULTS

Adsorption isotherms

Adsorption isotherms were measured for the binding of proteins to immobilised Cibacron Blue and to the immobilised monoclonal antibodies. The adsorption isotherm for the binding of lysozyme in 0.05 *M* Tris–HCl buffer, pH 7.2 to Cibacron Blue–Sepharose CL-6B is shown in Fig. 3. Favourable adsorption isotherms were found for all the systems investigated and the equilibrium values of q^* and c^* were found to fit expressions of the type described by eqn. 4. The values of the effective dissociation constants (K_d) and the maximum capacity of the adsorbent (q_m) for the affinity systems were determined as described in Experimental and are shown in Table I.

Blue-Sepharose appeared to have an equal maximum capacity for binding bovine serum albumin and lysozyme when measured on the basis of mg of protein found per ml of adsorbent although the capacity for lysozyme appeared to be greater when compared on a molar basis. Comparison of the molar values of K_d for the binding of lysozyme and bovine serum albumin to the adsorbent suggested that lysozyme had a five-fold greater affinity for the adsorbent. As far as we are aware, the

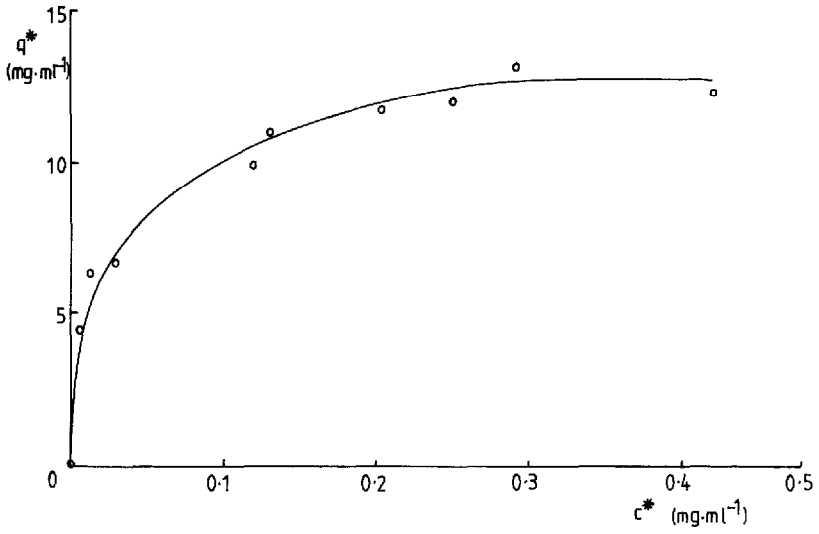

Fig. 3. Isotherm for the binding of lysozyme to Cibacron Blue–Sepharose CL-6B. The isotherm for the binding of lysozyme to Cibacron Blue Sepharose CL-6B in 0.05 *M* Tris HCl, pH 7.2 at 20°C was determined as described in Experimental.

TABLE I

MEASUREMENT OF ADSORPTION PARAMETERS IN BATCH EXPERIMENTS

Values of the effective dissociation constant for the binding reaction between adsorbate and adsorbent (K_d) and the maximum capacity of the adsorbent (q_m) were determined from isotherm experiments performed with small scale batch systems. Values of the overall rate constants for the adsorption and desorption processes were determined from kinetic data obtained from the batch experiments. All measurements were carried out at 20°C in 0.05 *M* Tris–HCl buffer, pH 7.2.

		Support material			
		Sepharose CL-6B	*Sepharose CL-6B*	*Sepharose 4B*	*Affinity adsorbent*
		Immobilised ligand			
		Cibacron Blue	*Cibacron Blue*	*Anti-β-galactosidase*	*Anti-β-galactosidase*
		Adsorbate			
		Lysozyme	*Bovine serum albumin*	*β-Galactosidase*	*β-Galactosidase*
q_m	mg ml^{-1}	14	14	2.9	2.2
	mol ml^{-1}	$1 \cdot 10^{-6}$	$0.21 \cdot 10^{-6}$	$6.2 \cdot 10^{-9}$	$4.7 \cdot 10^{-9}$
K_d	mg ml^{-1}	0.025	0.56	$6.5 \cdot 10^{-3}$	$2.2 \cdot 10^{-4}$
	M	$1.7 \cdot 10^{-6}$	$8.5 \cdot 10^{-6}$	$1.4 \cdot 10^{-8}$	$4.7 \cdot 10^{-10}$
k_1	ml mg^{-1} sec^{-1}	0.02	$1.2 \cdot 10^{-3}$	0.03	$5.5 \cdot 10^{-3}$
	M^{-1} sec^{-1}	286	82	$1.4 \cdot 10^{4}$	$2.6 \cdot 10^{3}$
k_2	sec^{-1}	$5 \cdot 10^{-4}$	$7 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$1.2 \cdot 10^{-6}$

affinity of lysozyme for Blue-Sepharose has not been reported before and, as the enzyme does not require an adenylyl containing co-factor, it would not necessarily be expected to have an affinity for this adsorbent. Experiments were carried out with underivatised Sepharose CL-6B to confirm that the lysozyme was not binding to polysaccharide moieties in the Sepharose itself. A number of other proteins, such as albumins, interferons and blood coagulation factors also have an unexpected affinity for Blue-Sepharose, although the mechanism of the interaction is not known[16].

Studies with the immobilised antibody systems indicated that the values of K_d and q_m depended on which support material the antibody had been coupled to. Although a slightly higher capacity was obtained for the system where the antibody was coupled to Sepharose rather than to a silica support, the former system was characterised by a 20-fold higher value of the effective dissociation constant (K_d) for the adsorption process. The values of K_d obtained were 100- to 2000-fold lower than those obtained for the Blue-Sepharose systems, confirming that the strengths of the interactions that occur in the antibody systems were much greater. The difference in the observed maximum capacities of the two adsorbents may merely reflect differences in the total amounts of protein that can be coupled to the supports. However, the difference in K_d values may be evidence for various alterations occurring in the antibody structure upon immobilisation and these alterations may effect the ability of the antibody to bind antigen to varying extents.

Kinetic studies

Values for the rate constants k_1 and k_2 for the forward and backward steps of the adsorption reactions in the affinity systems were measured in batch experiments using the techniques described in Experimental. Fig. 4 shows a typical comparison between the experimental results and a theoretical prediction of the kinetic performance using values of K_d and q_m previously obtained from isotherm experiments. The system under investigation in this example is the binding of β-galactosidase to antibody immobilised to the silica support, and the theoretical curve shown was generated with a value of k_1 which best fitted the experimental results. Similar experiments were carried out with a range of initial concentrations of adsorbate and with different amounts of adsorbent to ensure that the same values of k_1 were obtained under such conditions. It was always possible, by choosing an appropriate value of k_1, to obtain a good correspondence between the shapes of the experimental curves and the predicted results. A unique value of k_1 could be used to generate predictions that fitted all the experimental results obtained for each affinity system studied. The values of k_1 and k_2 found to be effective in the affinity systems investigated are shown in Table I. These results confirm that the rate of adsorption in an affinity system can indeed by described by a relationship of the form presented in eqn. 2. The equilibrium values of q^* and c^* were found to be accurately predicted by eqn. 8.

It is worth commenting on the different rates of adsorption that were observed in different systems. The forward rate of adsorption (k_1) of lysozyme to Blue-Sepharose was about four times faster than the rate of binding of bovine serum albumin, although the rates of the reverse reactions (k_2) were practically identical. Significant differences were also observed in the rates of adsorption and desorption effective in the two immobilised antibody systems. The binding of β-galactosidase to the antibody immobilised to Sepharose appeared to be five times faster than the rate of binding to the antibody immobilised to silica. However, the rate of the reverse re-

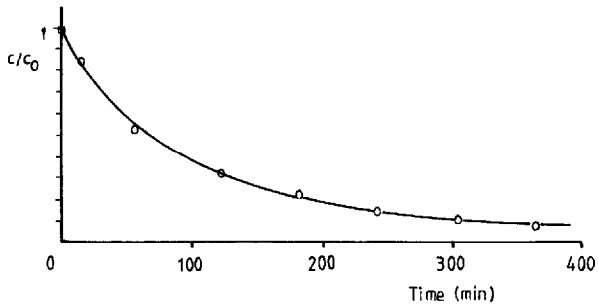

Fig. 4. Determination of adsorption rate constant (k_1) in a batch system. 1.5 ml (settled volume) of anti-β-galactosidase, attached to the silica support with a maximum binding capacity for β-galactosidase of $4.7 \cdot 10^{-9}$ mol.ml^{-1}, was added to 100 ml of 0.05 *M* Tris–HCl buffer, pH 7.2, containing β-galactosidase at a concentration of $3.4 \cdot 10^{-8}$ *M*. Samples were removed at intervals whilst the system was kept well mixed by agitation in a water bath, maintained at 20°C. The soluble phase of the samples was assayed for β-galactosidase activity as described in Experimental. An identical experiment, but without addition of affinity adsorbent, was performed simultaneously as a control to ensure that β-galactosidase activity did not decrease during the 6-h adsorption period. The figure shows the experimental points (○) and the solid line is the predicted performance of such a system assuming that the rate of mass transfer could be described by eqn. 2. The values of the parameters used in the prediction were $K_d = 4.7 \cdot 10^{-10}$ *M*, $q_m = 4.7 \cdot 10^{-9}$ mol ml^{-1}, and $k_1 = 2.6 \cdot 10^3$ M^{-1} sec^{-1}.

action was found to be almost 200 times faster with the Sepharose system. The difference in these observed rates may reflect differences in the mass transfer resistances that occur in the two systems.

Experiments with packed columns

A large number of experimental breakthrough curves were measured for packed column systems in order to study the effect on these curves of changing operating variables such as the volumetric flow-rate through the column, the inlet concentration of adsorbate and the size and shape of the column. The experimental data obtained has been compared with the predicted performance of the column, as described by eqn. 11. The values of the parameters used in calculating the predicted performance were the values of K_d, q_m and k_1, measured as described above in batch systems. In general, we have found good agreement between the experimental and the predicted results, thus confirming the validity of this approach. An example of the type of study undertaken is shown in Fig. 5, where a series of breakthrough curves was obtained for lysozyme binding to columns of Blue-Sepharose with constant cross-sectional area but with various lengths.

We have found that there was excellent agreement between the predicted and measured points on the breakthrough curves where a value of c/c_0 of 0.5 is obtained. This suggests that the values of K_d and q_m measured in batch systems are directly applicable for packed column operation. Isotherms, constructed from plots of the total amount of adsorbate bound by the column when at equilibrium with the inlet stream against inlet concentration of the adsorbate, determined from a series of experiments with different inlet concentrations, have also yielded similar values for K_d and q_m. However, we have found in some experiments, particularly at very low flow-rates, that the sharpness of the breakthrough curve was more consistent with that of a process governed by a value of k_1 somewhat lower than the value applicable to batch studies. This discrepancy may be due to the omission of any consideration of axial dispersion forces in the theoretical predictions. The magnitude of the effects

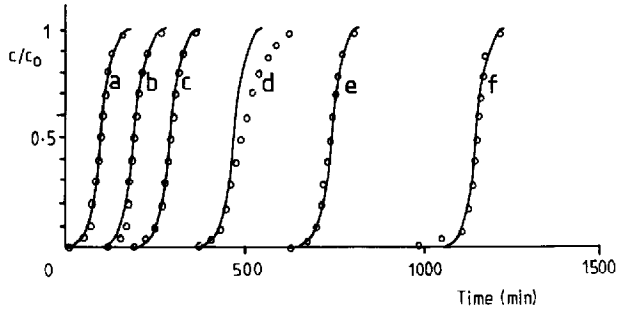

Fig. 5. Variation of shape and position of breakthrough curve with length of the packed column. A series of experiments was performed to study the adsorption of lysozyme to packed columns of Cibacron Blue Sepharose CL-6B. The column diameter (10 mm), the volumetric flow-rate (1 ml min^{-1}) and the inlet adsorbate concentration (0.1 mg ml^{-1}) were kept constant in each experiment. The column lengths used were (a) 14 mm; (b) 27 mm; (c) 41 mm; (d) 66 mm; (e) 104 mm; (f) 161 mm. The figure shows the experimental determinations of c/c_0 (○) and the solid lines are the predictions of the shape and position of the breakthrough curves as determined by use of eqn. 11. The parameters used in the theoretical predictions were: $K_d = 1.7 \cdot 10^{-6}$ M; $q_m = 1 \cdot 10^{-6}$ mol ml^{-1}; $k_1 = 286$ M^{-1} sec^{-1}.

of axial dispersion is considered later. Alternatively, it is possible that additional resistances to mass transfer may occur in packed columns but not in batch systems.

Factors determining the shape of breakthrough curves

In affinity systems where frontal methods of operation are adopted, the most efficient adsorption performance will be obtained when the shape of the breakthrough curve for the system is as sharp as possible. This point is illustrated in Fig. 6 which shows two breakthrough curves of different sharpnesses which could be obtained for the same packed column depending on the operating conditions. It is normal practice in preparative affinity chromatography to terminate the loading of the column when the level of adsorbate in the outflow from the column rises above a certain level. If loading of the column continues beyond this point, considerable amounts of adsorbate may pass through without being adsorbed. The amount of adsorbate that can be applied to the column before this level is exceeded will depend critically on the shape of the breakthrough curve. In practice, it is unlikely that the column will be loaded to its maximum capacity. It is clear from Fig. 6 that adsorption conditions that result in the sharper breakthrough curve would also result in more material being retained by the column.

We have been interested therefore in studying the influence of a number of factors on the shape of the breakthrough curve and hence the performance of the adsorption stage of packed column affinity processes.

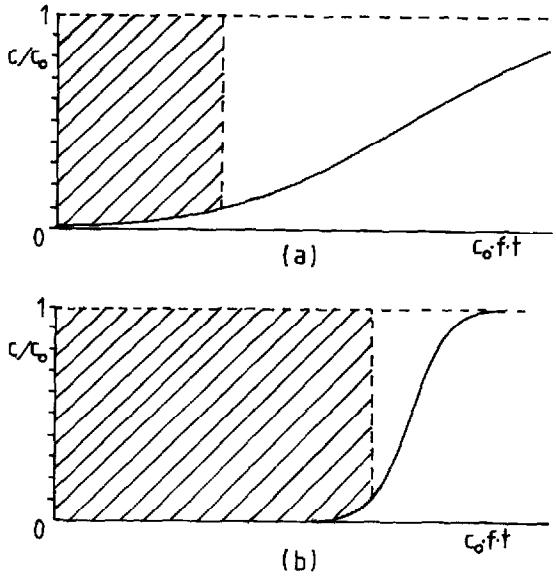

Fig. 6. The influence of the shape of the breakthrough curve on the adsorption performance of packed columns. The figure shows a plot of c/c_0, the concentration of adsorbate in the outlet of the column compared to the concentration in the inlet, against $c_0 \cdot f \cdot t$, the amount of adsorbate applied to the column. (a) A shallow breakthrough curve that might be obtained for the column and (b) a sharper curve obtained for the same column by an alteration in the operating conditions. The shaded areas indicate the amounts of adsorbate that would be bound to the column if loading were terminated when the value of c/c_0 reached 0.1.

The apparent success of the use of eqn. 11 for describing fixed column adsorption has enabled us to use computer methods to determine the influence of the variation of a number of factors on the shape of breakthrough curves. In each set of predictions, only one variable was altered with all others remaining constant. The "position" of the breakthrough curve is taken to be the amount of adsorbate that has to be applied for the value of c/c_0 to reach 0.5. The results that were obtained from these experiment predictions are shown in Fig. 7.

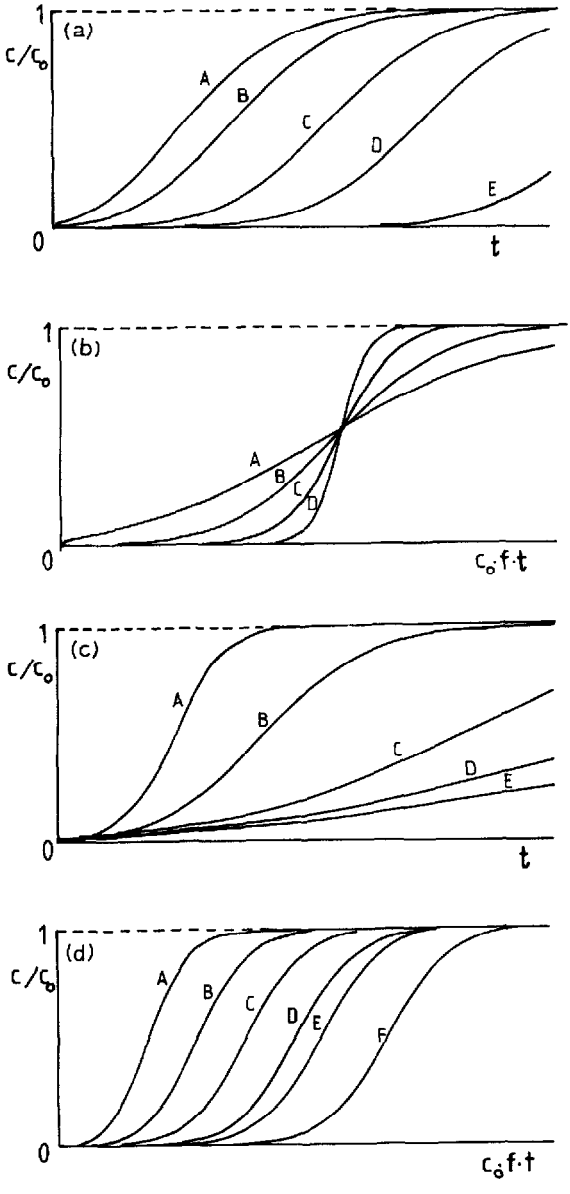

Fig. 7.

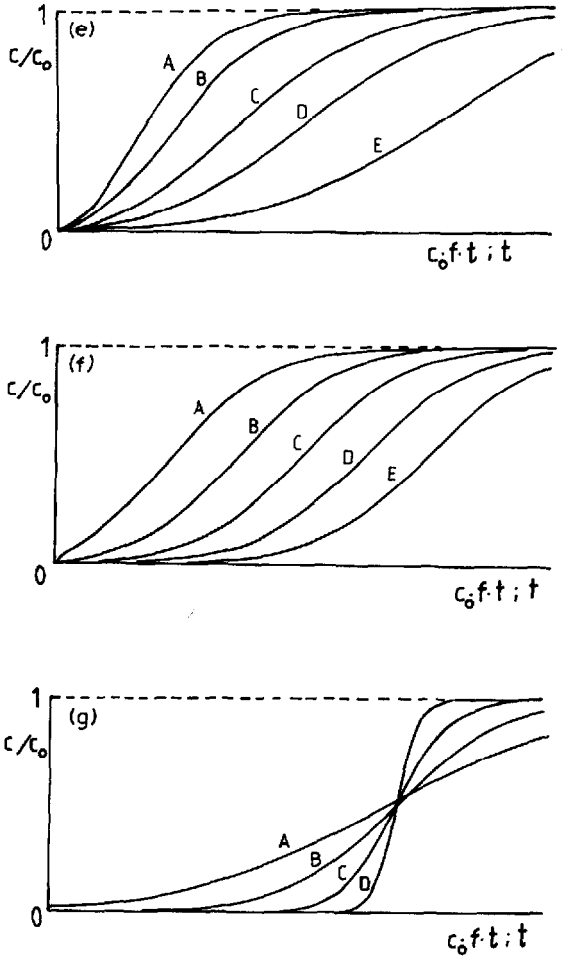

Fig. 7. Influence of parameters of the packed column on the shape and position of breakthrough curves. The figures show qualitatively the variation in the shape and position of adsorption breakthrough curves as one of the parameters associated with the packed column or its operation is altered. In each set of predicted curves, only one variable is altered, the others remaining constant. The predictions were made using eqn. 11. In each figure the value of c/c_0 in the column outlet is plotted either against the time of loading (t) or the amount of adsorbate applied to the bed ($c_0 \cdot f \cdot t$). In some sets, the shapes of the curves are the same regardless of whether c/c_0 is plotted against (t) or ($c_0 \cdot f \cdot t$). (a) Influence of flow-rate [plotted against time (t)]. The flow-rates are (arbitrary units): A, 1; B, 0.75; C, 0.5; D, 0.375; E, 0.25. (b) Influence of flow-rate [plotted against amount of adsorbate applied to the column ($c_0 \cdot f \cdot t$)]. The flow-rates are (arbitrary units): A, 1; B, 0.5; C, 0.25; D, 0.125. (c) Influence of concentration of adsorbate [plotted against time (t)]. The concentrations are (as multiples of the dissociation constant K_d): A, 10 × K_d; B, 5 × K_d; C, 2 × K_d; D, 1 × K_d; E, 0.5 × K_d. (d) Influence of concentration of adsorbate [plotted against the amount of adsorbate applied to the bed ($c_0 \cdot f \cdot t$)]. The concentrations are: A, 0.5 × K_d; B, 1 × K_d; C, 2 × K_d; D, 5 × K_d; E, 10 × K_d; F, 100 × K_d. (e) Influence of the dissociation constant K_d. The values of K_d are (as multiples of the inlet adsorbate concentration c_0): A, 3 × c_0; B, 2 × c_0; C, 1 × c_0; D, 0.5 × c_0; E, 0.1 × c_0. (f) Influence of the amount of adsorbent packed in the column. The amounts of adsorbent are (arbitrary units): A, 1; B, 2; C, 3; D, 4; E, 5. (g) Influence of the rate constant k_1. The values of k_1 are (arbitrary units): A, 1; B, 2; C, 4; D, 10.

A reduction in the rate of volumetric flow through the column has little effect on the sharpness of the breakthrough curve when plotted against time (Fig. 7a), but a marked improvement in performance is noticed when the curve is plotted against the amount of adsorbate applied to the column (Fig. 7b). Fig. 7b also shows that the position of the breakthrough curve is not affected by flow-rate. The use of low flow-rates increases the time that the adsorbate is in contact with the adsorbent, hence allowing more time for adsorption to occur and permitting a nearer approach to local equilibrium conditions. The concentration of the adsorbate in the input stream markedly effects the shape and position of the breakthrough curve when c/c_0 is plotted against time (Fig. 7c). However, when c/c_0 is plotted against the amount of adsorbent applied to the column (Fig. 7d), an effect is only noticed on the shape and position of the curve when c_0 is comparable to or smaller than K_d. The shape and position of the curve becomes constant when $c_0 \gg K_d$. The equilibrium nature of the interaction between adsorbate and adsorbent, as described by eqn. 4 will result in utilisation of the maximum capacity of the adsorbent only in situations where the inlet adsorbate concentration (c_0) is much greater than K_d. This point is explored in greater depth in the Discussion. The same effect on the breakthrough curves is likewise noticed in predictions of the influence of the value of the dissociation constant K_d (Fig. 7e), with the efficiency of adsorption performance increasing as the value of K_d is decreased. Fig. 7f shows that an increase in the amount of adsorbent in the column, and hence the number of available binding sites, increases the amount of adsorbate that can be retained by the column before a given level of breakthrough has been reached. Increasing the value of the forward rate constant for the adsorption reaction (k_1) also increases the sharpness of the breakthrough curve (Fig. 7g), thus improving adsorption efficiency. Faster rates of the forward adsorption reaction allows local equilibrium conditions to be approached more quickly. The present model also predicts that alteration of the relative geometry of the column, but retaining the same overall capacity and volumetric flow-rate would have no effect on the shape or position of the breakthrough curve. A series of experiments using the lysozyme–Blue Sepharose system has confirmed that this theoretical prediction is valid, provided that the diameter of the column is not large compared to the length. Under the latter circumstances, it is likely that the applied adsorbate did not become evenly distributed across the surface of the column during passage through the column.

DISCUSSION

Our observations that the adsorption isotherms obtained for the affinity systems studied were of a hyperbolic shape and obeyed a relationship described by eqn. 1, confirm that a simple equilibrium relationship can be used to describe the interactions that occur in affinity chromatography. The agreement between the experimental observations of the kinetics of batch adsorption and the results predicted if the rate of the adsorption process can be described by eqn. 2 suggests that this simplistic approach may satisfactorily model actual systems. Although we have at present restricted our investigation to immobilised dye and immobilised antibody systems, it is tempting to suggest that all affinity chromatography systems could be modelled by such an approach. However, our model assumes that the parameters describing the binding reaction are the same for all the adsorbate binding sites. Such

a situation may not occur in practice, and a more realistic approach, allowing for a distribution of binding parameters, may be more appropriate, although much more complicated mathematically. In particular, affinity systems known to have a heterogeneity of binding sites, such as occur in systems involving immobilised polyclonal antibodies, will probably not be able to be described by single values for the binding parameters. For simplicity, we have purposely restricted the present consideration to affinity systems where only one species can bind to the affinity adsorbent. The models described here do not take into account the effects that would be noticed if more than one species of molecule with an affinity for the adsorbent were present. Multicomponent liquid phase adsorption in fixed beds has been considered by other workers[17,18] and the methods used may also be applicable to affinity chromatography systems.

The work described here is an empirical approach to the prediction of affinity chromatography performance. The parameters that describe the adsorption reaction (K_d, q_m, k_1) have all been directly measured in small-scale experimental systems. We have made no attempt to predict or calculate these values from knowledge of the concentration of the immobilised ligand, the equilibrium constant of the reaction between the adsorbate and the soluble ligand, or the rate constants associated with the latter interaction. Although a comparison of the interactions involved in soluble systems with the interactions that occur in the equivalent immobilised system is also of interest, it is difficult to make the necessary allowances for factors, such as loss or impairment of the ability of the immobilised ligand to bind adsorbate and to calculate the additional mass transfer resistances that will inevitably occur in the solid phase. Previous attempts to predict affinity chromatography operation for batch[19] or fixed bed systems[20] have involved assumptions, that the total amount of immobilised ligand can participate in binding reactions and that local equilibrium conditions occur. The latter assumption eliminates the necessity to consider the rate of adsorption processes as these are assumed to be infinitely great. These assumptions are clearly not justified in the light of the results reported here.

It is important to realise that the values of the parameters reported here for the interaction between adsorbent and adsorbate are only applicable to the conditions under which they were measured. Different values of these parameters may well be obtained in studies on the adsorption of the same protein from other solutions. The values may be altered because of differences in the physical nature of the solution (temperature, pH, ionic strength, dielectric constant) or by the presence of other components in the solution interfering with the binding. Hence, it is important that these parameters should be measured under conditions identical to those that will be used in the final system. The results that were obtained when the same antibody was immobilised on two different support materials highlight the effects that the nature of the support and the method of immobilisation can have on the performance of an affinity system. These effects should be the subject of further study in order that the relative merits of different immobilisation procedures for the synthesis of immunoadsorbents can be meaningfully assessed. Kinetic and equilibrium studies of an immunoaffinity chromatography system involving the binding of insulin to immobilised antibodies against insulin have been carried out by Sportsman *et al.*[21,22]. However, different techniques for measuring the kinetic parameters were used in these studies and the value of the dissociation constant for the adsorption reaction

appeared to vary with the flow-rate used in fixed bed experiments. No such dependence on flow-rate of the value of K_d for the adsorption reaction was found in the study of immunoadsorbents described here.

The parameters describing the adsorption reaction in batch affinity systems also seem applicable to the operation of packed column adsorption systems. This has enabled the effects on the performance of packed columns caused by changing some of the variables in the system to be predicted by computer methods, saving the need to perform an extensive experimental programme. The computer methods can readily be used to design large-scale affinity separation processes and to develop efficient operating protocols. One of the major consequences of the fact that affinity adsorption is governed by an equilibrium relationship, is that the maximum binding capacity of the adsorbent will only be utilised if the inlet concentration of adsorbate is very much greater than the value of K_d for the adsorption reaction and if the column is loaded until it is in equilibrium with the inlet stream. Because the shape of the breakthrough curve broadens as the adsorbate concentration is lowered, the ratio of the inlet concentration of adsorbate (c_0) to the dissociation constant (K_d) should be arranged to be as high as possible. In situations where it is attempted to use fixed bed affinity methods to adsorb components from solutions in which they are present at concentrations significantly lower than the value of K_d, the breakthrough curves that will be displayed will be very broad and adsorption performance will be poor. Under such circumstances, only a fraction of the total capacity of the column will be utilised for binding adsorbate, even when the column is at equilibrium with the input stream. The amount of adsorbate that will be bound to the column at equilibrium can be calculated from eqn. 4 with the substitution of c_0 for c^*.

Although the results have indicated operating conditions that may be preferred on theoretical grounds for improving the adsorption performance of affinity system, the use of some of these conditions may not be desirable in practice. The use of low flow-rates will result in long overall process times, thus reducing the number of cycles that can be completed in a given time. This reduction of throughput may be of particular importance when large volumes of adsorbate at low concentration have to be processed. The desirability of using high inlet concentrations of adsorbate may necessitate the insertion of a concentration step before the affinity chromatography procedure, which may significantly increase the overall cost of the process. Although the use of columns with a high total capacity for binding adsorbate improves adsorption performance, this is at the expense of using more adsorbent, which could be an important consideration in large-scale applications. The finding that the shape of the column has no influence on the shape of the breakthrough curve, would indicate the use of short, wide columns to minimise the pressure drop across the column. However, it is not recommended that columns with much larger diameters to length are used because of the difficulties of ensuring an even flow distribution across the column cross section.

We have deliberately omitted any consideration of axial dispersive effects in our models of fixed bed performance in order to simplify the mathematical analysis of the system. We have however observed that when columns are loaded at very low flow-rates over the period of a number of hours, the shapes of the breakthrough curves obtained are broader than would be predicted. Axial dispersion effects would be expected to result in the broadening of the shape of a breakthrough curve and the

most likely source of axial dispersive forces at low flow-rates is molecular diffusion. The magnitude of the diffusion coefficient in water at 20°C for a protein of the size used in the experiments where the broadening was noticed is of the order of 10^{-6} cm^2/sec. The corresponding average distance traversed by molecular diffusion will be about 0.5–1 cm/h. Such diffusion effects could therefore account for the broadening of breakthrough curves that were measured over periods of a few hours. More sophisticated theoretical models should in future take account of these dispersive effects, and some attempts to derive models for fixed bed adsorption including dispersive effects have been published[23].

The work described here has primarily been concerned with the prediction of the adsorption stage of an affinity chromatography process. It is also important however, to have knowledge of how quickly specifically adsorbed material will be lost from the column during washing procedures. In some circumstances, when it is important that non-specifically bound contaminants are removed from the adsorbent before elution occurs, considerable periods of washing may be necessary. During such washing procedures, significant amounts of the specifically bound material may also be lost from the column. This loss is due to the reversible nature of the equilibrium interaction. Thomas[12] has obtained an alternative solution to the differential equations describing fixed bed performance using a set of boundary conditions appropriate to washing a fully loaded column. However, the use of this equation in predicting washing performance will not be of much general use as it is unlikely that in practice, columns will be loaded to saturation. The values of the parameters describing interactions between adsorbent and adsorbate during the washing stage of operation are likely to be similar to those applicable to the adsorption stage.

The prediction of the behaviour of affinity systems during the elution stage of the adsorption cycle requires knowledge of the parameters of the binding interaction in the presence of the eluent. Ideally, there should be no affinity between adsorbent and adsorbate in the presence of eluent and the rate of release of bound material from the adsorbent should be rapid under these conditions. Preliminary studies of the measurement of the parameters associated with the binding of proteins to Blue-Sepharose in the presence of 1 *M* potassium chloride (the eluent used in these studies) have indicated that the adsorbent does indeed have no affinity for the adsorbate under these conditions (*i.e.* the value of K_d becomes very large) and the rate of release of bound protein was too fast to follow using the techniques that have been described here. The latter observation implies that the value of the rate constant for the desorption reaction (k_2) becomes very much greater in the presence of eluent and that the eluent promotes the breakdown of the adsorbent–adsorbate complex rather than just preventing the dissociated complex from reforming.

It seems that it will not be possible to derive further analytical solutions to the equations describing fixed bed affinity chromatography performance that are able to describe the washing and elution stages of operation. The main reason for this inability is that the corresponding boundary conditions for such stages are not as simple as those applicable to the adsorption stage when the column is initially devoid of adsorbate. Hence we have recently had to resort to using computer-based numerical methods to simulate these stages.

The design criteria for efficient affinity chromatography performance apply equally well to affinity systems operated under conventional low-pressure techniques

or under HPLC techniques. HPLC methods have already been employed in affinity chromatography[24] and it is worth assessing what effect the application of these techniques could have on improving the performance of preparative affinity separations. Fig. 7f shows that one method of improving the adsorption efficiency of preparative procedures is to use a system that has a high rate constant for the forward direction of the adsorption reaction. The use of systems with high adsorption rate constants would allow the column to be loaded at high flow-rates thus shortening the overall process time. The rate constant (k_1) probably contains contributions from a number of types of resistance to mass transfer particularly those involving diffusion of adsorbate to binding sites within the pores of a porous adsorbent. One method of reducing the mass transfer resistances would be to confine the adsorption reaction to the outer surface of the particle by using non-porous support materials. In a comparison of support materials used in the synthesis of immunoadsorbents, Eveleigh and Levy[25] found that the use of non-porous particles gave the fastest adsorption rates. The major problem associated with the use of non-porous supports is the small amount of surface area available for adsorption per unit volume of the packed bed. For example, for the porous silica support material used in these studies (average particle size 150 μm), the specific surface area contributed by the pore surface was about $4 \cdot 10^6$ m^{-1}, whereas the specific area contributed solely by the outer surface of the beads is $4 \cdot 10^4$ m^{-1}, *i.e.* about 1% of the pore surface area. If it is assumed that the total pore surface area can be derivatised, then the adoption of non-porous particles of similar size would reduce the capacity of the column 100-fold. Hence the size of a column of equivalent total capacity to the porous column would be 100 times greater. This magnitude of increase would be unacceptable, mainly because of the concommitant increase in the void volume of the bed. However, as the surface area of non-porous spherical particles is inversely proportional to the diameter of the particle, a reduction in particle diameter to 5 μm would increase the outer surface area to 10^6 m^{-1}. Hence the column size would be only four times greater than the conventional porous column, and such an increase would be acceptable if the performance of the column were markedly increased. The use of small particles would greatly increase the pressure drop over the column if the same or higher flow-rate were to be used, and, hence, HPLC technology would have to be implemented to operate such systems. Particle sizes of 5 μm are frequently used in HPLC techniques and hence small non-porous particles could readily be used as supports in affinity chromatography. The use of high-pressure chromatography systems will substantially increase the cost of the chromatography equipment, particular for large-scale systems. We are currently measuring the values of K_d, q_m and k_1 for affinity systems involving non-porous support particles and other adsorbents suitable for use in HPLC procedures. The results of these studies should enable us to assess the extent of the increase in performance that the adoption of HPLC techniques would provide and to decide whether the additional equipment costs would be justified.

Although this study has been restricted to a consideration of preparative affinity chromatography based on frontal methods, there may be some circumstances when the use of elution development methods may be desirable. Such a situation could occur when more than one species of molecule has an affinity for the adsorbent, as is the case when immobilised co-factors or dye molecules are used in affinity separations. Operation under conventional frontal methods would result in co-purifi-

cation of these species. In order to separate these molecules, it is either necessary to use biospecific elution methods or to use the column in a truely chromatographic manner, where pulses of applied material are separated into bands containing only one species because of different degrees of partitioning to the solid phase. Under chromatographic conditions, only small pulses of material would be applied to the column in order to achieve good resolution. To avoid the use of very large columns, it would be necessary to adopt rapid cycling techniques to purify compounds on a macropreparative scale. The use of HPLC techniques with porous or non-porous supports under these circumstances could greatly decrease the time for a complete cycle of affinity operation[24].

NOTATION

A molecule of adsorbate
AB complex between adsorbate and adsorbent
A_c cross-sectional area of packed bed
B biospecific binding site on adsorbent
c concentration of adsorbate in solution
c_0 initial concentration of adsorbate; inlet concentration of adsorbate in packed bed system
D_x axial dispersion coefficient
f volumetric flow-rate through packed bed
h height of packed bed
J the function defined by Hiester and Vermeulen[13]
K_d dissociation constant for adsorbent · adsorbate complex
k_1 rate constant for the binding reaction between adsorbent and adsorbate
k_2 rate constant for the breakdown of adsorbent · adsorbate complex
l linear velocity of fluid flow through packed bed
q amount of adsorbate bound to adsorbent per unit volume of settled adsorbent
q_m maximum capacity of adsorbent for adsorbate per unit volume of settled adsorbent
R rate of interface mass transfer in packed bed
t time
V total volume of batch adsorption system
v settled volume of adsorbent
x axial distance along bed

Superscript
* value when system is at equilibrium

ACKNOWLEDGEMENTS

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