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MODELLING AFFINITY CHROMATOGRAPHY

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ABSTRACT

Affinity chromatography plays a significant role in the separation and purification of biologically active macromolecules in laboratory and large-scale applications. There is a need for models which could be used to predict accurately the dynamic behavior of affinity chromatography separations, in order to permit the design, optimization, control, and process scale-up of affinity chromatography systems. Furthermore, the construction and use of such models will contribute to a better fundamental understanding of the physicochemical and biospecific mechanisms involved in affinity chromatography processes. The parameters of the models should be obtainable by using information from a small number of experiments.

This work reviews the modeling of affinity chromatography, and presents general models that could be used to describe the dynamic behavior of the adsorption, wash, and elution stages of affinity chromatography systems. Certain model structures, modeling approaches and operational strategies for systems having porous or nonporous adsorbent particles are also suggested, and experiments are proposed whose data are necessary for parameter estimation and model discrimination studies in affinity chromatography.

Particular emphasis is given to the modeling of the intrinsic mechanisms of intraparticle diffusion, adsorption, and desorption, because the intrinsic mechanisms are normally independent of the mode of operation (i.e., batch, fixed bed, fluidized bed, continuous countercurrent, or others).

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1. INTRODUCTION

Affinity adsorption processes (affinity chromatography) are considered to be highly selective separation methods[1-10] in the downstream processing (bioseparation) of antigens, antibodies, proteins, and enzymes. Biospecific adsorption is also of great importance for the development of biocompatible materials, and of new types of biosensors.[11-14] Different mechanisms[11,15,16] of interaction may be involved in affinity adsorption processes. These mechanisms include specific bio-recognition[2] interactions as in immunoaffinity separations; electrostatic[10] interactions in ion-exchange methods; and general hydrophobic or hydrophilic interactions[4,5,7] in systems involving less specific adsorbents.

Affinity adsorption separations usually involve four stages (adsorption, wash, elution, and re-equilibration or regeneration), and these stages may be carried[11,15,16] in a finite bath (batch) system, a fixed bed, a periodic countercurrent bed, a continuous countercurrent bed, a fluidized bed, a radial flow system, or in a magnetically stabilized fluidized bed.[17-19] Industry has significant interest in the design, optimization, and control of large-scale affinity adsorption systems which are to be used for the purification of proteins for use as pharmaceuticals or in other applications where the purity of the product is a very important consideration. Certain fundamental mechanisms underlying the affinity adsorption separations have been identified and constitutive expressions which may be used to quantify these mechanisms and their effects, have been suggested and constructed.[2,3,5-11,14-16,20-45] The parameters characterizing the mechanisms involved in the different stages (i.e., adsorption; wash; elution) of affinity adsorption and in the different operational modes (i.e., batch; fixed bed; fluidized bed) could be estimated from proper correlations and/or by matching the predictions of appropriate models, which are developed to describe the behavior of affinity adsorption in the different stages and operational modes, with experimental data obtained over satisfactory and significantly large regions of the possible experimental space. It is well established that affinity adsorption experiments are tedious, time consuming, difficult, and

expensive. The number of experiments at the bench-scale and pilot-scale levels can be significantly reduced by developing and using mathematical models that would satisfactorily predict the behavior of the affinity adsorption stages under different operational modes. Such models may be used to guide the experiments[11,16,46] in regions of the experimental space where a better scientific understanding of the behavior of affinity adsorption mechanisms may be obtained, and even new and interesting phenomena might be observed. Furthermore, these models could be used in the complex tasks of design, optimization, control, and scale-up of affinity adsorption processes. It should be emphasized that there is nothing more practical than a mathematical model which can accurately predict the dynamic behavior, scale-up, and design of a process of interest, since such a model would obviate very many experiments.

The main aim of this work is to review the modeling of affinity chromatography, as well as to present general models that could be used to describe the dynamic behavior of the adsorption, wash, and elution stages of affinity chromatography systems. Certain model structures, modeling approaches, and operational strategies for systems having porous or nonporous adsorbent particles are also suggested, and experiments are proposed whose data are necessary in parameter estimation, model discrimination, and model development studies. The proper combination of experimental and theoretical research could develop mathematical models whose dynamic predictions may become more accurate, and which may also improve our understanding of the interactions of the various mass transfer and adsorption/desorption mechanisms involved in affinity chromatography processes.

2. ADSORPTION

Affinity adsorption may be divided into two main groups as follows[3]: (a) Single component adsorption; in this case a ligand is used which has a high biospecific recognition of only one species[1,3,4,11,32,47]. Therefore, such a ligand will adsorb only one component from a multicomponent mixture. Single component adsorption may also occur when group-specific or "general" ligands[1,3,47] are used and the composition of the feed solution is

such that only one species is adsorbed by the ligands. (b) Multicomponent adsorption; when group-specific[3,5,11,16,47] ligands are used, several closely related compounds may interact with the ligand. Less specific adsorbents are obtained when more general ligands are employed.

In the adsorption stage the following mass transfer and interaction steps may be considered to occur:

(i) The transport of adsorbate(s) from the bulk fluid to the external surface of the adsorbent particle.

(ii) The transport of adsorbate(s) within the porous particle; in case that the particle is nonporous, this mass transfer step does not occur.

(iii) The interaction between the adsorbate(s) and the immobilized ligand.

The interaction step (iii) may be composed of several substeps, depending on the complexity of the adsorbate-ligand interaction, and could include the binding of multivalent adsorbates to monovalent ligands.[3,11,15,16,47]

The most commonly used mode of operation in affinity chromatography separations is the fixed bed mode with axial or radial flow.[6,11,15,16] Batch adsorption systems would be appropriate where the fluid to be processed was of high viscosity or contains particulate material. Arve and Liapis[3,24] and Liapis[11,15,16] have indicated that, for a given affinity adsorption system, the parameters that characterize the intraparticle diffusion and adsorption mechanisms should be independent of the operational mode (e.g., batch; fixed bed; continuous countercurrent bed; fluidized bed; magnetically stabilized fluidized bed), and therefore, if these parameters are estimated by utilizing information obtained from batch experiments (batch experiments are easier to perform and analyze[3,11,15,16,40,41,48] than column experiments), then their values should characterize the intraparticle diffusion and adsorption mechanisms in other operational modes; this theoretical approach[3,11,15,16,24] has been shown to be valid by the data of Horstmann and Chase.[49]

2.1 Finite Bath

Adsorption is considered in a finite bath containing n components, and m($m<n$) solutes may compete for the available ligands which are immobilized on the internal surface of porous particles. It is also taken that $m+1 \leq i \leq \ell$($\ell<n$) solutes may bind to the support matrix by nonspecific adsorption, and that $\ell+1 \leq i \leq n$ solutes simply diffuse into the particles without interacting with the adsorbent. The porous particles are suspended in the liquid by agitation so that the liquid has free access, and the bulk phase concentration is taken to be uniform throughout the bath except in a thin film (film mass transfer resistance) of liquid surrounding each particle. The adsorption process is considered to be isothermal since the heats of adsorption apparently do not change the temperature[3,4,6,11,15,16,20,25] of the liquid phase even in large-scale systems; this occurs because the total amount of adsorbed material is small and the heat capacity of the liquid phase is high.

A differential mass balance for each component in the fluid phase of the bath gives

$$\frac{dC_{di}}{dt} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{fi}\left[C_{pi}(t,r_o)-C_{di}(t)\right], \quad i = 1,2,..m,...,\ell,..,n \quad (1)$$

Equation (1) can be used for particles having geometry of slab, cylinder, or sphere by putting α=0,1, or 2. The terms in equation (1) stand for accumulation in the fluid phase and transport from the fluid phase to the adsorbent particles by film mass transfer. The initial condition of equation (1) is given by

$$C_{di} = C_{doi} \qquad \text{at} \qquad t = 0 \quad (2)$$

In the following sections, the models describing the mass transfer and interaction mechanisms in porous and nonporous adsorbent particles are presented and discussed.

2.1.1 Porous Adsorbents

The mean pore diameter of most porous adsorbents employed in affinity adsorption systems lies[93] between 6 and 100 nm. In most

adsorbents, the relative contribution of intraparticle convection to the total intraparticle mass transport is considered to be not significant. But, for certain adsorbents with high porosities and large pore diameters[94] it is possible that the contribution of intraparticle convection to the total intraparticle mass transport may be significant, and the effect of intraparticle convection may be important.[95] It should be noted that adsorbent particles with large pore diameters will tend to have lower surface areas per unit volume and may have reduced adsorption capacities; therefore, some of the benefits obtained from intraparticle convection could be counterbalanced by the effect of lower capacity. In this review, the contribution of intraparticle convection is not considered.

The complex transport mechanisms of the adsorbates in the adsorbent are often simplified by assuming that the transport is governed either by the diffusion of the species in the pore fluid (pore diffusion) and/or by diffusion on the pore surfaces. All the transport mechanisms are taken to be one-dimensional and in particles that have an axis of symmetry. It is understood that in the case of the slab and the cylinder, the particles are of infinite extent or alternatively one must artifically assume that the ends of a finite cylinder or edges of a finite slab are sealed in order to keep the problem one-dimensional. The intrinsic transport mechanisms within the porous particles are normally the same in any mode of adsorption operation (batch, fixed bed, etc.).

Isothermal operation is considered for the reasons presented above, and the differential mass balance for each component i in the adsorbent particle is given by

$$\frac{\partial}{\partial t}(\epsilon_p C_{pi}) + \frac{\partial C_{si}}{\partial t} = \frac{1}{r^\alpha}\left\{\sum_{j=1}^{n}\frac{\partial}{\partial r}\left[r^\alpha \epsilon_p D_{pij}\frac{\partial C_{pj}}{\partial r}\right] + \sum_{j=1}^{n}\frac{\partial}{\partial r}\left[r^\alpha D_{sij}\frac{\partial C_{sj}}{\partial r}\right]\right\} \qquad (3)$$

In equation (3), the terms $\partial C_{si}/\partial t$ and $\partial C_{sj}/\partial r$ become equal to zero for species which do not bind by specific or non-specific adsorption.

The mixtures of biological macromolecules to be separated by affinity chromatography are usually very dilute, especially with respect to the component(s) of interest, and therefore, it may be possible to set the off- diagonal (D_{pij}, $i \neq j$; D_{sij}, $i \neq j$) elements of the effective pore diffusivity matrix, $\underset{\approx}{D}_p$, and of the surface diffusivity matrix, $\underset{\approx}{D}_s$, equal to zero.[3,11,15,16,50-53] In this case, equation (3) would take the following form:

$$\frac{\partial}{\partial t}(\epsilon_p C_{pi}) + \frac{\partial C_{si}}{\partial t} = \frac{1}{r^\alpha}\frac{\partial}{\partial r}\left[r^\alpha\left(\epsilon_p D_{pi}\frac{\partial C_{pi}}{\partial r} + D_{si}\frac{\partial C_{si}}{dr}\right)\right] \qquad (4)$$

In equation (4), D_{pi} and D_{si} represent the diagonal terms (D_{pij}, $i=j$; D_{sij}, $i=j$) of the diffusivity matrices $\underset{\approx}{D}_p$ and $\underset{\approx}{D}_s$. Surface diffusion is usually neglected, because the interaction between the adsorbate and ligand is considered to be strong.[3,4,11,14,15,16,20,25,41,47] It remains to be demonstrated[11,41] whether surface diffusion of the adsorbed macromolecules occurs, and whether or not it plays a significant role in protein transport. If the contribution of surface diffusion to mass transfer is insignificant, then equation (4) would become as follows:

$$\frac{\partial}{\partial t}(\epsilon_p C_{pi}) + \frac{\partial C_{si}}{\partial t} = \frac{1}{r^\alpha}\frac{\partial}{\partial r}\left[r^\alpha \epsilon_p D_{pi}\frac{\partial C_{pi}}{\partial r}\right] \qquad (5)$$

The initial and boundary conditions for equation (5) are

$$C_{pi} = 0 \text{ at } t = 0,\ 0 \le r \le r_o \qquad (6)$$

$$C_{si} = 0 \text{ at } t = 0,\ 0 \le r \le r_o \qquad (7)$$

$$\epsilon_p D_{pi}\left.\frac{\partial C_{pi}}{\partial r}\right|_{r=r_o} = K_{fi}\left(C_{di} - \left.C_{pi}\right|_{r=r_o}\right),\ t>0 \qquad (8)$$

$$\left.\frac{\partial C_{pi}}{\partial r}\right|_{r=0} = 0,\ t>0 \qquad (9)$$

It is clear that equation (5) cannot be solved if an appropriate expression for the term $\partial C_{si}/\partial t$ is not available.

This term represents the accumulation of the adsorbed species i on the internal surface of the porous particle, and it can be quantified if a mathematical model could be constructed that would describe the mechanism of adsorption for component i. Various mechanisms for the interaction between macromolecules and ligands have been suggested in the literature.[3,8,10,11,14,15,16,20,22,25,27-29,32,34-37,47,54] Most of the mechanisms proposed and examined in the literature involve the interaction of a single adsorbate with ligand; and in certain cases, the interaction between a multivalent adsorbate (single component) with a monovalent ligand has been considered.[3,11,15,23,27,47] It should be emphasized at this point that the theory for the construction of dynamic kinetic expressions which may describe the competitive adsorption of multicomponent mixtures of macromolecules onto immobilized ligands, is in its infancy.[3,11] The experimental multicomponent adsorption data of macromolecules reported in the literature,[14,28,29,55,56] have been correlated by using multicomponent isotherms or multicomponent dynamic kinetic expressions which have been constructed to correlate multicomponent adsorption data of small[57-59] molecules; but it is well established that the adsorption of biologically active macromolecules differs,[11,20,25,26,31] in a number of ways, from that of low molecular weight substances. The above discussion about competitive adsorption for systems involving multicomponent mixtures of biologically active macromolecules, indicates the significant need for research towards the formulation of a quantitative theory that would at least correlate (if it cannot predict) the dynamic[11] data of the mechanism of adsorption of such mixtures onto immobilized ligands. It should be noted that at this time there are no appropriate (in practice) predictive theories even for the mechanisms of multicomponent adsorption of mixtures of low molecular weight substances; the available expressions are correlative.[57-59]

In this review, the kinetic adsorption mechanisms describing only single component macromolecule interaction with immobilized ligands are presented and examined. A significant number of practical[3,4,5,10-13,20,25,31,41,47,49] affinity chromatography

systems involve single component adsorption. It may be argued[11,16,20,25] that even the theory concerning the mechanism of single component macromolecule interaction with immobilized ligands is in its infancy, but the experience[11,20,25] in analyzing single component adsorption data with kinetic adsorption models is much richer than that available for multicomponent adsorption mixtures of macromolecules.

2.1.2 Models of the Adsorption Mechanism

A solution containing only one type of macromolecule is considered, and it is assumed that biomolecules in general adsorb onto ligand in a bound monolayer.[3,8,11,20,25,26,31,49] If the adsorption is completely reversible and with no interaction between the adsorbed molecules, the interaction between unbound monovalent adsorbate (A) in the solution and vacant immobilized monovalent ligand (L) may be considered to be of the form[3]

$$A + L \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AL \tag{10}$$

where AL represents the non-covalent adsorbate-ligand complex. Then assuming elementary interactions, the rate of the adsorption step may be described by the following second-order reversible interaction:

$$\frac{\partial C_s}{\partial t} = k_1 C_p (C_T - C_s) - k_2 C_s \tag{11}$$

In equation (11), C_s represents the adsorbed concentration of the adsorbate (the concentration of AL) and C_T is the total concentration of available ligand; the subscript i in the term $\partial C_{si}/\partial t$ has been dropped since we only consider single component adsorption. Equation (11) represents one kinetic model which can be employed together with the continuity equation (5a)

$$\frac{\partial}{\partial t}(\epsilon_p C_p) + \frac{\partial C_s}{\partial t} = \frac{1}{r^\alpha}\frac{\partial}{\partial r}\left(r^\alpha \epsilon_p D_p \frac{\partial C_p}{\partial r}\right) \tag{5a}$$

in order to obtain[3,24] the dynamic profiles of C_p and C_s in the porous adsorbent particles. Arve and Liapis[3] and Liapis et al.[8]

studied two different immunoaffinity systems and found that the dynamics of the interaction expression given by equation (11) were of significant importance and together with the dynamics of the mass transfer mechanisms (film mass transfer and pore diffusion) determined the rate of adsorption of the adsorbate molecules onto immobilized ligands. It should be noted at this point that a constant value of C_T may be considered to suggest that the affinity ligand is distributed evenly (homogeneously) throughout the interior of the porous particles; but, if there is a gradient in the concentration of the affinity ligand along the radius of the adsorbent particles, then this could mean that C_T is a function of the radial distance r. In the last paragraph of section 2.1.4, an approach is suggested that could be used to estimate the distribution of the affinity ligand along the radial distance r of the adsorbent particle at the end of the process involving the immobilization of the ligand. For certain adsorbents, the distribution of ligand could be examined[49] experimentally.

The accumulation term, $\partial C_s/\partial t$, in equation (11) becomes equal to zero when adsorption equilibrium is established, and the following expression for the equilibrium isotherm is obtained:

$$C_s = \frac{C_T K_a C_p}{1+K_a C_p} \qquad (12)$$

In equation (12), K_a represents the equilibrium association constant ($K_a = k_1/k_2$). Equation (12) represents the Langmuir equilibrium adsorption model where C_T is supposed to represent a fixed number of surface sites and it should therefore be a temperature independent constant, while K_a should follow a van't Hoff equation[57-59]

$$K_a = K_{ao} \exp(-\Delta H/RT) \qquad (13)$$

where K_{ao} is a constant and ΔH represents the heat of adsorption. The experimental adsorption isotherms for a wide variety of affinity chromatography systems indicate that equation (12) is widely applicable.[4,8,10,11,49] Liapis et al.[8] have suggested that

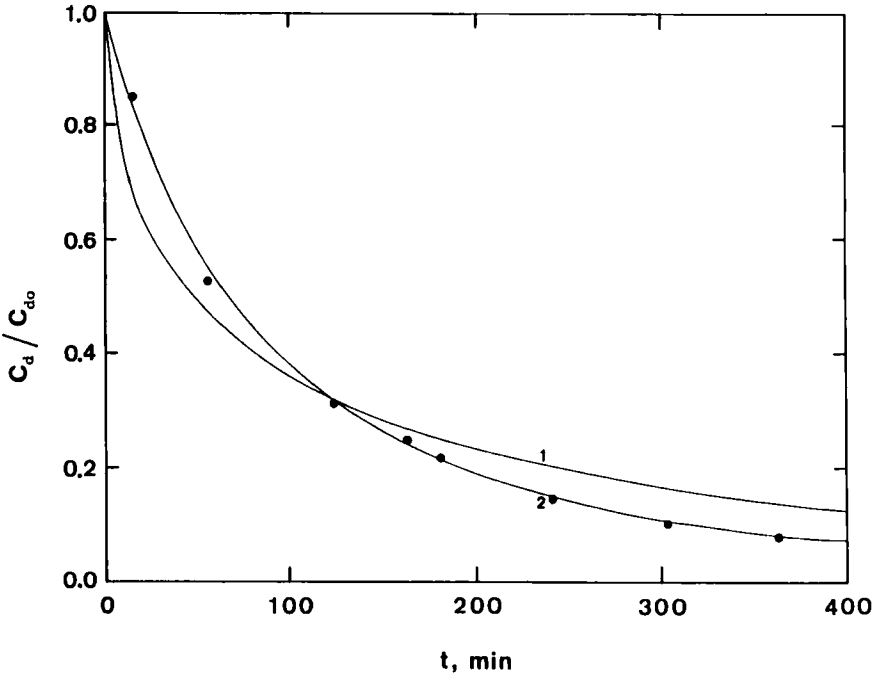

FIGURE 1

Time variation of the dimensionless concentration of β-galactosidase in the bulk fluid phase of a finite bath.[3,11]

- Experimental data.

Curve 1: Theoretical model prediction; local equilibrium between adsorbate and adsorbate-ligand complex at each point in pore.

Curve 2: Theoretical model prediction; interaction rate between adsorbate and ligand given by second-order kinetic expression (equation (11)).

in affinity adsorption systems the units of the experimental equilibrium adsorptivity should be in terms of the number of moles of adsorbate interacting per mole of ligand. This presentation of the experimental equilibrium adsorptivity may provide evidence about the possibility of non-specific adsorption on the surface of the adsorbent particles and/or suggestions about the mechanism of adsorption. If the interaction between the adsorbate and ligand occurs infinitely fast, then the adsorbate molecules in the pore

fluid and in the adsorbed phase are in equilibrium at every point in the pore and the term $\partial C_s/\partial t$ in equation (5a) would take the following form (equation (12) is employed):

$$\frac{\partial C_s}{\partial t} = \left(\frac{\partial C_s}{\partial C_p}\right)\left(\frac{\partial C_p}{\partial t}\right) = \left(\frac{C_T K_a}{(1+K_a C_p)^2}\right)\left(\frac{\partial C_p}{\partial t}\right) \qquad (14)$$

In Figure 1 the finite bath model[3,11,24] predictions are compared with the experimental data of the adsorption of β-galactosidase onto immobilized anti-β-galactosidase in a batch system; the anti-β-galactosidase is immobilized on porous silica particles. The theoretical results of curve 1 have been obtained from a batch model[3] where local equilibrium between the adsorbate and the adsorbate-ligand complex at each point in the pore was assumed (equations (1), (2), (5a), (6), (8), (9), and (14) were solved simultaneously). The results of curve 2 have been obtained with the same batch model,[3] but the assumption of local equilibrium has been dropped and the interaction rate is considered to be given by a second-order reversible expression (equations (1), (2), (5a), (6)-(9), and (11) were solved simultaneously). The results in Figure 1 suggest that for this adsorption system, the dynamics of the adsorption kinetics play a significant role in determining the overall mass-transfer resistance, and therefore, the adsorption kinetics (equation (11)) have to be considered together with the film mass transfer and pore diffusional resistances. The values of the interaction and mass transfer parameters for the two different curves in Figure 1 are as follows:

Curve 1: $K_f = 5.84 \times 10^{-4}$ cm/s, $D_p = 3.40 \times 10^{-8}$ cm^2/s, $K_a = 4.54 \times 10^{3}$ cm^3/mg.

Curve 2: $K_f = 5.84 \times 10^{-4}$ cm/s, $D_p = 6.9 \times 10^{-8}$ cm^2/s, $k_1 = 2.35 \times 10^{-2}$ cm^3/(mg)(s), $k_2 = 5.18 \times 10^{-6}$ s^{-1}.

The procedures employed for the estimation of the above parameters are presented elsewhere.[3] Johnston and Hearn[92] compared the experimental dynamic (batch) adsorption data of the binding of several proteins (with different molecular geometries) to several ion-exchange and dye-affinity chromatographic resins, with the theoretical predictions of different models. They found that the batch model[3] described by equations (1), (2), (5a), (6)-(9), and

(11) provided the best agreement between experiment and theory, and furthermore, the values of the kinetic parameters estimated by matching the theoretical predictions of this model with the experimental data, were found[92] to be consistent with enzyme kinetic theory.

Some of the reasons that have been presented in the literature in order to explain the rather slow rate of interaction between the adsorbate and ligands of certain affinity adsorption systems, are:

(a) The difficulty of the macromolecule in finding the proper orientation for binding within the confined spaces of the pores;

(b) The active site(s) of the macromolecule is located at such a position in its three dimensional structure that even in the absence of any hindrance from the pore surfaces, it requires certain time interval to elapse in order to make properly available its active site for interaction with the active site of the ligand;

(c) The ligand molecules may have been immobilized on the surface in such a way that the active site(s) of a certain fraction of the vacant ligands are not available for binding at any given time.

(d) The adsorbate molecules are reversibly adsorbed in one conformation but may change conformation to a second irreversibly bound form. Experimental evidence[25] indicates that spreading of the adsorbing molecule at the sorbent surface may occur, and the new conformation needs a larger area on the surface and this may lead to a decrease on the number of available vacant ligands per unit surface area as well as to the introduction of an extra desorption of molecules.

For the adsorption of immunoglobulin G to Protein A immobilized to agarose matrices, it was found[49] that the rate of adsorption was controlled by film mass transfer and pore diffusion while the interaction between immunoglobulin G and Protein A was fast; for this system, equation (12) described satisfactorily the equilibrium data (adsorption isotherms), and equation (14) was

employed for the term $\partial C_s/\partial t$ in equation (5a) since film mass transfer and pore diffusion were found to be the mass transfer resistances controlling the rate of adsorption.

In equation (12), $C_s \rightarrow C_T$ as $C_p \rightarrow \infty$, while at low adsorbate concentrations ($C_p \rightarrow o$) Henry's law is approached

$$\lim_{C_p \rightarrow o} \left(\frac{C_s}{C_p}\right) = C_T K_a = K_H \tag{15}$$

and thus, the expression of linear adsorption equilibrium has the form

$$C_s = K_H C_p \tag{15a}$$

Experimental equilibrium adsorption data obtained at very low adsorbate concentrations (Henry's law region of linear equilibrium) and different temperatures, could be used to estimate the values of K_H and ΔH. This information would be especially useful in examining equilibrium theories of binary adsorption (competitive adsorption of two-component mixtures of macromolecules), since such theories should have to be thermodynamically consistent and reduce to Henry's law at low coverage. It has been suggested[11,20] that the heat of adsorption, ΔH, for a given affinity adsorption system, should be obtained directly by (micro) calorimetry. The measurement of ΔH at different temperatures (constant pressure system) by (micro) calorimetry, would indicate the change upon adsorption of the apparent heat capacity, $(\Delta C_p)_{app}$, of the system

$$(\Delta C_p)_{app} = \left[\frac{\partial(\Delta H)}{\partial T}\right]_p \tag{16}$$

$(\Delta C_p)_{app}$ may be interpreted in terms of change in configurations of the components that are involved in the process.[20] For example, loss of secondary structure (i.e., β-sheet, α-helix) which allows a greater rotational mobility along the polypeptide chain of the protein, would lead to a larger value[20] of the heat capacity. It has been pointed out[31] that the measurement of ΔH and $(\Delta C_p)_{app}$ under appropriate conditions, may often lead to an understanding[31]

of the dominant types of forces (e.g., hydrophobic; electrostatic; van der Waals; hydrogen bonds) involved in adsorption. If the dominant forces of interaction for an affinity adsorption system are known, then the selection of an effective eluent for use in the elution[21,22] stage of the affinity chromatography process, could be made with minimal experimentation.

In the linear equilibrium region, Gorbunov et al.[34] used the Monte Carlo technique to calculate numerically a distribution coefficient which is equal to the ratio of the equilibrium macromolecule concentration in the chromatographic stationary phase to the concentration in the mobile phase. The calculation of the distribution coefficient consists in calculating[34] the partition function for the macromolecule in the stationary phase and in the mobile phase. The results of this study suggest that two factors are essential in determining retention in hydrophobic interaction chromatography of proteins. Protein retention depends on (a) the hydrophobic protein surface area, and (b) the mutual arrangement of the exposed hydrophobic groups. Their results suggest that a correlation may be possible between retention and the structural characteristics of the surface of the protein molecule; X-ray analysis[60] may provide information about these characteristics. Although Gorbunov et al.[34] left the nature of the adsorption interactions unspecified, with the energy of interaction varying over a wide range, their modeling approach might provide some qualitative insight about hydrophobic interaction chromatography of proteins.

The Langmuir isotherm was developed by assuming that the adsorption is (i) completely reversible, (ii) the adsorption takes place on fixed sites, (iii) the molecule does not change conformation upon adsorption, (iv) lateral interaction between the adsorbed molecules may be ignored, and (v) at most monolayer coverage could occur. Experimental evidence obtained from different techniques[11,20,25,31] suggests that in the adsorption of proteins the existence of a second layer may be improbable. There is experimental evidence[20,25,31,54] which indicates that certain protein molecules usually change conformation upon adsorption, and that lateral interaction may not be ignored in some systems.

Certain adsorbed protein molecules take time to develop their contact points with the surface,[3,23] which suggests that the degree of reversibility or exchangeability of a given molecule decreases with time. Furthermore, in certain systems[25] there is evidence for macromolecule-induced exchange interactions on the sorbent surface, whereby an already adsorbed molecule is exchanged with a protein molecule from the solution; this process occurs even if the spontaneous desorption of biomolecules is very small. These findings[11,20,25,31,54] suggest that if an experimental isotherm for a protein does fit the Langmuir or the modified[58] Langmuir expression (Frumkin-Fowler-Guggenheim equilibrium model), this may be fortuitous[20] since, in the case of certain affinity adsorption systems, some of the Langmuir assumptions may not be satisfied.

Lundstrom et al.[25] have suggested a simple kinetic model which considers several of the experimental observations. This model may be considered for systems where the volume of the immobilized ligand is smaller than the volume of the adsorbate molecule. It is assumed that a biomolecule adsorbs on the surface forming one type of adsorbate-ligand complex ("form a"), and that after adsorption it may change conformation or orientation ("form b"). An adsorbed molecule in "form a" is considered to occupy an area A_a on the surface, with $A_b/A_a = \delta$ ($\delta>1$). The adsorbed molecules of "form a" and "form b" are competing for the same area on the surface, and it is assumed that both exchange interactions and spontaneous desorption take place on the surface. The exchange interactions are modelled as a desorption, which depends on the concentration of the adsorbate, $C_p(t,r)$, in the pore fluid. If C_T now represents the available adsorption sites for molecules of "form a", then the interaction rate expressions for this physical model are

$$\frac{\partial C_{sa}}{\partial t} = (k_1 C_p - k_3 C_{sa})(C_T - C_{sa} - \delta C_{sb}) - k_4 C_p C_{sa} - k_2 C_{sa} \qquad (17)$$

$$\frac{\partial C_{sb}}{\partial t} = k_3 C_{sa}(C_T - C_{sa} - \delta C_{sb}) - k_6 C_p C_{sb} - k_5 C_{sb} \qquad (18)$$

where C_{sa} and C_{sb} represent the concentrations of the adsorbate--ligand complexes of "form a" and "form b", respectively. The

parameters k_i (i = 1, 2, .., 6) are interaction rate constants. In equations (17) and (18) the exchange due to molecules of "form b" which may be present in the solution is neglected[25] (the qualitative result will not change because of this omission). The accumulation term, $\partial C_s/\partial t$, in equation (5a) is obtained from the terms $\partial C_{sa}/\partial t$ and $\partial C_{sb}/\partial t$. It should be noted that the equilibrium expressions for C_{sa} and C_{sb} are obtained[11,25] from equations (17) and (18) by setting the accumulation terms ($\partial C_{sa}/\partial t$, $\partial C_{sb}/\partial t$) equal to zero.

In certain biospecific adsorption systems[20,25] the experimental results suggest that, with time, some of the adsorbed molecules become unexchangeable. This may be modelled[25] by an equation of the form

$$\frac{\partial C_{sb,irr}}{\partial t} = k_7(C_{sb} - C_{sb,irr}) \qquad (19)$$

where it has been assumed that only molecules of "form b" become totally irreversibly adsorbed on the surface (represented by $C_{sb,irr}$). One may also regard the irreversibility as a decrease in k_6 with time.[25] The equilibrium expressions ($\partial C_{sa}/\partial t = \partial C_{sb}/\partial t = 0$) of equations (17) and (18) have been found[25] to describe satisfactorily the equilibrium affinity adsorption behavior of certain experimental systems. It should be emphasized at this point that in this simple model with only two possible conformations or orientations of the protein molecules, there are several parameters that have to be estimated for a given affinity adsorption system. McCoy and Liapis[61] have estimated from experimental data certain kinetic parameters of the adsorption mechanism proposed by Lundstrom et al.[25], for the system involving the adsorption of β-galactosidase onto monoclonal antibody immobilized on porous silica particles.

It is important to note that in order to perform studies in parameter estimation, model discrimination, and optimal design of experiments[46] for affinity adsorption systems, the investigator needs a number of kinetic models for the interaction mechanism(s) between the adsorbate and ligand. Equations (11) and (17)-(19) represent two different nonlinear kinetic models for the

interaction of a monovalent adsorbate with a monovalent ligand.

In certain affinity adsorption systems, multivalent[3,11,15,23,27,32,47] adsorbates are adsorbed onto monovalent ligands. Yon[47] has presented an equilibrium model of affinity partitioning for such systems. This equilibrium model is based on two essential postulates: (a) the immobilized ligands are distributed singly or in clusters within spherical bounds of the size of the protein molecule, and cluster concentrations follow a Poisson distribution; and (b) interactions within a cluster are highly cooperative. The assumption of a truly random distribution (Poisson distribution) of immobilized ligands is questionable, since a number of mass transfer and reaction mechanisms[48,62,63] operating during the immobilization of the ligands may produce non-random ligand distributions. Furthermore, the spatial distribution of protein binding-sites would restrict the number of ligands within the bounds of a cluster that may bind to protein. The equilibrium model[47] currently assumes that all ligands within the bounds of a cluster can bind to protein. Yon[47] found by using this equilibrium model that, when the total concentration of accessible immobilized ligand is 10^{-3}M (practical upper limit for immobilized ligand concentration), a protein (four-site protein molecule) of radius 4 nm would "see" over 95% of all the accessible ligand as isolated single ligand-groups. For lower values of the concentration of accessible immobilized ligand (realistic values), single ligands are overwhelmingly predominant. The interesting result obtained from this equilibrium model, is that unless the concentration of the adsorbate (protein) is well below the micromolar range (since the concentration of accessible immobilzied ligand, in most cases, will be well below 10^{-3}M), the partitioning will seem to be monovalent, i.e. interaction between a monovalent adsorbate and a monovalent ligand. Notwithstanding the difficulties associated with the assumptions employed in the development of this equilibrium model, the cluster model has provided certain interesting implications regarding the equilibrium interactions of a multivalent adsorbate with immobilized monovalent ligands.

For a single adsorbate (A) having a maximum number of z available sites, the following simple kinetic model of

interactions with monovalent vacant ligands (L) may be considered[3,23]

$$\begin{array}{lll} & \overset{k_{1,1}}{\underset{k_{2,1}}{\rightleftarrows}} & AL_1 \\ A + L & \overset{k_{3,1}}{\underset{k_{4,1}}{\rightleftarrows}} & AL_2 \\ AL_1 + L_{adj} & \vdots & \\ & \overset{k_{2z-1,1}}{\underset{k_{2z,1}}{\rightleftarrows}} & AL_z \\ AL_{z-1} + L_{adj} & & \end{array} \qquad (20)$$

where L_{adj} represents an adjacent vacant ligand and AL_1, AL_2, ..., AL_z represent the adsorbate-ligand complexes. By adopting an argument similar to that of Hougen and Watson[64] for the evaluation of the concentration of adjacent vacant active sites (ligands), and by considering each interaction in expression (20) to be elementary, Arve and Liapis[3] developed the rate equations for the adsorption mechanism shown in expression (20). The rate equations are given in Reference 3, and are employed in the evaluation of the accumulation terms $\partial C_{sAL_1}/\partial t$, $\partial C_{sAL_2}/\partial t$, ..., $\partial C_{sAL_z}/\partial t$; these terms are then used to obtain $\partial C_s/\partial t$ in equation (5a). It may take time[3,15,23] for the adsorbate molecules to form complexes AL_1, AL_2, ..., AL_z. These multiple interactions form relatively strong bonds and may lead to progressively smaller probabilities for exchange or desorption of the molecule. This would mean that the degree of reversibility or exchangeability of the adsorbate molecule may decrease with time. The kinetic model in expression (20) may be extended by considering (i) exchange interactions between the complexes and adsorbate molecules in the solution, (ii) conformational changes occurring in complexes AL_1, AL_2, .., AL_z, (iii) spontaneous desorption of the complexes (sequential desorption is already considered in expression (20)), and (iv) some of the adsorbed molecules in complexes AL_1, AL_2, .., AL_z become unexchangeable. This extension could be made by expanding on the ideas and expressions (equations (17)-(19)) presented above. A primary and difficult task in the modelling of affinity adsorption systems involving a monovalent or a multivalent

adsorbate, should be to estimate the kinetic parameters characterizing the interaction mechanisms. Furthermore, there is need for the construction of additional dynamic kinetic models for describing the interaction between adsorbate molecules (monovalent or multivalent) and immobilized ligands, so that accurate model discrimination and parameter estimation studies[46] can be made.

An interesting feature of macromolecular surfaces is the degree of irregularity[8,65-68] of protein surfaces and its effect on macromolecular interactions. Future model studies of equilibrium isotherms and of dynamic interaction models, may have to examine the possibility that the interaction of molecules having fractal surfaces might contribute to adsorptivities that are different than those associated with apparent active sites.

2.1.3 Film Mass Transfer

For certain batch systems, the value of the film mass transfer coefficient, K_{fi} (equations (1) and (8)), of the adsorbate may be estimated from literature correlations.[3,54,61,69] One such correlation for K_f (the subscript i is dropped for single component mass transfer) is given by the following expression[69]

$$K_f = \frac{2D_{mf}}{d_p} + 0.31 \left[\frac{(\Delta\rho)\mu g}{\rho^2}\right]^{1/3} \left[\frac{\mu}{\rho D_{mf}}\right]^{-2/3} \qquad (21)$$

where D_{mf} denotes the diffusion coefficient of the macromolecule in free solution; d_p is the mean diameter of the adsorbent particles; $\Delta\rho$ is the density difference between the particulate and continuous phases; ρ is the density of the liquid solution; $g = 9.80665\ m/s^2$; and μ is the viscosity. The value of the film mass transfer coefficient depends on the solution environment, and the physicochemical solution environment would be different for the stages of adsorption, wash, and elution, and therefore, the value of K_f may be different for each stage. Equation (21) may be used to estimate the film mass transfer coefficient, K_f, in batch systems employing porous or nonporous adsorbent particles. It has been found[61] that when the estimated value (from equation (21)) of K_f is varied by ±20%, the effect on the dynamic behavior of the batch systems appears to be not significant.

It should be emphasized that literature correlations may not be applicable to some systems involving very large adsorbate molecules, and thus, they should be used with caution. Experimental and theoretical studies may be needed, in order to develop expressions from which the film mass transfer coefficient of biological macromolecules can be accurately estimated for different physicochemical environments and various modes of operation.

2.1.4 Intraparticle Diffusion

The effective pore diffusivity, D_p, in equation (5a), may be obtained by using parameter estimation methods[3,11,16,46,61] which would be employed to match in an optimal way the predictions of the batch (finite bath) models with sets of experimental batch data,[3,49,61] as shown in the information flow chart[3,11] of Figure 2. It has been found[61] that when the estimated value of the effective pore diffusivity is varied by ±20%, the effect on the dynamic behavior of the batch and column systems can be appreciable.

In affinity systems involving adsorbed species whose molecular diameter is not much smaller than the pore diameter, the effect of restricted[11,16,40,48,62] pore diffusion on the mass flux of the adsorbate, during the adsorption, wash, and elution stages, should be considered. It is worth noting at this point that the term "pore diameter" denotes the diameter of the pore after the immobilization of the ligand molecules. There are different techniques[70-72] for measuring the pore size distribution of adsorbent particles. If these techniques[70-72] are not applicable for measuring the pore size distribution after the immobilization of the ligands, then one may use the pore size distribution of the inert matrix (the pore size distribution of the porous particle before the immobilization of the ligands), the reaction[62] kinetics for forming the covalent bond between the ligands and the activated surface of the porous particle, and a restricted diffusion model[40,48] in order to estimate the pore size distribution and ϵ_p of the adsorbent particles resulting after the immobilization of the ligands.

Petropoulos et al.[40,48] have constructed a restricted pore diffusion model, which may be used to estimate the permeability of

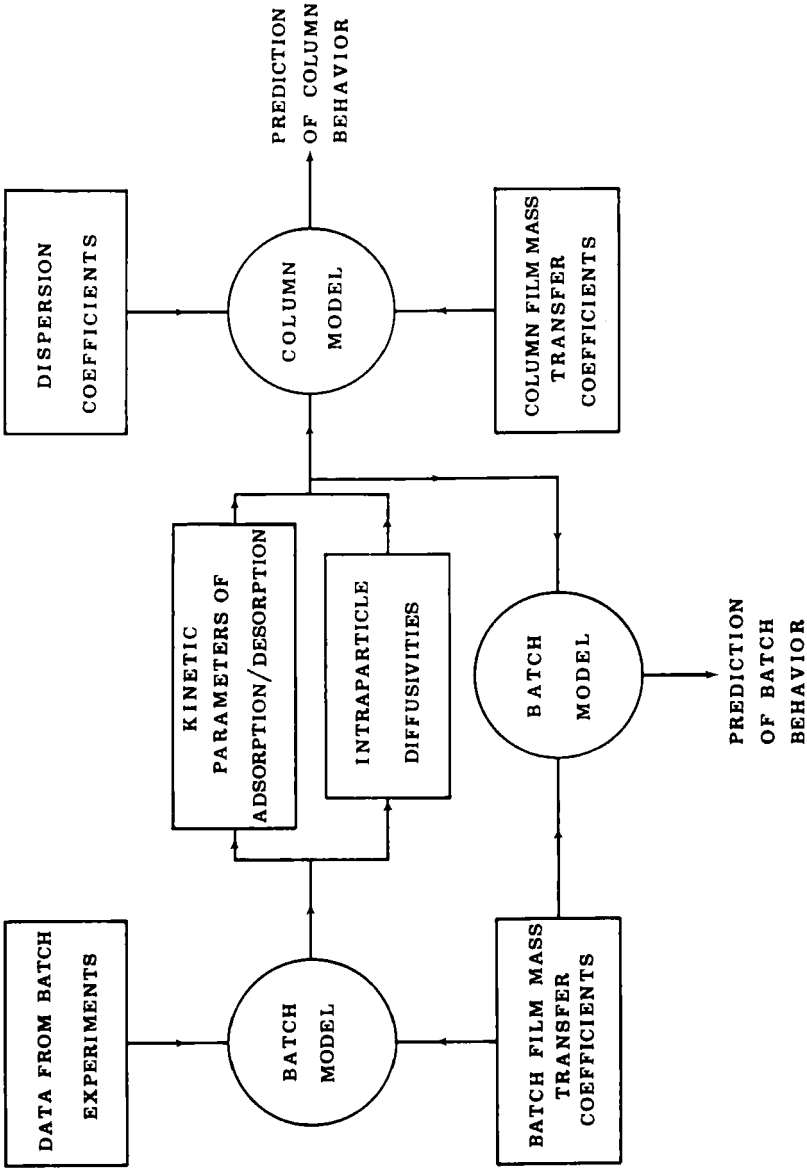

FIGURE 2. Information flow of data used in batch and column models to predict the dynamic behavior of affinity adsorption systems.[3,11,18]

the adsorbate within the pores of an affinity chromatography adsorbent as the fractional saturation of the ligands changes. Their model considers the combined effects of steric hindrance at the entrance to the pores and frictional resistance within the pores, as well as the effects of pore size distribution, pore connectivity of the adsorbent, amount of immobilized ligand, molecular size of the adsorbate and ligand, and the fractional saturation of adsorption sites (ligands); the fractional saturation of ligands is related to the variable C_s in equation (5a). In the affinity adsorption of immunoglobulin G to Protein A immobilized to agarose matrices,[49] the effective pore diffusivity of immunoglobulin G in the porous adsorbent particle varied with matrix type, Protein A loading, and the buffer system used; the buffer system affects the free molecular diffusion coefficient of the adsorbate (the parameter D_{Ao} in equations (7), (11), and (18) of Petropoulos et al.[48]). The data of Johnston and Hearn[92] suggest that restricted pore diffusion may occur in the DEAE Fractogel 65/ferritin system, and in the dye-affinity Fractogel HW55/HSA system; the effective pore duffusivity was estimated[92] to be up to 100-fold smaller than the free molecular diffusivity.

The restricted pore diffusion model[40,48] also provides estimates of the conditions for which the percolation threshold is obtained. In Figure 3, the relative permeability[11,48], $P_R(\theta)$, versus the fractional saturation of active sites (ligands), θ (θ is related to the variable C_s in equation (5)), is presented for different affinity adsorbents having high[48] ligand concentrations; $P_R(\theta) = P_{mac}(\theta)/P_{mac}(\theta=0)$; P_{mac} denotes the macroscopic permeability of the adsorbate in the porous affinity adsorbent, and $P_{mac} = \epsilon_p D_p$; D_p represents the effective pore diffusion coefficient of the adsorbate in the porous affinity adsorbent; ϵ_p denotes the particle porosity; and θ represents the fractional saturation of ligands, $0 \leq \theta \leq 1$. The parameters α' and β' represent the effective dimensionless radii of the adsorbate and ligand molecules, respectively;[48] n_T denotes the pore connectivity; σ denotes the width of the pore size distributions C and D, while σ_1 and σ_2 represent the widths of distribution G; distributions C, D, and G are presented in Reference 48. The results in Figure 3

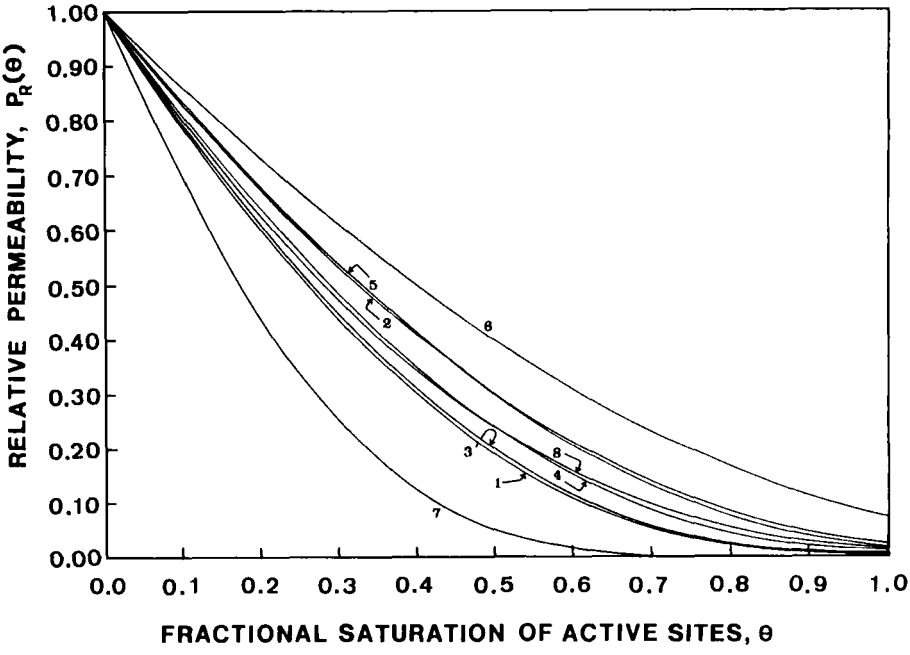

FIGURE 3

Relative permeability, $P_R(\theta)$, versus fractional saturation of active sites (ligands), θ, for concentrated ligand systems treated by the t approach,[11,48] with networks having pore radius distributions C, D, and G; $\alpha' = 0.4$, $\beta' = 0.0$; Curves 1, 3, 5, 7: $n_T = 4$; Curves 2, 4, 6, 8: $n_T = 18$; 1, 2:D, $\sigma = 0.5$; 3, 4: D, $\sigma = 0.25$; 5, 6: G, $\sigma_1 = 0.25$, $\sigma_2 = 0.75$; 7,8: C, $\sigma = 0.5$ (the theoretical model and the details for all parameters are given in Reference 48).

suggest that significant reductions in the macroscopic effective pore diffusivity or macroscopic network permeability may indeed occur during the adsorption stage of affinity chromatography. Furthermore, the results for the system represented by curve 7 in Figure 3, indicate that the percolation threshold is attained for values of θ greater or equal to 0.67. This means that when $\theta \geq 0.67$ there is no continuous conducting pathway through the porous network of the system represented by curve 7, in consequence of

which the macroscopic network permeability, P_{mac}, vanishes. In Reference 48, the expressions which may be used to estimate P_R, ϵ_p, and D_p for a given affinity adsorption system, are presented; systems with dilute and high ligand concentrations have been studied.[48]

The restricted pore diffusion model,[40,48] with a certain modification, may also be used in studies involving the immobilization of ligand on the activated surface of a porous matrix (covalent interaction). In this case, the expression of the adsorption mechanism (e.g., equation (11)) is replaced by the kinetics of the interaction between the ligand and the activated surface of the porous medium. This modification would result in a restricted pore diffusion model which could be incorporated as a constitutive expression in a dynamic material balance equation describing the process of ligand immobilization. Thus, the ligand concentration distribution on the surface of the porous matrix could then be predicted. In fact, the ligand concentration distribution at the end of the ligand immobilization process is of paramount importance for determining properly the pore size distribution of an affinity adsorbent, especially when the molecular diameter of the ligand is comparable to the radius of the pores of the original porous matrix. It is also important for determining the number of active sites per unit surface area of pore walls along the radial direction of an adsorbent particle. If there is a gradient in the concentration of the affinity ligand along the radius of the adsorbent particle, then this could mean, as mentioned earlier, that C_T is a function of the radial distance r. It has been found that for certain adsorbent particles the distribution of the ligand along the radial distance r, could be examined[49] experimentally; in such cases, the experimental data could be compared with the theoretical results obtained from the restricted pore diffusion model[40,48] and the expression that describes the process of ligand immobilization.

2.1.5 Parameter Estimation

In Figure 2, it is shown that from (i) experimental data obtained from batch experiments, (ii) appropriate values for the batch film mass transfer coefficient, and (iii) a proper batch

model (i.e., equations (1), (2), (5a), and (6)-(9)) which incorporates satisfactory effective pore diffusion and interaction (adsorption mechanism of adsorbate interacting with ligand) models, estimates for the effective pore diffusivity and for the parameters characterizing the adsorption mechanism may be obtained. It is necessary to note that for the accurate estimation of the parameters many sets of experimental batch data would be required,[11,16,46,48] and a number of different[61] dynamic adsorption mechanisms and intraparticle pore diffusion models may have to be available for proper studies of parameter estimation, model discrimination, and optimal design of experiments.[46] It should also be noted that the estimation procedure may be complex and difficult, because from the measurement of an overall mass-transfer resistance estimates for the kinetic parameters of the adsorption mechanism and for the intraparticle diffusivity have to be obtained. The results of McCoy and Liapis[61] indicate that while it is a necessary condition for a kinetic model to describe properly the experimental overall mass-transfer resistance, this is not also a sufficient condition for the accurate determination of the adsorption mechanism and for the accurate estimation of the values of the rate constants and of the pore diffusivity. Experiments have been suggested[61] which could provide information that could significantly improve the model discrimination and parameter estimation studies for the determination of a proper mechanism for the dynamics of the adsorption step and of an accurate estimate for the value of the pore diffusivity.

The estimation problem becomes less complex if certain parameters of the adsorption mechanism may be estimated from experimental adsorption data which would mainly represent the kinetics of interaction between adsorbate and ligand, and may have been measured by different experimental techniques[11,20,25] (e.g., optical; spectroscopic; electrochemical; thermodynamic methods; radio labeling). Furthermore, Lightfoot et al.[41] have indicated that pulse field-gradient spin-echo NMR may be used to measure a solute diffusivity at a single solute concentration and investigate the possibility of using labeled proteins in order to

measure protein diffusivities, both in the pore liquid and in the adsorbed state on the surface of the sorbent, as a function of the protein concentration, sorbent morphology, and the sorption chemistry. The availability of experimental results of this kind, would be useful for estimating the parameters of the restricted[40,48] pore diffusion model, and thus, values for the effective pore diffusivity, D_p, could be obtained; in this case, the dynamic batch adsorption data would be used to estimate only the kinetic parameters of the interaction model(s) (adsorption mechanism(s)) by the approach indicated in Figure 2, and thus, the degree of complexity of the parameter estimation and model discrimination problem[46] would be significantly reduced. It is also expected that the experimental data obtained from the above mentioned NMR studies,[41] might indicate the occurrence or not of surface diffusion, and whether or not surface diffusion plays a significant role in protein transport. If it is found that in certain affinity adsorption systems surface diffusion cannot be neglected, then equation (4) should represent the continuity equation of the adsorbate in the adsorbent particle, and values for the surface diffusion coefficient and the effective pore diffusion coefficient have to be estimated.

The kinetics of the interaction of adsorbate with ligand and the intraparticle diffusivity are considered to represent the intrinsic[3] mechanisms of a given affinity adsorption system, and thus, the values of the parameters characterizing these mechanisms should be independent[3] of the operational mode. The information flow diagram shown in Figure 2[3,11] suggests the use of batch experiments. Batch experiments are easier to perform, less time consuming, less expensive, and easier to analyze[3,11,15,16,40,48] and interpret than column experiments; it should be mentioned that certain batch experiments may be difficult to perform[11,41] for the small particles of interest in some HPLC systems, which may have very short time scales of equilibration (in such systems an alternate approach[8] could be used). The experimental adsorption data should be obtained under various temperature and pH values, either in batch or column systems. It has been shown[39] that the temperature may have a significant effect on the value of the

equilibrium dissociation constant for a complex between an antigen and a monoclonal antibody, and it has been observed[20] that the influence of temperature on the adsorption of macromolecules depends on the pH of the adsorption; temperature effects are discussed in detail in the section on the elution stage. The procedure in Figure 2 could provide the information about the intrinsic mechanisms occurring in the porous adsorbent particles. This information would be necessary for predicting the performance of any mode of operation (other than that of a finite bath or a fixed bed), assuming that a proper continuity equation is available for the given mode (e.g., fluidized bed operation) and the process uses porous adsorbent particles. Horstmann and Chase[49] studied the affinity adsorption of immunoglobulin G to Protein A immobilized to agarose matrices, in batch and column (fixed bed) systems. They used the approach of Figure 2, and found that mass transfer parameters determined from batch experiments could be used in a column model and predict satisfactorily the performance (experimental data) of the adsorption and wash stages of fixed bed systems.

2.1.6 Nonporous Adsorbents

In the previous sections porous adsorbents were considered, since it is common to use porous particles in order to obtain high macromolecule (e.g., protein, hormone) adsorption capacities per unit volume. But the porous adsorbent particles, for a given mode of operation, would have a higher overall mass-transfer resistance (because of the intraparticle mass transfer resistance) than that encountered in nonporous adsorbent particles of the same dimension. In nonporous adsorbents the ligands are immobilized on the outer surface of the particle.

For single component adsorption in a finite bath with nonporous adsorbent particles, equation (1) assumes the following form:[54,61]

$$\frac{dC_d}{dt} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_f\left(C_{dp}-C_d\right) \qquad (22)$$

In equation (22), C_{dp} denotes the concentration of the adsorbate in the liquid layer adjacent to the surface of the nonporous

adsorbent particle. Since $dC_d/dt = -\left[(1-\epsilon)/\epsilon\right]\left[dC_s/dt\right]$, the term dC_s/dt would be given by equation (23)

$$\frac{dC_s}{dt} = \left[\frac{\alpha+1}{r_o}\right] K_f\left[C_d - C_{dp}\right] \tag{23}$$

where C_{dp} is related to C_s (see below). The initial conditions for equations (22) and (23) are:

$$C_d = C_{do} \quad \text{at} \quad t=0 \tag{24}$$

$$C_s = 0 \quad \text{at} \quad t=0 \tag{25}$$

The only remaining step is an equation for C_{dp}. It is apparent that in order to develop an expression for C_{dp}, one has to consider the controlling mechanisms of the adsorption process. The following two possibilities may be considered:[61]

(a) It is assumed that adsorption is controlled by film mass transfer, and therefore, C_{dp} is taken to be in equilibrium with the adsorbate-ligand complex concentration, C_s, at every point on the surface of the particle. For the Langmuir equilibrium isotherm (equation (12))

$$C_{dp} = \frac{C_s}{K_a(C_T-C_s)} \tag{26}$$

and the right-hand-side of equation (26) should replace C_{dp} in equations (22) and (23). Equations (22) and (23) would have to be integrated by a numerical method.

(b) Adsorption is controlled by film mass transfer and the interaction mechanism between the adsorbate and the ligands. In this case, the concentrations C_{dp} and C_s are not in equilibrium, and the dynamic interaction mechanism between the adsorbate and the ligands has to be considered. If, for instance, the second-order reversible interaction mechanism (equation (11)) is applicable for a given system, then

$$C_{dp} = \frac{(\gamma C_d + k_2 C_s)}{(k_1(C_T-C_s)+\gamma)} \tag{27}$$

where

$$\gamma = \left(\frac{\alpha+1}{r_o}\right) K_f \qquad (28)$$

The right-hand-side of equation (27) should replace C_{dp} in equations (22) and (23), and the resulting nonlinear differential equations will have to be integrated numerically.

The approach for estimating the film mass transfer coefficient, K_f, was presented in a previous section. If the available correlations are not applicable for a given affinity adsorption system, then the film mass transfer coefficient may be estimated together with the parameters of the interaction mechanism by matching sets of experimental batch data with the predictions of an appropriate batch model. It is apparent that the procedures of parameter estimation,[46] model discrimination, and optimal design of experiments would be less complex for systems having nonporous adsorbent particles (there is no need for estimating intraparticle mass transfer parameters). It should be of interest to estimate the parameters of different interaction mechanisms of an affinity adsorption system from (i) data obtained when the ligands are immobilized on nonporous particles, and (ii) data obtained when the same ligands are immobilized on porous particles (in both cases, the particles should be made of the same material and should have the same size (r_o)). Such a study may suggest that (a) there are no differences in the mechanism of interaction for cases (i) and (ii), and the differences in the values of the parameters of the interaction mechanism are within acceptable bounds; (b) there are no differences in the mechanisms of interaction, but the differences in the values of the parameters of the mechanism are significant; and (c) different mechanisms of interaction describe the adsorption of adsorbate onto immobilized ligand for cases (i) and (ii). If a proper model for intraparticle diffusion was used, then cases (b) and (c) may suggest that the porous structure affects the adsorbate macromolecule in finding the proper orientation for binding within the confined spaces of the pores, and/or the porous structure affects the immobilization of the ligands in such a way that the

active site(s) of a certain fraction of the vacant ligands are not available for binding at any given time; in such cases, other porous matrices may be considered for the immobilization of the ligands so that the above mentioned difficulties are avoided and high adsorption capacities per unit volume may be obtained.

Finally, it should be mentioned at this point that in affinity adsorption systems involving a distribution of particle sizes (porous or nonporous adsorbent particles), the continuity equations for the adsorbent particles presented in all the above sections may be used together with the particle size distribution[73] to predict the affinity adsorption course in the adsorbent particles.

2.2 Fixed Bed

Adsorption is considered to take place from a flowing liquid stream in a fixed bed of particles under isothermal conditions, and the concentration gradients in the radial direction of the bed are considered to be not significant.[8,11,23,42] The feed solution to the bed is considered to contain n components, and m ($m<n$) solutes may compete for the available ligands and $m+1 \leq i \leq \ell$ ($\ell<n$) solutes may be nonspecifically adsorbed. It is also taken that $\ell+1 \leq i \leq n$ solutes simply diffuse into the pores of the particles without interacting with the adsorbent.[3,23] A differential mass balance for each component in the flowing fluid stream gives

$$\frac{\partial C_{di}}{\partial t} - D_{Li}\frac{\partial^2 C_{di}}{\partial x^2} + \frac{V_f}{\epsilon}\frac{\partial C_{di}}{\partial x} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right)K_{fi}\left(C_{pi}\Big|_{r=r_o} - C_{di}\right), \quad i=1,2,..,n \tag{29}$$

In equation (29) the velocity of the fluid stream, V_f, is taken to be independent of the space variable x, because the liquid solutions encountered in affinity chromatography systems are very dilute and the main component of the solution is the carrier fluid (for non-dilute solutions a material balance, as shown in Reference 74, would provide the expression for $\partial V_f/\partial x$). The pressure drop through the fixed bed, which is important in the

design of an affinity adsorption packed bed (fixed bed) system, can be determined by the methods reported by Geankoplis.[69] The initial and boundary conditions of equation (29) are as follows:

$$C_{di} = 0 \text{ at } t=0 \ , \ 0 \le x \le L \ , i=1,2,\ldots n \tag{30}$$

$$\frac{V_f}{\epsilon} C_{di} - D_{Li} \frac{\partial C_{di}}{\partial x} = \frac{V_f}{\epsilon} C_{di,in} \text{ at } x=0,\ t>0,\ i=1,2,..,n \tag{31}$$

$$\frac{\partial C_{di}}{\partial x} = 0 \text{ at } x = L,\ t>0,\ i=1,2,..,n \tag{32}$$

In certain systems the axial dispersion is so low that by setting its value equal to zero the error introduced in the prediction of the behavior of an affinity adsorption system is not significant.[42,43] When the axial dispersion coefficient is set equal to zero, equation (31) (with $D_{Li}=0$) becomes

$$C_{di} = C_{di,in} \text{ at } x=0,\ t>0,\ i=1,2,..,n \tag{33}$$

The intraparticle diffusion mechanism and the interaction mechanism(s) between adsorbate(s) and ligands for an affinity adsorption system in a fixed bed, should be the same as those in a finite bath (for the reasons discussed earlier). If the adsorbent particles in the fixed bed are porous, then equation (29) has to be solved simultaneously[23] with the continuity equations of the porous adsorbent particles (e.g., equation (4) or equation (5a) when single component adsorption is considered). For nonporous adsorbent particles, C_{dp} replaces $C_{pi}\big|_{r=r_o}$ in equation (29) for single component adsorption, and the resulting equation is solved together[8] with equation (23). For this case, in equation (23) the term dC_s/dt is replaced by the partial derivative $\partial C_s/\partial t$.

Experimental column data may be matched[8,11,49,61] with the predictions of the column model in order to obtain estimates for the parameters that characterize the intrinsic mechanisms (intraparticle diffusion and the interaction mechanism of the adsorbate and ligand). These parameters, as it was discussed earlier, may often be obtained from batch experiments which are

less expensive and easier to perform and analyze than column experiments. For affinity adsorption systems with small particles having very short time scales of equilibration,[11,41] it may be necessary to estimate the parameters that characterize these intraparticle intrinsic mechanisms by matching the predictions of the fixed bed model with data obtained from properly designed column experiments.

The film mass transfer coefficient, K_f (the subscript i is dropped for single component adsorption), may be estimated from the following expression:[75]

$$Sh = 2+0.51 \left(\epsilon_d^{1/3} d_p^{4/3}/\nu\right)^{0.60} Sc^{1/3} \qquad (34)$$

$$0.2 < \left(\epsilon_d^{1/3} d_p^{4/3}/\nu\right) < 4{,}600; \quad 505 < Sc < 70{,}600$$

Equation (34) appears to provide reasonable estimates[8,11,61,75] for the film mass transfer coefficient, K_f, in column systems employing porous or nonporous adsorbent particles; it has been found[61] that when the estimated value (from equation (34)) of the film mass transfer coefficient is varied by ±20%, the effect on the dynamic behavior of the column systems appears to be not significant. In equation (34), Sh denotes the Sherwood number ($Sh = K_f d_p/D_{mf}$); Sc is the Schmidt number ($Sc = \nu/D_{mf}$); d_p denotes the particle diameter; ν is the kinemetic viscosity of the solution; and ϵ_d represents the energy dissipation rate per unit mass of liquid. Kikuchi[76] indicates that equation (34) may also be applicable to systems with values for the specific power group, $\left(\epsilon_d^{1/3} d_p^{4/3}/\nu\right)$, lower than 0.2 and greater than 0.08. Equation (34) does not account for the abnormal decrease[77] in K_f in the very low Reynolds number region, which may be due to micro-nonuniformities in flow distribution.[76] Furthermore, there is another difficulty in the characterization of the film mass transfer mechanism, such as the nature of the variation[41] of the mass transfer resistance over the surface of a packing particle. Thus, there is need for experimental and theoretical studies which would result in the development of expressions from which the film mass transfer coefficient of biological macromolecules can be accurately

estimated (better estimates than those obtained from equation (34)) for different physicochemical environments in fixed beds.

The axial dispersion coefficient, D_L (i is dropped for single component adsorption), in equation (29), may be estimated by the procedure reported in Arnold et al.[43] These calculations[8,43] suggest that in affinity separations involving long narrow packed beds of small particles at low Reynolds numbers, the effect of axial dispersion may be neglected. However, many scale-dependent factors have not been analyzed[41] because they are important only in large columns, and therefore, cannot be properly investigated in small column experiments.[8,43] Axial dispersion caused by non-uniform packing density and inadequate header design requires studies in large column experiments. Furthermore, for concentrated protein solutions involved in certain preparative affinity chromatography systems, the effect of viscous fingering[41] (viscosity-induced dynamic instabilities), which may be responsible for poorer performance,[41] has not been studied in large column experiments. For large-scale columns, design and operational approaches should be developed so that low dispersion in the flow direction and good uniform lateral flow distribution[41] are obtained.

Liapis et al.[8] used the fixed bed model to predict the affinity adsorption of lysozyme onto monoclonal antibody ligand immobilized on nonporous silica particles. Two different densities of immobilized antibody were considered, and the agreement between theory and experiment is good for the initial phases of breakthrough, where the mechanism of biospecific adsorption is dominant. In the later phase (saturation neighborhood) of breakthrough, the effects of nonspecific interactions appear to be greater in the low-density ligand system. The kinetics of the nonspecific interactions were estimated from the data of the later phase of breakthrough and were found to be considerably slower than those attributed to biospecific adsorption. In Figure 4 the results are shown for a system with low density anti-lysozyme ligand, while the data in Figure 5 are those of a system with high density anti-lysozyme

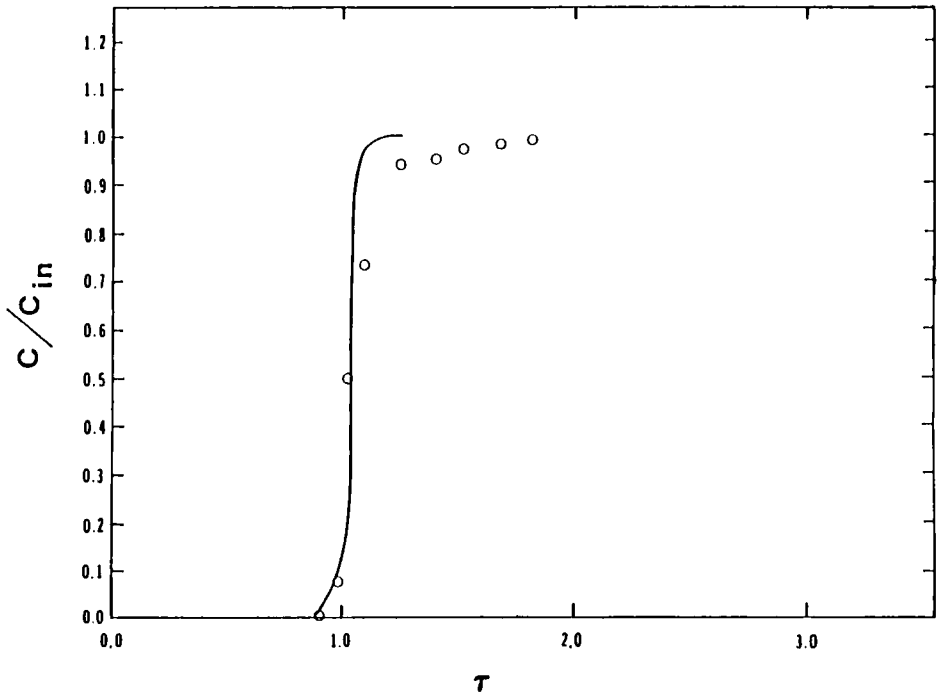

FIGURE 4

Column breakthrough curve for system with low-density anti-lysozyme ligand[8] (C/C_{in} is the dimensionless effluent concentration of the adsorbate, and τ represents the dimensionless time; the experimental conditions and the values of the parameters of the theoretical model are reported in Figure 5 of Reference 8).

o Experimental data

— Theoretical model prediction

ligand. It should be noted that it is the earlier part of the breakthrough curve that is of most interest, since the adsorption stage of an actual process would be terminated at less than 50% breakthrough; for such a condition, it is clearly observed from Figures 4 and 5 that the agreement between experiment and theory is indeed good. Horstmann and Chase[49] used the fixed bed model to predict the affinity adsorption of immunoglobulin G to Protein A

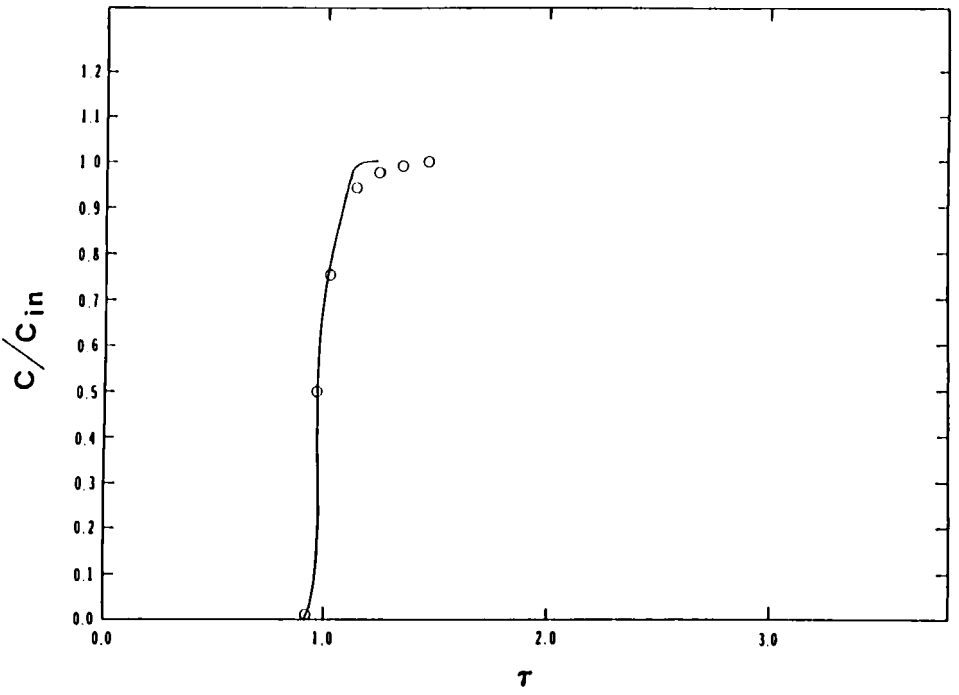

FIGURE 5

Column breakthrough curve for system with high-density anti-lysozyme ligand[8] (C/C_{in} is the dimensionless effluent concentration of the adsorbate, and τ represents the dimensionless time; the experimental conditions and the values of the parameters of the theoretical model are reported in Figure 7 of Reference 8).

o Experimental data

— Theoretical model prediction

immobilized to agarose matrices (porous particles). They found that the agreement between theory and experiment was reasonably good, particularly for the early part of the breakthrough curve.

In Table I[23] the percentage increase is shown in the amount of adsorbate adsorbed in fixed bed systems where local equilibrium between the adsorbate and the adsorbate-ligand complex at each point in the pore exists, relative to the amount of adsorbate adsorbed in the same systems when the rate of the interaction

TABLE I

Percentage increase in the amount of adsorbate adsorbed in the case where local equilibrium exists between the adsorbate and adsorbate-ligand complex relative to the amount adsorbed in the case where the adsorbate-ligand interaction is described by a second-order reversible rate, when the adsorbate concentration in the effluent stream has reached 1% of its inlet concentration (for details, see Reference 23).

Bed Length (m)	$\frac{\text{Amount adsorbed (lc*)-Amount adsorbed (sorr**)}}{\text{Amount adsorbed (sorr**)}} \times 100\%$
1.00	8%
0.50	16%
0.25	52%
0.10	1880%

* lc = local equilibrium between the adsorbate and adsorbate--ligand complex

** sorr = second-order reversible rate

between the adsorbate and ligand is finite and second-order. The film mass transfer coefficient and pore diffusivity have the same values in both cases, and the results represent the conditions in the columns when the adsorbate concentration in the effluent has reached 1% of its value in the feed. It is observed in Table I that for all four bed lengths, more product is adsorbed for the system where local equilibrium exists between the adsorbate and the adsorbate-ligand complex at each point in the pores. For a bed length of 0.1 m the amount adsorbed is substantially larger in the case where local equilibrium exists between the adsorbate and

the complex. When the bed length is 1.0 m, the relative difference in the amounts adsorbed for the two systems is rather small. The substantial difference in the adsorbed amounts of adsorbate between the two cases for the shortest bed length may be very important in the design of affinity adsorption systems, since short fat beds[4,43] are often used in order to minimize the pressure drop across the column. The results in Table I suggest that when short beds are employed in an affinity chromatography system, then the choice of ligand with respect to its rate of interaction with the adsorbate may be of significant importance. Furthermore, the results suggest that if the adsorbent particles are not affected in any significant way by higher pressure drops and if the economics of the system dictate the use of ligands which are not interacting infinitely fast with the adsorbate molecules (local equilibrium does not exist), then long columns may be employed in order to obtain efficient utilization of the immobilized ligands.

In certain single component affinity adsorption systems whose adsorbate is bivalent and the immobilized ligand molecules are monovalent, the adsorbate from the one-site complex is displaced and re-enters the flowing fluid stream.[23,78] This displacement increases the concentration of the adsorbate at the exit of the column above its inlet concentration;[23] this phenomenon occurs in competitive adsorption involving binary[79] mixtures where the exit concentration of the least preferentially adsorbed species can exceed its inlet concentration. It is very interesting that this phenomenon may occur in an affinity adsorption system involving a single adsorbate having two sites for interaction with monovalent ligands. An important implication of this result is that the adsorption stage may have to be terminated before this phenomenon occurs, so that (i) less amount of adsorbate leaves the column with the effluent stream, and (ii) the adsorbate molecules have formed mostly one-site complexes with the ligands. This may make the dissociation of the adsorbate-ligand complex easier during the elution stage.

2.3 Periodic Countercurrent Beds

The employment of affinity chromatography as an efficient and competitive separation process, when compared to other separation methods, requires the effective use of the immobilized ligands. It has been shown[23,80] that the utilization of an adsorbent may be substantially increased if periodic countercurrent operation is employed in the adsorption process. The most efficient mode of operation would theoretically be the continuous[80] countercurrent operation where the adsorbent particles move in a direction opposite to the direction of motion of the flowing fluid stream; the model equations for the continuous countercurrent bed are given in Reference 80. However, this mode would have practical problems because of mechanical complexity of the equipment, gradual attrition of the solid adsorbent, and channeling (nonuniform flow) of either fluid or solid. Therefore, it may be easier to use a periodic countercurrent mode of operation since, if a column is divided into an infinite number of infinitesimal-in-size beds operating in a periodic countercurrent mode, this would give the same results as the continuous countercurrent mode of operation. In practice one has to deal with finite bed sizes, and therefore, the original column is subdivided into a number of smaller-in-size columns that operate in a periodic countercurrent mode. In Figure 6 a column of length L has been divided into two columns, each of length L/2, that operate in a periodic countercurrent mode during the adsorption stage. Three columns, each of length L/2, are shown in the system of Figure 6 since one column of length L/2 is always under regeneration. In a periodic countercurrent operation a column switch occurs, as in the case of fixed bed operation, when the outlet concentration of the adsorbate reaches a certain percentage of its inlet value. Figure 7 shows the relationship between ligand utilization and column length for fixed bed and periodic countercurrent bed operation. The ligand utilization in Figure 7 is defined as the ratio of the amount of ligands that have formed adsorbate-ligand complexes at the end of the adsorption stage to the total amount of ligands available at t=0 for each column length. The adsorbate and the ligands of the system in Figure 7 are monovalent; the

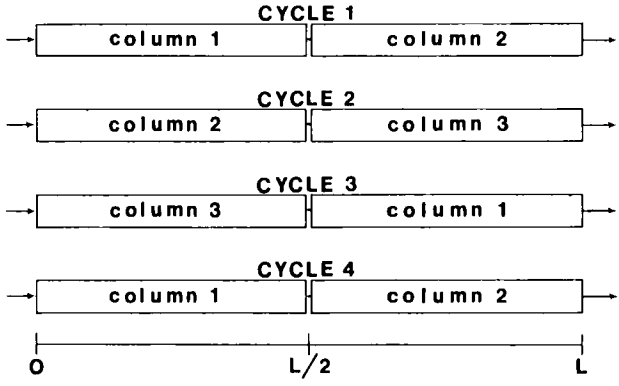

FIGURE 6

Principal arrangement[11,16] of two columns, each of length L/2, in periodic countercurrent operation where L is the total operating length. The system employs three columns, but one is always under regeneration.

values of the parameters of the affinity adsorption system in Figure 7, are given in Reference 23. It is observed in Figure 7 that the periodic countercurrent mode of operation results in a higher utilization of the ligands for all bed lengths compared to the fixed bed operation. In fact, for beds of length 0.5 m and larger the utilization is very close to 100% when the columns are operated in a periodic countercurrent mode. It is also observed in Figure 7 that for bed lengths shorter than 0.5 m there is a substantial difference in the utilization for the two modes of operation. In fact, for a bed length of 0.1 m the ligand utilization is almost four times higher when the column is operated in a periodic countercurrent mode. The results of Figure 7 suggest that the periodic countercurrent bed operation could be one of the desired modes employed in practice, especially when short beds are used.

McCormick[6] presented some aspects of radial flow chromatography where the liquid stream instead of moving along the axis of a fixed bed, it is applied to the column's outer wall and travels along the radius of the bed. Liapis[11,16] has discussed

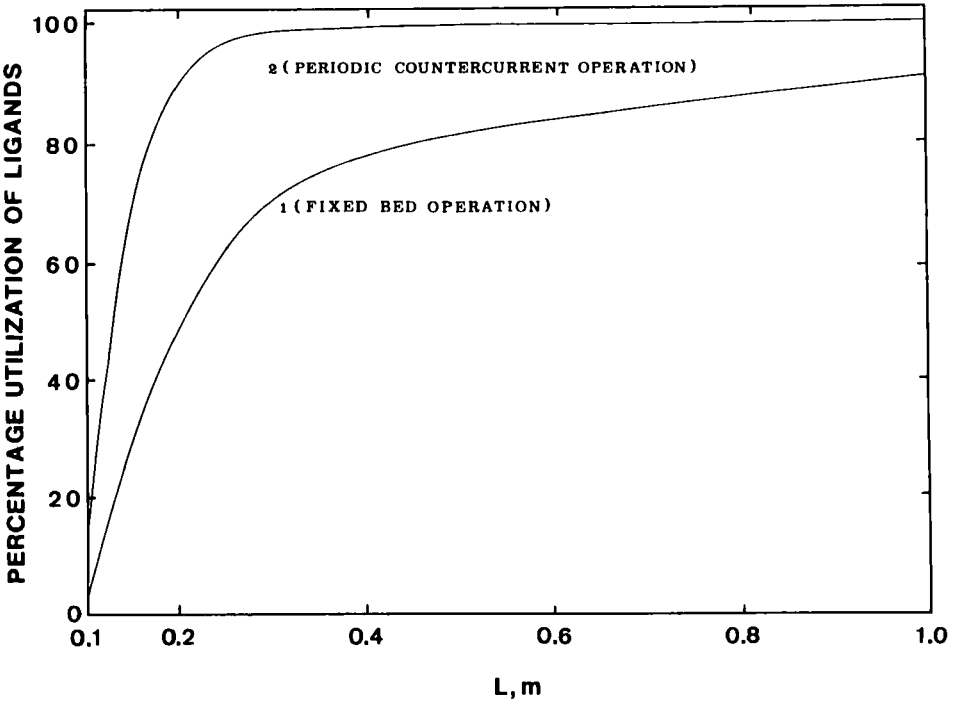

FIGURE 7

Percentage of ligand utilization[11,16,23] versus column length for a system involving a monovalent adsorbate and ligand (for details, see Reference 23).
Curve 1: Fixed bed operation with a column of length L.
Curve 2: Periodic countercurrent bed operation with two columns, each of length L/2, and total operating length L.

the relative advantages and disadvantages of radial flow and axial flow chromatography.

3. WASH

The contaminants may be divided into four groups according to their interaction with the porous adsorbent:[3]

1. Contaminants that do not diffuse into the adsorbent, such as cell debris.
2. Species that simply diffuse in the fluid of the pores but

which are not adsorbed by either nonspecific or biospecific adsorption.

3. Contaminants that diffuse into the particles and are adsorbed nonspecifically to the solid support matrix.
4. Species that diffuse into the particles and compete with the solutes(s) of interest for the ligands.

In nonporous adsorbent particles intraparticle diffusion of the contaminants does not occur, and the contaminants either may not interact with the surface of the adsorbent particles, or may be adsorbed on the surface of the adsorbent particles by nonspecific or specific interactions.

Contaminants belonging to the first group may be separated from the adsorbent particles by centrifugation and filtration or by extraction in an aqueous two-phase system.[81] Removal of contaminants that belong to the second group is accomplished by resuspending the adsorbent particles in a weak buffer solution which allows the contaminants to diffuse from the porous particles into the surrounding bulk phase solution. If the total amount of contaminants present within the pores of the adsorbent particles is large and a high product purity is required, then more than one wash may be necessary in order to reduce the concentrations of the contaminants to a low level. For the removal of contaminants that belong to the third or fourth group, washing with a weak buffer solution may not be sufficient and more effective methods, which may be equivalent to mild elution, may have to be employed.[4] The wash stage may take place in a finite bath, or a fixed bed, or another appropriate mode of operation. For systems having only contaminants of group 2, the model expressions describing the wash stage are required to be developed only for porous adsorbent particles.[3,23] For systems having contaminants of group 3 and/or 4, the model expressions of the wash stage can be developed for porous and nonporous adsorbent particles.

3.1 Finite Bath

The washing medium may affect the film mass transfer and effective pore diffusion coefficients of the adsorbate(s) and contaminants (e.g., by affecting their free molecular diffusion coefficients), as well as the interaction mechanisms involved in

the adsorption of the adsorbate(s) and of the contaminants (contaminants of group 3 and/or 4; contaminants of group 2 are not adsorbed) on the surface of the adsorbent particles. These effects may be quantified by matching the experimental wash data (sets of data) obtained in batch (the procedure of Figure 2 applied to the wash stage) or fixed bed systems with the theoretical predictions of appropriate finite bath or column models of the wash stage. If the effect of the washing medium on the free molecular diffusion coefficients may be satisfactorily estimated by the semi-empirical equation of Polson[69,82] (or from another expression), then the film mass transfer and effective pore diffusion coefficients in the wash stage may be estimated from the model equations discussed in the sections of the adsorption stage presented above; in this case, the matching of the theoretical predictions with the sets of the experimental wash data would provide estimates of the parameters characterizing the adsorption mechanisms of the adsorbates and of the contaminants, in the presence of the washing medium. For the reasons discussed in the adsorption stage, it would be desirable to estimate the parameters of the pore diffusion and/or interaction mechanisms from wash data obtained from batch experiments. It is apparent that the complexity of the parameter estimation procedure may be significantly reduced if the particles are nonporous (there is no intraparticle diffusion mechanism).

The continuity equations of the components during the wash stage, can be described by the same material balance equations and boundary conditions used to model the adsorption stage (i.e., equations (1), (5), (8), and (9)); of course, the values of K_{fi} and D_{pi} (i=1,2,..,n), as well as the interaction mechanism from which the term $\partial C_{si}/\partial t$ (i=1,2,..,ℓ) in equation (5) is obtained, may be different in the wash stage than those employed in the adsorption stage because of the influence of the washing medium. The initial conditions of the expressions that describe the dynamic behavior of the solutes in the fluid of the finite bath during the wash stage, become

$$C_{di} = 0 \quad \text{at} \quad t_w = 0, \; i = 1,2,..,m,..,\ell,..,n \tag{35}$$

The initial conditions (equations (6) and (7)) of the pore diffusion model (equation (5)), are as follows for the wash stage:

$$C_{pi} = \Gamma_i(r) \text{ at } t_w = 0, \quad 0 \le r \le r_o, \quad i = 1, 2, .., m, .. \ell, .., n \qquad (36)$$

$$C_{si} = H_i(r) \text{ at } t_w = 0, \quad 0 \le r \le r_o, \quad i = 1, 2, .., m, .. \ell \qquad (37)$$

The functions $\Gamma_i(r)$ and $H_i(r)$ provide the concentration profiles at the end of the adsorption stage for solutes in the pore fluid and for adsorbed components, respectively. The concentration profiles $\Gamma_i(r)$ and $H_i(r)$ are obtained from the solution of the continuity equations of the adsorption stage. In the case of a multivalent adsorbate having z(z>1) active sites and interacting with monovalent ligands (e.g., equation (20)), there will be a maximum number of z adsorbed concentration profiles for the single adsorbate at the start of the wash stage (end of the adsorption stage).

If nonporous adsorbents are employed and the contaminants are of group 3 and/or 4, then the material balance equations (22) and (23) should be developed for the adsorption of multiple solutes ($C_{dpi}(i=1,2,...m,m+1,...,\ell)$ may be evaluated by the two approaches indicated earlier, when appropriate multicomponent equilibrium and dynamic models for the adsorption mechanisms are available). The solution of the continuity equations of multiple solutes would provide at the end of the adsorption stage, the initial values for the adsorbed concentrations $C_{si}(i=1,2,...,m,m+1,..,\ell)$ of the wash stage (that is, initial conditions for the continuity equations of the adsorbed species in the wash stage). In the wash stage, the material balance equations developed for the adsorption stage may be used. The values of the film mass transfer and interaction parameters in the continuity equations of the wash stage, should be estimated from experimental wash data and appropriate expressions (as discussed above), while equation (35) will provide the initial conditions for the continuity equations of the species in the fluid of the finite bath.

The quantitative study[3,24] of the wash stage in a finite bath, for a system having a single macromolecule adsorbate

interacting with immobilized ligands and a contaminant of group 2, shows that in order to satisfy a specified high level of product (adsorbate of interest) purity more than one wash may be required, and the number of washes, the overall wash time, and the amount of product entering the fluid of the finite bath during the wash stage depend on the criterion used to terminate each wash in the wash stage, for a given washing medium. The different operational possibilities in the wash stage, could lead to certain interesting optimization problems.

3.2 Fixed Bed

The wash stage in a fixed bed[23] of porous adsorbent particles can be described by the same mass balance equations (equations (5) and (29)) used in the modeling of the adsorption stage. Equations (8), (9), and (32) remain unchanged, but the values of the film mass transfer, axial dispersion, and effective pore diffusion coefficients, as well as the values of the parameters of the interaction mechanisms that are active during the wash stage, should be estimated from experimental wash data and appropriate expressions (as discussed above). However, the initial condition and the boundary condition at $x = 0$ of equation (29), become as follows in the wash stage:

$$C_{di} = Q_i(x) \quad \text{at} \quad t_w=0\ , \quad 0 \le x \le L,\ i = 1, 2, .., n \tag{38}$$

$$\frac{V_f}{\epsilon} C_{di} - D_{Li} \frac{\partial C_{di}}{\partial x} = 0 \text{ at } x = 0,\ t_w>0,\ i=1,2,..,n \tag{39}$$

The function $Q_i(x)$ provides the concentration profile of component i in the flowing fluid stream at the end of the adsorption stage. In systems where D_{Li} may be taken to be approximately equal to zero, equation (39) takes the form

$$C_{di} = 0 \quad \text{at} \quad x=0,\ \ t_w>0,\ \ i=1,2,..,n \tag{39a}$$

The initial conditions of the continuity equations for the solutes within the porous particles of the fixed bed are as follows in the wash stage:

$$C_{pi} = B_i(r,x) \text{ at } t_w=0,\ 0 \le r \le r_o,\ 0 \le x \le L,\ i=1,2,..,n \tag{40}$$

$$C_{si} = E_i(r,x) \text{ at } t_w=0,\ 0 \le r \le r_o,\ 0 \le x \le L,$$

$$i=1,2,..,m,m+1,..,\ell \qquad (41)$$

where $B_i(r,x)$ and $E_i(r,x)$ represent the concentration profiles within the porous particles along the length of the bed at the end of the adsorption stage for solutes in the pore fluid and adsorbed phase, respectively. In the case of a multivalent[23] adsorbate interacting with monovalent ligands, there will be a number of adsorbed concentration profiles for the single adsorbate at the start of the wash stage, as discussed above. The dynamic behavior of the wash stage in columns of various lengths packed with porous adsorbent particles has been analyzed.[23,78]

If nonporous adsorbents are used and the contaminants are of group 3 and/or 4, then equation (29), with the term $C_{pi}\big|_{r=r_o}$ replaced by the variable C_{dpi}, may be used together with the continuity equations of the adsorbed species (these equations were discussed for the wash stage in a finite bath), in order to describe the wash stage in a fixed bed of nonporous adsorbent particles.

4. ELUTION

The dissociation of the adsorbate-ligand complex when non-selective and selective eluents are employed in the elution stage, has been recently modelled.[15,21,22] The non-selective eluents[4] change the physicochemical properties of the solution in contact with the adsorbent particles so that the avidity of the binding between ligand and adsorbate is reduced and the dissociation of the adsorbate-ligand complex is promoted. In selective elution, the adsorbate-ligand complex is exposed to a solution containing high concentration of free ligand. This selective eluent (ligand) has also a significant affinity for the adsorbate, and may be the same ligand as that attached on the internal surface of the porous support matrix or may be different than the immobilized ligand. There is competition[11,15,21,22]

between the soluble and the immobilized ligands and, if the soluble ligand is in significant excess, the adsorbate will partition almost exclusively[4] into the soluble phase[21,22], which is then separated from the soluble ligand by exploiting the difference in molecular size of the two species.

Although the elution step is of paramount importance[4,11,21,22] in affinity chromatography processes, experimental elution data are very scarce in the literature, and the reported experimental elution studies have been performed with insufficiently defined systems. Furthermore, very few works[15,21,22] have considered the theoretical modeling, and the qualitative and quantitative analysis of the elution kinetics and the dynamic behavior of the elution stage. In the following sections, models describing the elution stage in a finite bath or a fixed bed are presented and discussed. Non-selective and selective elution is considered, and the adsorbent particles can be porous or nonporous.

4.1 Finite Bath

At the beginning of the elution stage it is considered that m ($1 \leq m < n$) adsorbates are adsorbed onto ligands by specific interactions. It is also taken that the concentrations of the contaminants (species $m+1,\ldots,\ell,..,n$) have been reduced to a specified low level during the wash stage, so that the product purity requirements are satisfied.[3,21-24]

4.1.1 Non-Selective Elution

The eluting agent is taken to represent the n+1 species of the system, and the eluent is considered not to be adsorbed either by specific or non-specific interactions.

The continuity equations for the adsorbates in the finite bath are given by equation (1) for i=1,2,..,m. The initial condition of equation (1) in the elution stage is as follows:

$$C_{di} = 0 \quad \text{at} \quad t_e = 0 \quad , \quad i=1,2,..,m \tag{42}$$

In the section of the adsorption stage above entitled "2.1.2 Models of the Adsorption Mechanism", it was discussed that the selection of an effective eluent for use in non-selective elution may be made with minimal experimentation, if the dominant forces

of interaction for the formation of the adsorbate-ligand complex, during the adsorption stage, are known. The differential mass balance for the eluent in the bulk fluid phase of the finite bath, has the following form:

$$\frac{dC_{di}}{dt_e} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{fi}(C_{pi}(t_e,r_o) - C_{di}(t_e)), \quad i = n+1 \tag{43}$$

The initial condition of equation (43) is given by

$$C_{di} = C_{doi} \quad \text{at} \quad t_e = 0 \quad , \quad i = n+1 \tag{44}$$

In the following two sections, porous and nonporous adsorbent particles are considered. It is important to note that the interaction between the adsorbate and ligand is considered to be strong[3,4,11,14,15,16,20,25,41,47], and surface diffusion of the adsorbed species is taken to be insignificant. Thus, the eluent has to diffuse in the pore fluid of the porous adsorbent particles in order to facilitate the desorption of the adsorbate from the adsorbate-ligand complex. Of course, if for an affinity adsorption system the surface diffusion flux (e.g., equation (4)) of the adsorbed species is significant (this would be an unusual affinity adsorption system), then it could be possible for the eluent to facilitate the desorption of the adsorbate from the complex without the eluent having to enter substantially into the pores of the adsorbent particles.

4.1.1.1 Porous Adsorbents

The continuity equation for the eluent in the porous adsorbent particles becomes

$$\frac{\partial}{\partial t_e}(\epsilon_p C_{pi}) = \frac{1}{r^\alpha}\frac{\partial}{\partial r}\left(r^\alpha \epsilon_p D_{pi}\frac{\partial C_{pi}}{\partial r}\right) \quad , \quad i = n+1 \tag{45}$$

The initial and boundary conditions of equation (45) are

$$C_{pi} = 0 \quad \text{at} \quad t_e = 0 \ , \ 0 \le r \le r_o \quad , \quad i = n+1 \tag{46}$$

$$\epsilon_p D_{pi} \left.\frac{\partial C_{pi}}{\partial r}\right|_{r=r_o} = K_{fi}\left(C_{di} - \left. C_{pi}\right|_{r=r_o}\right), \quad t_e > 0, \quad i = n+1 \tag{47}$$

$$\left.\frac{\partial C_{pi}}{\partial r}\right|_{r=r_o} = 0 \quad , \quad t_e > 0 \quad , \quad i = n+1 \tag{48}$$

The continuity equations for the adsorbates within the porous particles and their boundary conditions, are given by equations (5), (8), and (9) for i = 1, 2,..., m (equation (4) is used if surface diffusion occurs). Their initial conditions in the elution stage are

$$C_{pi} = D_i(r) \quad \text{at} \quad t_e = 0, \quad 0 \le r \le r_o, \quad i = 1,2,3..,m \tag{49}$$

$$C_{si} = M_i(r) \quad \text{at} \quad t_e = 0, \quad 0 \le r \le r_o, \quad i = 1,2,3..,m \tag{50}$$

The functions $D_i(r)$ and $M_i(r)$ provide the concentration profiles at the end of the wash stage for adsorbates in the pore fluid and in the adsorbed phase, respectively. These concentration profiles are obtained by first solving the model equations that describe the dynamic behavior of the adsorption stage and then solving the model equations that describe the wash stage in a finite bath.[3,78]

It is considered that desorption of the adsorbate from the adsorbate-ligand complex starts at a particular position in the porous particles when the concentration of the eluent reaches a certain critical value at this particular position. This suggests that once the concentration of the eluent reaches the critical level at a particular position in the adsorbent, the interaction mechanism between the adsorbate and ligand switches from conditions favoring adsorption to conditions promoting desorption of adsorbate. The critical eluent concentration refers to the required level of eluent concentration that will reduce the avidity of the binding between ligand and adsorbate and promote the dissociation of the adsorbate-ligand complex without impairing the stability of ligand and adsorbate. It is also important that whenever the concentration of the eluent in the pore fluid and the

finite bath is higher than the critical eluent concentration, there is no impairment of the stability of adsorbate and ligand. The critical eluent concentration and the maximum eluent concentration allowed (so that stability of adsorbate and ligand is preserved) for a given affinity chromatography system may be determined by the experimental procedures and methods presented by Kennedy and Barnes[83] and Sada et al.[84]

A constitutive expression (desorption mechanism) describing the adsorbate-ligand interaction in the presence of a non-selective eluent is necessary, in order to evaluate the term $\partial C_{si}/\partial t$ ($i = 1,2,..,m$) in equation (5) during the elution stage. In certain systems the desorption of adsorbate may take place at a much higher rate than the rates of mass transfer through the pore fluid and the liquid film. The desorption may be complete, meaning that the concentration of the adsorbate-ligand complex becomes zero. It may then be assumed that the desorption of adsorbate occurs infinitely fast at a certain position in the particle where the concentration of the eluent is equal to or greater than the critical value. Then the adsorbed phase concentrations will be given by

$$C_{si}(r) = 0 \quad \text{if } C_{pn+1}(r) \geq C_{pn+1}^{ci} \quad , \quad i = 1,2,..,m \qquad (51)$$

$$C_{si}(r) \neq 0 \quad \text{if } C_{pn+1}(r) < C_{pn+1}^{ci} \quad , \quad i = 1,2,..,m \qquad (52)$$

where C_{pn+1}^{ci} represents the critical eluent concentration for solute i ($i = 1,2,..,m$). It should be noted that since the relative contributions of the different types of forces involved (e.g., electrostatic, hydrophobic, etc.) in determining the overall strength[11,20,25,31,54] of the binding would be different for different adsorbate-ligand complexes, the critical value of the eluent concentration at which desorption of a particular species is promoted may be different for different adsorbates. The concentrations $C_{si}(r)\neq 0$ of the adsorbate-ligand complexes in expression (52) have been calculated from the solution of the dynamic equations of the wash stage,[3,22] and are given by equation (50).

For systems where the desorption rate of the adsorbate(s) is not infinitely fast relative to the rates of mass transfer in the pore fluid and the liquid film, the following irreversible rate expression[22] may be used to describe the dynamics of the desorption step of the adsorbate from the adsorbate-ligand complex:

$$\frac{\partial C_{si}}{\partial t_e} = - k_{2i,e} C_{si} \quad \text{if } C_{pn+1}(r) \geq C^{ci}_{pn+1},\ i = 1,2,..,m \tag{53}$$

Equation (52) remains the same for positions within the particle where $C_{pn+1}(r) < C^{ci}_{pn+1}$. The initial condition for expression (53) is given by equation (50).

In certain systems, complete desorption may be difficult to achieve experimentally and at equilibrium some material may still be bound to the ligands. These systems may be described by a second-order reversible interaction expression[22] given by

$$\frac{\partial C_{si}}{\partial t_e} = k_{1i,e} C_{pi} (C_T - \sum_{j=1}^{m} C_{sj}) - k_{2i,e} C_{si} \quad \text{if}$$

$$C_{pn+1}(r) \geq C^{ci}_{pn+1}, i = 1,2,..,m \tag{54}$$

For positions within the particle where $C_{pn+1}(r) < C^{ci}_{pn+1}$, equation (52) would provide $C_{si}(r)$ as discussed above. Furthermore, the initial condition of equation (54) is given by expression (50).

In the elution stage, it would be desirable to have very high values for the parameter $k_{2i,e}$ of the adsorbate(s) of interest (in equations (53) and (54)); of course, in equation (54) it would be desirable to have $k_{1i,e} \ll k_{2i,e}$ so that $(k_{2i,e}/k_{1i,e}) \gg 1$. The film mass transfer coefficients of the adsorbate(s) and eluent may be estimated from equation (21) or from another appropriate correlation. The effective pore diffusion coefficients of the eluent and adsorbate(s), as well as the kinetic parameters characterizing the desorption mechanism of the adsorbate-ligand complex (e.g., $k_{1i,e}$ and $k_{2i,e}$ in equations (53) and (54)) may be estimated by matching the predictions of the batch or fixed bed models for the elution stage with sets of experimental elution

data obtained from batch (the procedure of Figure 2 applied to the elution stage) or fixed bed experiments. It should be noted that desorption mechanisms other than those proposed above, may have to be developed and used in the parameter estimation,[46] model discrimination, and optimal design of experiments studies of the elution stage of a given affinity chromatography system. Furthermore, expressions (51)-(54) may be considered to be more applicable to systems involving the desorption of a single adsorbate, since the mechanism(s) of the desorption of multiple solutes (by the eluent) may be more involved (complex) than those indicated in equations (51)-(54). But the theory on the desorption of multiple macromolecule solutes by a non-selective eluent, is in its infancy (as is the case with the theory on multicomponent adsorption of macromolecules, discussed earlier). Thus, equations (51)-(54) may be considered to represent approximations for the desorption mechanisms of systems involving multiple macromolecule adsorbed solutes, and may be useful in first-level design, scale-up, and dynamic behavior studies of the elution stage of affinity chromatography systems having multiple adsorbed solutes. It is also worth noting that the model expressions for the desorption step presented above, may be useful in describing the removal of nonspecifically adsorbed contaminants in mild eluting solutions during the wash stage.

The effects of the film mass transfer and effective pore diffusion coefficients of the adsorbate (product of interest) and eluent, as well as the effects of the desorption parameters (C^{ci}_{pn+1}, $k_{1i,e}$, and $k_{2i,e}$) in equations (51)-(54) on the dynamic behavior of the elution stage in a finite bath, have been studied by Arve and Liapis[22] for an affinity adsorption system involving a single adsorbate. It is important to note at this point that the expressions and values of the parameters that characterize the pore diffusion and desorption mechanisms (intrinsic mechanisms) in the elution stage, should be normally independent of the mode of operation (e.g., batch; fixed bed; fluidized bed, etc.) employed in the elution stage.

It has been shown by Frankel[39] that the value of the equilibrium dissociation constant for an antigen-monoclonal

antibody complex increases by two orders of magnitude when the temperature is increased from 4°C to 43°C. This finding and other[8,11,20,31,54,61] results indicate that the strength of the interaction between the adsorbate and the adsorbent varies as the temperature changes. Thus, the temperature of the solution may be used as a control variable, so that, at a certain operating temperature during the adsorption stage, significant adsorption could occur, while the elution stage would operate at a different temperature which could facilitate the desorption of the adsorbate from the adsorbate-ligand complex. The temperature interval of variation should be selected in such a way that both the adsorbate(s) and the ligands are stable[37] for any value of the temperature in the interval. The above discussion suggests that in certain affinity chromatography systems, high desorption rates could be achieved by utilizing a proper non-selective eluent and operating the elution stage at a temperature which facilitates elution. Furthermore, it should be emphasized that the values of the parameters which characterize the interaction mechanism between adsorbate(s) and ligands during the adsorption, wash, and elution stages, should be estimated from dynamic experimental adsorption, wash, and elution data obtained under various temperature and pH values, either in batch or fixed bed systems. It is important to obtain data for different pH values, since it has been observed[20] that the influence of temperature on the adsorption of macromolecules depends on the pH of the adsorption. An Arrhenius expression[11,15,45] may, for instance, be used to describe the temperature dependence of the rate constants characterizing the interaction mechanism between the adsorbate(s) and ligands in each stage (adsorption; wash; elution) of a given affinity chromatography system. The estimated values of the activation energy and pre-exponential factor might provide some useful suggestions with regard to the nature of the interaction mechanisms occurring in the adsorption, wash, and elution stages. It is apparent that the above mentioned studies may be carried with porous or nonporous adsorbents.

4.1.1.2 Nonporous Adsorbents

When the adsorbent particles are nonporous, there is no intraparticle diffusion for the eluent and the desorbed

adsorbate(s). Desorption is considered to occur when the eluent concentration, $C_{dn+1,p}$, in the liquid layer adjacent to the surface of the adsorbent particle, has reached a certain critical value, C^c_{dn+1}. This critical eluent concentration (C^c_{dn+1}) would represent the lowest value of the eluent concentration required to facilitate the desorption of that adsorbate which has the highest strength of binding with ligand, for a given affinity adsorption system. The concentration of the eluent in the finite bath is taken to be equal to C_{don+1} at $t_e = 0$. The eluent is transported to the surface of the nonporous particles by film mass transfer. The total volume of the liquid films surrounding all the particles is very much smaller than the volume of the liquid phase in the batch system, and thus, one may consider that the accumulation term, dC_{dn+1}/dt_e, of the eluent concentration in the finite bath may be approximately equal to zero. A dynamic material balance for the average concentration, $\bar{C}_{dn+1}$, in the liquid film surrounding each particle (it is considered that $\bar{C}_{dn+1} \simeq (C_{dn+1,p} + C_{dn+1})/2$; $dC_{dn+1}/dt_e \simeq 0$; and $C_{dn+1} \simeq C_{don+1}$ for $t_e > 0$), may provide an expression from which an estimate of the time, t^c_e, required for the eluent concentration $C_{dn+1,p}$ to become equal to C^c_{dn+1} ($C_{dn+1,p} = C^c_{dn+1}$) could be obtained. The form of the expression t^c_e is as follows for spherical particles:

$$t^c_e = \left(\frac{(r_o\delta_1(r_o+\delta_1)+(\delta_1^3/3))}{2K_{fn+1}(r_o+\delta_1)^2}\right)\left(\ln\left(\frac{C_{don+1}}{C_{don+1}-C^c_{dn+1}}\right)\right) \qquad (55)$$

In equation (55), δ_1 represents the thickness of the liquid film. For an example, one may consider a system with spherical particles of radius $r_o = 4.5\times10^{-5}$m, and an eluent with $K_{fn+1} = 9.0\times10^{-6}$m/s, $D_{fn+1} = 7.0\times10^{-11}m^2/s$ (D_{fn+1} is the diffusion coefficient of the eluent in free solution), and $C_{don+1} = 1.01C^c_{dn+1}$; for this system δ_1 is estimated to be approximately equal to 7.77×10^{-6}m. These parameter values are used in equation (55) and one obtains that $t^c_e = 1.71$s; of course, if $C_{don+1} > 1.01\ C^c_{dn+1}$ then the value of t^c_e will be smaller than 1.71s. In general, one could assume with reasonable accuracy that the time required for $C_{dn+1,p}$ to be equal to C^c_{dn+1} would be very short, and the initial concentration,

C_{don+1}, of the eluent in the finite bath has to be only slightly higher than the critical eluent concentration, C^c_{dn+1}.

The eluent concentration in the fluid phase of the finite bath is considered to be, in effect, equal to C_{don+1} for $t_e>0$. The initial condition for the adsorbed species is given by

$$C_{si} = \lambda_i \quad \text{at} \quad t_e = 0 \quad , \; i = 1,2,\ldots,m \tag{56}$$

The λ_i are the values of the concentrations of the adsorbed species at the end of the wash stage. The λ_i are obtained by first solving the model equations that describe the dynamic behavior of the adsorption stage and then solving the model equations that describe the wash stage in a finite bath. The desorption mechanisms described by equations (51), (53), and (54) become as follows in the case where nonporous adsorbent particles are employed:

$$C_{si} = 0 \quad \text{if} \quad C_{dn+1,p} \geq C^c_{dn+1} \quad , \; i = 1,2,..,m \tag{51a}$$

$$\frac{dC_{si}}{dt_e} = - k_{2i,e}C_{si} \quad \text{if } C_{dn+1,p} \geq C^c_{dn+1} \; , \; i = 1,2,..,m \tag{53a}$$

$$\frac{dC_{si}}{dt_e} = - k_{1i,e}C_{di,p}\left(C_T - \sum_{j=1}^{m} C_{sj}\right) - k_{2i,e}C_{si}$$

$$\text{if } C_{dn+1,p} \geq C^c_{dn+1}, \quad i = 1,2,..,m \tag{54a}$$

In equation (54a), $C_{di,p}$ denotes the concentration of the desorbed adsorbate i in the liquid layer adjacent to the surface of the nonporous particle. The continuity equations for the adsorbates in the finite bath, are given by equation (1) with the variable $C_{pi}(t,r_o)$ replaced by $C_{di,p}$; the initial condition is given by equation (42). The expression for $C_{di,p}(i=1,2,..,m)$ has the following form when the desorption is described by equation (53a) or equation (54a):

(a) The desorption of adsorbate i is described by equation (53a);

then $C_{di,p}$ is given by

$$C_{di,p} = \frac{(\gamma_i C_{di} + k_{2i,e} C_{si})}{\gamma_i}, \qquad i=1,2,..,m \tag{57}$$

where

$$\gamma_i = (\frac{\alpha+1}{r_o}) K_{fi}, \qquad i = 1,2,..,m \tag{58}$$

(b) The desorption of adsorbate i is described by equation (54a); then $C_{di,p}$ has the form

$$C_{di,p} = \frac{(\gamma_i C_{di} + k_{2i,e} C_{si})}{(k_{1i,e}(C_T - \sum_{j=1}^{m} C_{sj}) + \gamma_i)}, \qquad i=1,2,..,m \tag{59}$$

where γ_i is given by equation (58). It is apparent that if the desorption of adsorbate i (i=1,2,..,m) occurs infinitely fast (equation 51a), then the desorption will be complete and the duration of the elution stage will be short.

The discussion in the section of the porous adsorbents above, regarding the estimation of the parameters $k_{1i,e}$ and $k_{2i,e}$, is also appropriate for the case where nonporous adsorbents are used.

4.1.2 Selective Elution

It is considered that a single adsorbate (product of interest) was adsorbed onto the immobilized ligands during the adsorption stage, and the selective eluent (e.g., a free ligand) is taken to interact only with unbound adsorbate present in the pore fluid and unbound adsorbate in the bulk fluid phase of the finite bath.

The following interactions may then be considered:

$$A + L \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AL \tag{60}$$

$$A + E \underset{k_4}{\overset{k_3}{\rightleftharpoons}} AE \tag{61}$$

where A represents unbound adsorbate; E is the selective eluent; L represents the immobilized vacant ligand; AL is the adsorbate--ligand complex; and AE denotes the adsorbate-eluent complex. Equation (60) is valid only for interactions within the pores of the porous adsorbent particles, and expression (61) holds for interactions in the bulk fluid phase of the finite bath and within the pore fluid. Assuming elementary interactions for the forward and reverse steps of expressions (60) and (61), the continuity equations for species A (component 1), E (component 2), and AE (component 3) in the bulk fluid phase of the finite bath, are as follows:

$$\frac{dC_{d1}}{dt_e} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{f1}\left(C_{p1}\Big|_{r=r_o} - C_{d1}\right) - k_3 C_{d1} C_{d2} + k_4 C_{d3} \tag{62}$$

$$\frac{dC_{d2}}{dt_e} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{f2}\left(C_{p2}\Big|_{r=r_o} - C_{d2}\right) - k_3 C_{d1} C_{d2} + k_4 C_{d3} \tag{63}$$

$$\frac{dC_{d3}}{dt_e} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{f3}\left(C_{p3}\Big|_{r=r_o} - C_{d3}\right) + k_3 C_{d1} C_{d2} - k_4 C_{d3} \tag{64}$$

The initial conditions for equations (62)-(64) are

$$C_{d1} = 0 \quad \text{at} \quad t_e = 0 \tag{65}$$

$$C_{d2} = C_{do2} \quad \text{at} \quad t_e = 0 \tag{66}$$

$$C_{d3} = 0 \quad \text{at} \quad t_e = 0 \tag{66}$$

The continuity equations for components 1, 2, and 3 in the porous adsorbent particles have the following forms (surface diffusion is not considered for the reasons discussed earlier):

$$\frac{\partial}{\partial t_e}(\epsilon_p C_{p1}) = \frac{1}{r^\alpha}\frac{\partial}{\partial r}\left(r^\alpha \epsilon_p D_{p1} \frac{\partial C_{p1}}{\partial r}\right) - \left(k_1 C_{p1}(C_T - C_{AL}) - k_2 C_{AL}\right) -$$

$$\epsilon_p(k_3 C_{p1} C_{p2} - k_4 C_{p3}) \tag{68}$$

$$\frac{\partial}{\partial t_e}(\epsilon_p C_{p2}) = \frac{1}{r^\alpha}\frac{\partial}{\partial r}\left(r^\alpha \epsilon_p D_{p2}\frac{\partial C_{p2}}{\partial r}\right) - \epsilon_p(k_3 C_{p1}C_{p2} - k_4 C_{p3}) \quad (69)$$

$$\frac{\partial}{\partial t_e}(\epsilon_p C_{p3}) = \frac{1}{r^\alpha}\frac{\partial}{\partial r}\left(r^\alpha \epsilon_p D_{p3}\frac{\partial C_{p3}}{\partial r}\right) + \epsilon_p(k_3 C_{p1}C_{p2} - k_4 C_{p3}) \quad (70)$$

The concentration C_{AL} of the adsorbate-ligand complex would be obtained by solving the following expression:

$$\frac{\partial C_{AL}}{\partial t_e} = k_1 C_{p1}(C_T - C_{AL}) - k_2 C_{AL} \quad (71)$$

The initial conditions for equations (68)-(71) are given by

$$C_{p1} = \kappa_1(r) \qquad \text{at} \qquad t_e = 0,\ 0 \le r \le r_o \quad (72)$$

$$C_{pi} = 0 \qquad \text{at} \qquad t_e = 0,\ 0 \le r \le r_o,\ \text{for } i = 2,3 \quad (73)$$

$$C_{AL} = \kappa_2(r) \qquad \text{at} \qquad t_e = 0,\ 0 \le r \le r_o \quad (74)$$

The functions $\kappa_1(r)$ and $\kappa_2(r)$ provide the concentration profiles at the end of the wash stage for the adsorbate in the pore fluid and the adsorbate-ligand complex, respectively. The boundary conditions for equations (68)-(71) are

$$\epsilon_p D_{pi}\frac{\partial C_{pi}}{\partial r} = K_{fi}\left(C_{di} - C_{pi}\Big|_{r=r_o}\right) \text{ at } r = r_o,\ t_e > 0\ \ i = 1,2,3 \quad (75)$$

$$\frac{\partial C_{pi}}{\partial r} = 0 \qquad \text{at} \quad r = 0,\ t_e > 0,\ i = 1,2,3 \quad (76)$$

In order to solve equations (62)-(76), the mass transfer parameters K_{fi} and D_{pi} (i=1,2,3), as well as the kinetic parameters k_1, k_2, k_3, and k_4 have to be known. The film mass transfer coefficients may be estimated from equation (21) or another appropriate expression. The parameters k_1 and k_2 could have been estimated from adsorption data; assuming, of course, that the presence of species E and AE, during elution, does not

affect in any significant way the values of k_1 and k_2 estimated from adsorption data. Otherwise, k_1 and k_2 should be estimated from elution data. The parameters k_3 and k_4 could be estimated from experimental data obtained in a batch system, where species A and E interact in the fluid phase (there are no adsorbent particles in this batch system) and the concentrations of A, E, and AE are measured at different times. The effective pore diffusion coefficients D_{p1}, D_{p2}, and D_{p3} could be estimated from the expressions[3,40,48] and estimation procedures discussed earlier in detail (e.g., batch elution data and procedure shown in Figure 2, or by matching fixed bed model predictions with elution data obtained from column experiments). It is worth noting that mechanisms other than those shown in expressions (60) and (61) may occur in selective elution.

Arve and Liapis[22] studied the dynamic behavior of the elution stage in a finite bath, when a selective eluent is employed for the elution of an adsorbate from a complex. They found that the amount of adsorbate recovered in the form of the adsorbate-eluent complex is greatly dependent on the initial concentration of the eluent and the values of the equilibrium dissociation constants of the adsorbate-ligand and adsorbate-eluent complexes; porous adsorbents were employed in their studies.

In the case where the adsorbent particles are nonporous, there is no intraparticle diffusion for components A, E, and AE. In equation (71), C_{p1} is replaced by C_{dp1} which represents the concentration of component 1 in the liquid layer adjacent to the surface of the nonporous particle; the initial condition of the resulting equation is given by

$$C_{AL} = \kappa \qquad \text{at} \qquad t_e = 0 \tag{77}$$

where κ represents the value of the concentration of the adsorbed species at the end of the wash stage. The continuity equations for components 1, 2, and 3 in the finite bath, are obtained from equations (62)-(64) by replacing $C_{p1}\big|_{r=r_o}$, $C_{p2}\big|_{r=r_o}$, and $C_{p3}\big|_{r=r_o}$ with C_{dp1}, C_{dp2}, and C_{dp3} which represent the concentrations of components 1, 2, and 3 in the liquid layer adjacent to the surface

of the nonporous adsorbent particles; appropriate expressions for C_{dp1}, C_{dp2}, and C_{dp3} can be derived. The forms of the initial conditions given by equations (65)-(67) remain unchanged. The simultaneous solution of the above mentioned equations, would provide information about the dynamic behavior of the elution stage in a finite bath, when a selective eluent (E) is used and equations (60) and (61) are satisfied; furthermore, the affinity system has a single adsorbate (A) and uses nonporous adsorbent particles.

4.2. Fixed Bed

At the start of the elution stage m ($1 \leq m < n$) adsorbates are considered to be adsorbed onto ligands by specific interactions. Furthermore, it is taken that the concentrations of the contaminants were reduced to a specified low level during the wash stage, so that the product purity requirements are satisfied.

4.2.1 Non-Selective Elution

The eluting agent is taken to represent the n+1 species of the system, and the eluent is considered not to be adsorbed either by specific or non-specific interactions. The transport of the eluent is governed by film mass transfer, pore diffusion, axial dispersion, and convective flow. Of course, there is no intraparticle diffusion when nonporous adsorbent particles are employed. A differential mass balance for the eluent in the following fluid stream of the fixed bed gives the following expression:

$$\frac{\partial C_{di}}{\partial t_e} - D_{Li} \frac{\partial^2 C_{di}}{\partial x^2} + \frac{V_f}{\epsilon} \frac{\partial C_{di}}{\partial x} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{fi}\left(C_{pi}\Big|_{r=r} - C_{di}\right), \quad i=n+1 \quad (78)$$

The initial and boundary conditions of equation (78) are

$$C_{di} = 0 \quad \text{at} \quad t_e = 0 \quad , \quad 0 \leq x \leq L, \; i = n+1 \quad (79)$$

$$\frac{V_f}{\epsilon} C_{di} - D_{Li} \frac{\partial C_{di}}{\partial x} = \frac{V_f}{\epsilon} C_{di,in} \quad \text{at } x = 0, \; t_e > 0 \quad i = n+1 \quad (80)$$

$$\frac{\partial C_{di}}{\partial x} = 0 \quad \text{at} \quad x = L \quad , \quad t_e > 0, \quad i = n+1 \quad (81)$$

In systems where the contribution of the axial dispersion term can be neglected,[23] equation (80) takes the form (with $D_{Li} = 0$):

$$C_{di} = C_{di,in} \quad \text{at} \quad x = 0 \quad , \quad t_e > 0, \; i = n+1 \tag{82}$$

The continuity equations for the adsorbates in the flowing fluid stream of the bed retain in the elution stage the same forms as in the adsorption and wash stages (equation (29)), and their boundary conditions are given by equations (32) and (39). Their initial conditions in the elution stage are

$$C_{di} = \beta_i(x) \quad \text{at} \quad t_e = 0, \quad 0 \le x \le L, \; i = 1,2,..,m \tag{83}$$

where the functions $\beta_i(x)$ provide the concentration profiles at the end of the wash stage for the adsorbates in the flowing stream.

In the following two sections the model equations for systems having porous and nonporous adsorbent particles, are considered.

4.2.1.1 Porous Adsorbents

The continuity equation for the eluent in the porous adsorbent particles and its initial and boundary conditions are given by equations (45)-(48).

The continuity equations for the adsorbates within the porous particles and their boundary conditions, are given by equations (5), (8), and (9) for $i = 1,2,..,m$ (equation (4) is employed if surface diffusion occurs). Their initial conditions in the elution stage are

$$C_{pi} = u_i(x,r) \text{ at } t_e = 0, \; 0 \le x \le L, \; 0 \le r \le r_o, \; i = 1,2,..,m \tag{84}$$

$$C_{si} = y_i(x,r) \text{ at } t_e = 0, \; 0 \le x \le L, \; 0 \le r \le r_o, \; i = 1,2,..,m \tag{85}$$

The functions $u_i(x,r)$ and $y_i(x,r)$ provide the concentration profiles within the porous particles along the length of the bed at the end of the wash stage for solutes in the pore fluid and adsorbed phase, respectively. These concentration profiles are

TABLE II

Total time duration, T_e, of the elution stage and values of the concentration factor, CF, for systems with $Po_{el}^{ir} = 10$ and $Po_{el}^{ir} = 1000$ (for details, see Reference 21).

Bed Length (m)	Amount of Adsorbate Recovered (kg)	$Po_{el}^{ir} = 10$		$Po_{el}^{ir} = 1000$	
		T_e(min)	CF	T_e(min)	CF
0.10	0.002	47.0	0.05	9.1	0.26
0.25	0.101	110.1	1.0	20.8	5.4
0.50	0.269	135.6	2.2	34.0	8.7
1.00	0.604	167.0	4.0	58.0	11.5

obtained by first solving the model equations that describe the dynamic behavior of the adsorption stage and then solving the model equations that describe the wash stage in a fixed bed.[21,23]

Equations (51)-(54) are considered to describe different mechanisms of desorption. An expression for the desorption mechanism is required in order to solve the continuity equations of the adsorbates in the porous particles (as discussed above for the elution stage in a finite bath).

Arve and Liapis[21] studied the dynamic behavior of the elution stage in fixed beds of porous adsorbent particles. Their results have shown that the concentrating effect of the elution stage on the adsorbate (product of interest) increases as the bed length and the value of the kinetic parameter that characterizes the mechanism of the dissociation (i.e., $k_{2i,e}$ in equations (53) and (54)) of the adsorbate from the adsorbate-ligand complex increase. The concentrating effect of the elution stage is represented by the concentration factor, CF. This factor for a system with a single adsorbate is given by[21]

$$CF = \frac{C_{dl,e}}{C_{dl,in}} \tag{86}$$

where $C_{dl,e}$ represents the concentration of the adsorbate in the

final bulk solution that was established by continuously collecting the effluent stream through the duration[21] of the elution stage, and $C_{di,in}$ denotes the inlet concentration of the adsorbate during the adsorption stage. In Table II, it can be observed that the value of CF increases significantly[21] as the bed length and the Porath[3,21-23] parameter, Po_{el}^{ir}, for non-selective irreversible elution increase; the Porath parameter, Po_{el}^{ir}, is dimensionless and is given by

$$Po_{el}^{ir} = \frac{k_{21,e}(4\pi r_o^2)}{\epsilon_p D_{pl}} \tag{87}$$

where $k_{21,e}$ is the kinetic parameter of the desorption mechanism described by equation (53). In Table II, the value of Po_{el}^{ir} is increased by increasing only the value of $k_{21,e}$, while the values of ϵ_p, D_{pl}, and r_o remain unchanged. Furthermore, the total time of the duration of the elution stage, T_e, for a given bed length, decreases as $k_{21,e}$ increases. For both non-selective and selective elution methods, a shorter total elution time, T_e, is obtained when the direction of flow in the elution stage is opposite (as compared to being the same) to that employed in the adsorption and wash stages.[21] The advantage gained with a reversed flow increases as the bed length decreases. It was also shown[21] that as the required critical eluent concentration increases, the finite mass transfer rate of the eluent in the pore fluid may have an increasingly significant effect on the duration of the elution stage.

In the section above on the elution stage in a finite bath, it was indicated that it may be possible to utilize temperature effects in order to facilitate the desorption of the adsorbate from the adsorbate-ligand complex. In affinity chromatography systems involving the adsorption of multiple adsorbates (a system of m adsorbates, with $m \geq 2$) onto immobilized ligands, it would be expected that the effect of the temperature on the interaction mechanism(s) responsible for the formation of each of the adsorbate-ligand complexes (maximum of m complexes), would be different. In this case, it is thought that the variable-

-temperature stepwise desorption (VTSD) method[85,86,96] may be used, during the elution stage, in order to increase the relative separation of the desorbed solutes. The VTSD method[85,86,96] could significantly improve the overall separation obtained in affinity chromatography systems involving multiple adsorbed components, and having either porous or nonporous adsorbent particles.

4.2.1.2 Nonporous Adsorbents

When nonporous particles are employed in a fixed bed, there is no intraparticle diffusion for the eluent and the desorbed adsorbate(s). The continuity equations for the adsorbates and the eluent in the flowing fluid stream of the bed, are obtained from equations (29) and (78) by replacing the variables $C_{pi}\Big|_{r=r_o}$ $(i = 1,2,..,m)$ and $C_{pn+1}\Big|_{r=r_o}$ with $C_{di,p}$ (from equation (57) or (59), whichever is appropriate) and $C_{dn+1,p}$ (see the section above on non selective elution in a finite bath for systems having nonporous adsorbent particles). The boundary conditions for the material balance equations of the adsorbates, are given by equations (32) and (39) for $i = 1,2,..,m$; their initial conditions in the elution stage are

$$C_{di} = \psi_i(x) \quad \text{at} \quad t_e = 0\ ,\ 0 \le x \le L,\ i = 1,2,..,m \qquad (88)$$

where the functions $\psi_i(x)$ provide the concentration profiles at the end of the wash stage for the adsorbates in the flowing fluid stream. The initial and boundary conditions of the continuity equation for the eluent, are given by equations (79)-(81).

The above mentioned equations are coupled with the expressions describing the dynamics of the desorption of the adsorbates from the adsorbate-ligand complexes. Equation (51a), or (53a), or (54a) (whichever is appropriate for a given system) may be used to describe the desorption of the adsorbates from the complexes. The estimation of the parameters encountered in all the above mentioned equations of this section, has been discussed in earlier sections.

4.2.2 Selective Elution

The mechanism of desorption during selective elution in a fixed bed is considered to be the same as in the case of selective

elution in a finite bath (presented and discussed above), and thus, the interactions between A, E, and L shown in expressions (60) and (61) are considered for the elution of a single monovalent adsorbate in a fixed bed of porous adsorbent particles. The continuity equations for component 1 (A), component 2 (E), and component 3 (AE) in the flowing fluid stream of the bed are as follows:

$$\frac{\partial C_{d1}}{\partial t_e} - D_{L1}\frac{\partial^2 C_{d1}}{\partial x^2} + \frac{V_f}{\epsilon}\frac{\partial C_{d1}}{\partial x} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{f1}\left(C_{p1}\Big|_{r=r_o} - C_{d1}\right) - (k_3 C_{d1} C_{d2} - k_4 C_{d3}) \tag{89}$$

$$\frac{\partial C_{d2}}{\partial t_e} - D_{L2}\frac{\partial^2 C_{d2}}{\partial x^2} + \frac{V_f}{\epsilon}\frac{\partial C_{d2}}{\partial x} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{f2}\left(C_{p2}\Big|_{r=r_o} - C_{d2}\right) - (k_3 C_{d1} C_{d2} - k_4 C_{d3}) \tag{90}$$

$$\frac{\partial C_{d3}}{\partial t_e} - D_{L3}\frac{\partial^2 C_{d3}}{\partial x^2} + \frac{V_f}{\epsilon}\frac{\partial C_{d3}}{\partial x} = \left(\frac{1-\epsilon}{\epsilon}\right)\left(\frac{\alpha+1}{r_o}\right) K_{f3}\left(C_{p3}\Big|_{r=r_o} - C_{d3}\right) + (k_3 C_{d1} C_{d2} - k_4 C_{d3}) \tag{91}$$

The initial and boundary conditions for equations (89)-(91) are given by

$$C_{d1} = \chi_1(x) \quad \text{at} \quad t_e = 0\,, \quad 0 \le x \le L \tag{92}$$

$$C_{di} = 0 \quad \text{at} \quad t_e = 0\,, \quad 0 \le x \le L, \quad i = 2,3 \tag{93}$$

$$\frac{V_f}{\epsilon} C_{di} - D_{Li}\frac{\partial C_{di}}{\partial x} = 0 \quad \text{at } x = 0,\ t_e > 0,\ i = 1,3 \tag{94}$$

$$\frac{V_f}{\epsilon} C_{d2} - D_{L2}\frac{\partial C_{d2}}{\partial x} = \frac{V_f}{\epsilon} C_{d2,in} \quad \text{at } x = 0,\ t_e > 0 \tag{95}$$

$$\frac{\partial C_{di}}{\partial x} = 0 \quad \text{at} \quad x = L,\ t_e > 0,\ i = 1,2,3 \tag{96}$$

The function $\chi_1(x)$ provides the concentration profile of the adsorbate in the flowing fluid stream at the end of the wash stage. In systems where the contribution of the axial dispersion term can be neglected,[11,23] equations (94) and (95) become (with $D_{Li} = 0$, $i = 1,2,3$)

$$C_{di} = 0 \quad \text{at} \quad x = 0, \quad t_e > 0, \quad i = 1,3 \tag{97}$$

$$C_{d2} = C_{d2,in} \quad \text{at } x = 0, \quad t_e > 0 \tag{98}$$

The differential mass balance equations for components 1, 2, and 3 within the porous adsorbent particles of the bed and their initial and boundary conditions have the same form as in the finite bath system (equations (68)-(71), (73), and (75-(76)). The initial conditions for C_{p1} and C_{AL} in a fixed bed system, are given by

$$C_{p1} = \kappa_3(x,r) \quad \text{at} \quad t_e = 0, \quad 0 \le x \le L, \quad 0 \le r \le r_o \tag{99}$$

$$C_{AL} = \kappa_4(x,r) \quad \text{at} \quad t_e = 0, \quad 0 \le x \le L, \quad 0 \le r \le r_o \tag{100}$$

where the functions $\kappa_3(x,r)$ and $\kappa_4(x,r)$ provide the concentration profiles within the porous particles along the length of the bed at the end of the wash stage for species A (adsorbate) and AL (adsorbate-ligand complex).

Arve and Liapis[21] studied the dynamic behavior of the elution stage in a fixed bed of porous adsorbent particles, when a selective eluent (E) facilitates the elution of an adsorbate (A) from the adsorbate-ligand complex (AL). They found that the dynamic behavior of the adsorbate-eluent complex in the effluent stream during selective elution, is similar to that of the desorbed adsorbate during non-selective elution. However, the amount of tailing and peak spreading is larger in selective elution, and this is due to the finite rate of formation of the adsorbate-eluent complex and the low values of the diffusional coefficients associated with the mass transfer of the selective eluent and the adsorbate-eluent complex. It was also shown[21] that the duration of the elution stage for a given bed length is

shorter when the direction of flow is opposite (as compared to being the same) in the elution stage than that employed in the adsorption and wash stages. The advantage obtained by using a reversed flow in the elution stage increases as the bed length decreases.

When nonporous particles are employed in the fixed bed, there is no intraparticle diffusion for components A, E, and AE. In equation (71), C_{p1} is replaced by C_{dp1} which represents the concentration of component 1 in the liquid layer adjacent to the surface of the nonporous particle; the initial condition of the resulting equation is given by

$$C_{AL} = \omega(x) \quad \text{at} \quad t_e = 0, \quad 0 \leq x \leq L \tag{101}$$

where $\omega(x)$ represents the concentration profile of the adsorbate-ligand complex (AL) along the length of the bed at the end of the wash stage. The continuity equations for components 1, 2, and 3 in the flowing fluid stream, are obtained from equations (89)-(91) by replacing $C_{p1}\Big|_{r=r_o}$, $C_{p2}\Big|_{r=r_o}$, and $C_{p3}\Big|_{r=r_o}$ with C_{dp1}, C_{dp2}, and C_{dp3} which represent the concentrations of components 1, 2, and 3 in the liquid layer adjacent to the surface of the nonporous adsorbent particles; appropriate expressions for C_{dp1}, C_{dp2}, and C_{dp3} can be derived. The forms of the initial and boundary conditions given by equations (92)-(96) remain unchanged. The simultaneous solution of the above mentioned equations, would provide information about the dynamic behavior of selective elution in a fixed bed of nonporous adsorbent particles.

At this point, we have come to the end of the presentation and discussion on the modeling of the elution stage (non-selective and selective elution) in a finite bath or a fixed bed having porous or nonporous adsorbent particles. It is worth noting that the models of the intraparticle diffusion and desorption mechanisms (intrinsic mechanisms) could be used in an affinity chromatography system whose mode of operation is different (e.g., fluidized bed) than that of a finite bath or a fixed bed. It is also important to emphasize that there is a tremendous need for

systematic experimental elution studies with well defined affinity adsorption systems, that could provide dynamic and equilibrium elution data obtained in batch or column systems. The experimental elution data would be used in studies of parameter estimation, model discrimination, and optimal design of elution experiments. Such studies could be expected to elucidate the salient features of the different elution mechanisms, and provide values for the parameters of the constitutive equations of the elution models.

5. COMPUTATIONAL METHODS

For affinity chromatography batch systems having porous adsorbent particles, the method of orthogonal collocation[57,78,87] was applied[3,21-24] with respect to the space variable r of the partial differential equations that describe the mass transfer of the components in the porous adsorbent particles. The resulting ordinary differential equations were successfully integrated together with the ordinary differential equations of the material balances in the finite bath,[3,22] by using either a third-order semiimplicit Runge-Kutta method,[57,78,88] or by Gear's method.[57,61] Jacobi orthogonal polynomials,[57] $P_N^{(o,o)}(r)$, were used[3,22,78] and it was found that an approximation order N=8 proved to be sufficient in obtaining solute concentrations differing only in the fourth decimal place when compared with those obtained by higher approximations.[61,78] For fixed bed systems having porous adsorbent particles and non-zero axial dispersion ($D_{Li} \neq 0$, i=1,2,..,n), the method of orthogonal collocation was applied[21,23,61,78] with respect to the space variable x of the partial differential equations of the components in the flowing fluid stream, as well as with respect to the space variable r of the partial differential equations that describe the mass transfer of the components in the porous adsorbent particles. The resulting sets of ordinary differential equations were integrated by using either a third-order semiimplicit Runge-Kutta method, or by Gear's method. Jacobi orthogonal polynomials ($P_N^{(o,o)}(x)$; $P_N^{(o,o)}(r)$) were used, and it was found[78] that for short beds, reasonably good numerical results could be obtained with approximation orders (for

the polynomials used to collocate in the x and r space variables) that were lower than those required in the numerical calculations involving long columns (this was particularly the case with the approximation order of the polynomials used to collocate with respect to the x space variable). When the axial dispersion is taken to be zero ($D_{Li}=0$, i=1,2,..,n), the method of characteristics[57,61,78,89] was applied to the continuity equations of the components in the flowing fluid stream, and the method of orthogonal collocation was used with respect to the space variable r of the partial differential equations that describe the mass transfer of the components in the porous adsorbent particles. The resulting sets of ordinary differential equations were solved with the same third-order semiimplicit Runge-Kutte method, or with Gear's method, as in the case where $D_{Li}\neq 0$ (i=1,2,..,n).

Batch systems with nonporous adsorbent particles are described only by ordinary differential equations. These equations can be solved[61] by various numerical integration methods[57,90] (e.g., third-order semiimplicit Runge-Kutta; Gear's method). In affinity chromatography systems having fixed beds with nonporous adsorbent particles,[8,61] the partial differential equations of the components in the flowing fluid stream can be solved by the method of orthogonal collocation if $D_{Li}\neq 0$ (i=1,2,..,n), or by the method of characteristics if $D_{Li}=0$ (i=1,2,..n), together with an appropriate numerical integration method of ordinary differential equations; the numerical integration method of ordinary differential equations will also be used to integrate the equations that describe the differential material balances of the components in the adsorbed phase.

It is important to note that the partial differential equations encountered in affinity chromatography systems employing porous or nonporous adsorbent particles, may also be solved by using finite difference methods[57,90,91] for spatial discretization, and appropriate numerical integration (time integrator) methods (e.g., trapezoidal rule; Runge-Kutta methods; Gear's method). Therefore, it should be emphasized that numerical methods other[57,90,91] than those mentioned in the above

paragraphs, could be used to solve the partial differential equations and the ordinary differential equations encountered in the models of affinity chromatography systems.

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NOMENCLATURE

A_a - area occupied by adsorbed molecule in "form a"

A_b - area occupied by adsorbed molecule in "form b"

C_{di} - concentration of component i in the bulk fluid phase (finite bath), or in the flowing fluid stream (fixed bed)

$C_{di,in}$ - concentration of component i at $x < 0$ when $D_{Li} \neq 0$, or at $x = 0$ when $D_{Li} = 0$

C_{doi} - initial concentration of component i in bulk fluid phase

C_{don+1} - initial concentration of non-selective eluent in bulk fluid phase

C^c_{dn+1} - critical non-selective eluent concentration

C_{dpi} - concentration of component i in the liquid layer adjacent to the surface of a nonporous adsorbent particle

C_{pi} - concentration of component i in pore fluid

C_{pn+1} - concentration of non-selective eluent in pore fluid

C^{ci}_{pn+1} - critical non-selective eluent concentration for component i

C_{si} - concentration of component i in adsorbed phase

C_T - total concentration of available (accessible) ligand

CF - concentration factor defined in equation (86)

D_{Li} - axial dispersion coefficient of component i

$\underset{\approx}{D}_p$ - effective pore diffusivity matrix

D_{pi} - effective pore diffusion coefficient of component i ($D_{pi} = D_{pii}$ of $\underset{\approx}{D}_p$)

$\underset{\approx}{D}_s$ - surface diffusivity matrix

D_{si} - surface diffusion coefficient of component i ($D_{si} = D_{sii}$ of $\underset{\approx}{D}_s$)

D_{pij} - terms of the effective pore diffusivity matrix $\underset{\approx}{D}_p$

D_{sij} - terms of the surface diffusivity matrix $\underset{\approx}{D}_s$

K_{fi} - film mass transfer coefficient of component i

K_{fn+1} - film mass transfer coefficient of non-selective eluent

L - length of fixed bed

Po_{el}^{ir} - parameter defined in equation (87)

r - radial distance in adsorbent particle

r_o - radius of adsorbent particle

R - universal gas constant

t - time in adsorption stage

t_w - time in wash stage

t_e - time in elution stage

T - temperature

t_e - total time duration of the elution stage

V_f - superficial fluid velocity

x - axial distance

Greek Symbols

α - form factor; 0, 1, and 2 for slab, cylinder, and sphere, respectively

ϵ - void fraction in finite bath, or fixed bed

ϵ_p - void fraction in porous adsorbent particles

Subscripts

i - integer

j - integer

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