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# Analysis and Simulation of Batch Affinity Processes Applied to Separation of Biomolecules

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**Abstract:** The scale-up and optimization of large-scale affinity chromatographic operations is of major industrial importance. In this work, a transport model which includes pore diffusion, external film resistance, and finite kinetic rate, was used to mathematically describe the performance of a batch affinity adsorption system. Experimental data from literature describing the adsorption of β-galactosidase onto anti-β-galactosidase immobilized on porous silica was used as a model system. The mathematical model was solved using the numerical method of lines (MOL) in a MATLAB platform. The use of the transport model is a unique way to predict batch affinity performance as well as to obtain a better understanding of the fundamental mechanisms involved in the bioseparations.
**Keywords**: Mathematical modeling; batch affinity chromatography; biomolecules.

**Resumen:** El escalamiento y optimización de las operaciones cromatográficas de afinidad presentan un gran interés industrial. En este trabajo se utilizó un modelo de transporte que incluye la difusión en el poro, la resistencia en la película y una cinética de adsorción finita, para describir matemáticamente el comportamiento de un sistema de adsorción por afinidad en un tanque perfectamente agitado. Como sistema modelo se utilizaron datos experimentales de la literatura que describen la adsorción por afinidad de β-galactosidasa en anti-β-galactosidasa inmovilizada en partículas de sílice porosa. El modelo matemático fue resuelto utilizando el método numérico de líneas (MOL) utilizando una plataforma MATLAB. El uso del modelo de transporte es una forma única para predecir el comportamiento de la adsorción por afinidad, así como para lograr un mejor entendimiento de los mecanismos fundamentales de las bioseparaciones.
**Palabras clave**: Modelación matemática; cromatografía de afinidad por lotes; biomoléculas

## Introduction

Affinity chromatography is nowadays an industrial standard method used to purify high value proteins present at very low concentrations in complex biological fluids such as liquid culture media and sera [1-4]. Recently, affinity separations have been considered for large-scale separations of plasmid DNA for gene therapy and DNA vaccine applications [5]. In this chromatographic mode an affinity solid matrix is prepared by immobilizing a ligand that interact specifically with the solute of interest. Among the molecules used as biospecific or pseudospecific ligands are proteins, DNA, antibodies, amino acids, inhibitors, cofactors, dyes and quelated metal ions [6-10].

The conventional operating format for affinity separations is a packed column of porous adsorbent. However, in some cases it may be convenient to perform the adsorption step in a batch mode using well-stirred tanks, for example, when crude extracts are purified, to avoid column clogging problems [1].

The scale-up and optimization of affinity chromatographic operations is of major industrial importance [11-14]. The development of mathematical models to describe affinity chromatographic processes, is an engineering approach that can help to successfully attain these bioprocess engineering tasks [12,15]. A thorough understanding of the fundamental mechanisms underlying such separations is needed in order to develop realistic models based on basic physical and chemical principles. The equations obtained through this approach generally involve non-linear partial differential equations (PDE) that are not amenable of analytical solutions. Computer programs need to be developed to solve these models.

Several efforts have been made to model and simulate batch affinity adsorption. Chase [16] used a simplified model known as the lumped parameters model to describe biospecific separations. Arnold *et al.* [1] derived a transport model assuming that pore diffusion was the rate controlling step. Arve and Liapis [17] considered that the adsorption of a solute involves three discrete steps which contribute resistance to the mass-transfer: film diffusion, pore diffusion and reaction kinetics. Hortsmann and Chase [18] used a two resistances model with an infinity fast reaction supposition. Numerical solution of the governing differential equations was carried out by a finite difference method using second order approximations in the space derivative. However, the method is impracticable to solve more complex systems. The use of advanced numerical methods is still required to solve these models.

There are two main strategies for the solution of adsorption PDE: global methods and methods of lines. Both time and spatial derivatives are discretized in the so called global methods. The numerical method of lines (MOL) evolved recently and is tied to the development of robust algorithms for the solution of initial value ordinary differential equation, namely the stiff ones [19-21]. The solution of the partial differential equations is performed in two steps: the boundary value problem is solved using a discretization technique, and then the remaining initial value problem is handled with an appropriate integrator. Although there are several software packages that use this methodology to solve two-dimensional problems, is not usually easy to cast the adsorption equations into these packages, since adsorption description is not given by truly two-dimensional but rather by two region model [22].

In this work a transport model that considers three consecutive transport rate resistances to ideal equilibrium separation: external film resistance, particle internal diffusion and finite kinetic rate, is used for simulation of batch affinity adsorption. The solution of the model was obtained using the numerical method of lines and the MATLAB platform. The solution was compared with the analytic solution of the lumped parameters batch affinity model and [16, 23, 24], with experimental data from the literature of the batch adsorption of β-galactosidase to anti-β-galactosidase immobilized onto porous silica matrix [17]. The transport model was used to perform a parametric analysis of the experimental batch system. The influence of both process parameters as well as physical parameters on the affinity process was investigated.

## Batch Affinity Adsorption Process Description

Much of the information needed to evaluate affinity adsorption performance is contained in the typical plots of solute concentration versus time. These curves can be used to determine: 1) how much of the adsorbent capacity has been utilized, 2) how much solute is lost in an operation cycle, and 3) the processing time. A mathematical model which can be used to accurately predict this dynamic behavior provides a practical way to obviate many experiments in the design and scale-up of an affinity process.

### Transport model

In this study the batch affinity model is based on the isothermal sorption of a single solute to spherical adsorbent particles with an average radius, $r_m$, and a porosity, $\varepsilon_i$. The operation is conducted in a well-stirred tank with a total system volume, *Vs*. The liquid volume external to the adsorbent matrix is, $V = \varepsilon_b Vs$, and the adsorbent volume, $v = (1 - \varepsilon_b)Vs$. The initial and the transient solute concentration in the liquid are, $c_o$ and *c(t)*, respectively. The solute concentrations in the fluid and solid phase of the adsorbent pores are $c_i$ and $q_i$, respectively.

To achieve a mathematical description of a batch affinity chromatographic process, three consecutive transport rate resistances to ideal equilibrium separation are incorporated: film mass-transfer, porous diffusion and kinetic solute-ligand interaction. The transport of solute is considered to involve the interfacial transport of solute to the outer surface of the adsorbent particles from the bulk liquid through the adsorbent surrounding stagnant film characterized by the film coefficient, $k_f$, the diffusion in the pore fluid described by a diffusion coefficient, $D_i$, and the adsorption step of the solute with active sites on the surface of the adsorbent. The intrinsic adsorption rate can be described by different kinetic models. In this study an adsorption-desorption model of the Langmuir type is used. It is generally assumed that surface diffusion can be neglected in affinity adsorption processes due to the strong interaction between the ligand and the solute developed in these systems [11].

Due to the nonlinear equilibrium that characterizes affinity chromatography, adsorption behavior is best described by rate theories. This engineering approach to modeling involves the use of conservation equations, equilibrium laws at interfaces, kinetic laws of transport and adsorption and, initial and boundary conditions.

To describe the concentration change of solute with time in the bulk fluid, the following equation can be derived by a solute mass balance in that phase,

$$\frac{dc}{dt} = -\frac{3}{r_m}\left(\frac{1-\varepsilon_b}{\varepsilon_b}\right) k_f \left(c - c_i\right)\Big|_{r=r_m} \tag{1}$$

The equation to describe the change of concentration of solute in the fluid of the adsorbent pores can be obtained by a solute mass balance in the particle,

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

To describe the complex interactions between solute and affinity adsorbent simplified models are often used [1,16, 18]. In general a second-order reversible adsorption reaction is considered, where the solute is assumed to interact with the adsorbent by a monovalent interaction and characteristic constant binding energy,

$$P + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PS$$

where *P* is the solute in solution, S is the ligand adsorption site; and *PS* is the solute-ligand complex.

The rate of adsorption of this type of interaction is usually represented by the Langmuir model,

$$\frac{\partial q_i}{\partial t} = k_1 c_i \left(q_m - q_i\right) - k_{-1} q_i \tag{3}$$

where $q_m$ is the maximum solute concentration in the affinity adsorbent, and $k_1$ and $k_{-1}$ are the specific kinetic constants of adsorption and desorption.

At the beginning of the operation the protein concentration in the solution is $c_o$ and there is no solute present in the adsorbent, therefore the following initial conditions are used:

$$\text{at t} = 0, \qquad c = c_o \tag{4}$$

$$\text{at t} = 0, \qquad c_i = 0, \qquad 0 \le r < r_m \tag{5}$$

$$\text{at t} = 0, \qquad q_i = 0, \qquad 0 \le r < r_m \tag{6}$$

Due to particle symmetry the following boundary condition is imposed on the system:

$$\text{at } r = 0, \qquad \left.\frac{\partial c_i}{\partial r}\right|_{r=0} = 0 \qquad t > 0 \tag{7}$$

The second boundary condition can be derived by a solute mass balance at the mouth of a particle pore,

$$\text{at } r = r_m \qquad k_f (c - c_i)\big|_{r=r_m} = \varepsilon_i D_i \left.\frac{\partial c_i}{\partial r}\right|_{r=r_m} \qquad t > 0 \tag{8}$$

**Lumped parameters batch affinity model**

The lumped parameters model [16, 24] takes an empirical approach to the adsorption process and assumes that all the rate limiting processes can be represented by kinetic rate constants. In such an approach, the rate of mass-transfer of solute to the adsorbent is assumed to be described by eq. (3) above. For batch affinity adsorption in a well-stirred tank, the solute concentration in solution at time, $t$, is given by the following analytical solution of the model:

$$\frac{c}{c_0} = 1 - \left(\frac{1-\varepsilon_b}{\varepsilon_b c_0}\right)\left\{\frac{(b+a)\left[1-\exp\left(-\frac{2a(1-\varepsilon_b)}{\varepsilon_b}k_{11}t\right)\right]}{\left(\frac{b+a}{b-a}\right)-\exp\left(-\frac{2a(1-\varepsilon_b)}{\varepsilon_b}k_{11}t\right)}\right\} \tag{9}$$

where:

$$a^2 = b^2 - \left[\frac{c_0\varepsilon_b}{(1-\varepsilon_b)}\right]q_{ma} \tag{10}$$

$$b = \frac{1}{2}\left[\frac{c_o\varepsilon_b}{(1-\varepsilon_b)} + q_{ma} + \frac{K_d\varepsilon_b}{(1-\varepsilon_b)}\right] \tag{11}$$

**Input data for the study**

A fundamental requirement for the use of the mathematical models of affinity adsorption to predict the behavior, scale or design a process of interest, it is the precise estimate, in experimental form or by means of correlations, of the parameters associated to the model.

The equilibrium system parameters: maximum capacity of adsorption, $q_m$, and the dissociation equilibrium constant, $K_d = k_{-1}/k_1$, can be estimated from equilibrium data. The kinetic parameter, $k_1$, can be calculated using dynamic adsorption data obtained in batch or column systems [16,18].

The effective pore diffusivity, $D_i$, can be estimated through experimental uptake results obtained in batch or column systems, according to detailed reported procedures in the literature [4]. This parameter has also been estimated in precise form using models generated for adsorbent of porous structure obtained by means of the pore network theory. This theory properly represents the discreet nature of the porous structures of chromatographic media and also properly describes phenomena of restricted pore diffusion and dynamic loading effects on intraparticle convection and diffusion [25-27]. The parameters that characterize intraparticle pore diffusion have been obtained also by combining the experimental method of confocal scanning laser microscopy with theoretical models [28-33].

The molecular diffusivity of β-galactosidase in free aqueous solution, $D_{AB}$, can be estimated by using the following semi-empirical equation [34]:

$$D_{AB} = 9.4\times10^{-15}\frac{T}{\mu\left(M_A\right)^{\frac{1}{3}}} \tag{12}$$

Where $M_A$ is the relative molecular weight of substance A. Taking 464,000 g/mol as the relative molecular weight of β-galactosidase, the absolute temperature T = 298 ºK and the liquid viscosity $\mu = 1 \times 10^{-3}$ Kg/m –s, the value of molecular diffusion coefficients is $D_{AB} = 3.62 \times 10^{-11}$ m$^2$/s. The effective pore diffusivity is given by the ratio of the molecular diffusivity to the tortuosity coefficient. According to Arve and Liapis [17] the tortuosity of the experimental system is 5.23 then $D_i = 6.9 \times 10^{-12}$ m$^2$/s.

The correlation used to estimate the liquid film mass transfer coefficient, $k_f$, of the protein to the adsorbent particles in stirred tank experiments is given by [35]:

$$k_f = \frac{2D_{AB}}{d_P} + 0.31\left(\frac{\mu}{\rho_C D_{AB}}\right)^{-\frac{2}{3}}\left(\frac{\Delta\rho\,\mu\,g}{\rho_C^2}\right)^{\frac{1}{3}} \tag{13}$$

Where $p_c$ = 1000 Kg/m$^3$ is the liquid density, Δρ = 402 Kg/m$^3$ is the density difference between the adsorbent particle and the liquid and $g$ = 9.8 m/s$^2$ is the gravity acceleration. For a particle diameter $dp = 150 \times 10^{-6}$m, the value of the mass-transfer coefficient is $k_f = 5.84 \times 10^{-6}$ m/s.

When the values of the parameters that characterize the mass transfer and the mechanisms of adsorption of the solute on the affinity matrix have been determined, the mathematical model can be used to predict and study the dynamic behavior and performance of the affinity system for different design and operating conditions.

Experimental data from the literature for the adsorption of β-galactosidase onto anti-β-galactosidase immobilized on porous silica matrix was chosen as model system [17]. The values of the parameters utilized to conduct the simulation studies are presented in Table I.

**Numerical solution of the transport model**

The transport model to describe the batch affinity adsorption of proteins in a perfectly stirred tank is given by the eqs. (1-8). This model can only be solved by advanced numerical techniques. In this study the solution of the model was obtained

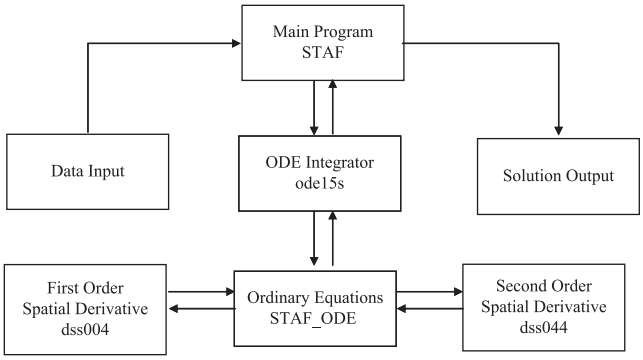


**Fig. 1**. Numerical method of lines solution (MOL) scheme of the batch affinity transport model in a MATLAB platform.

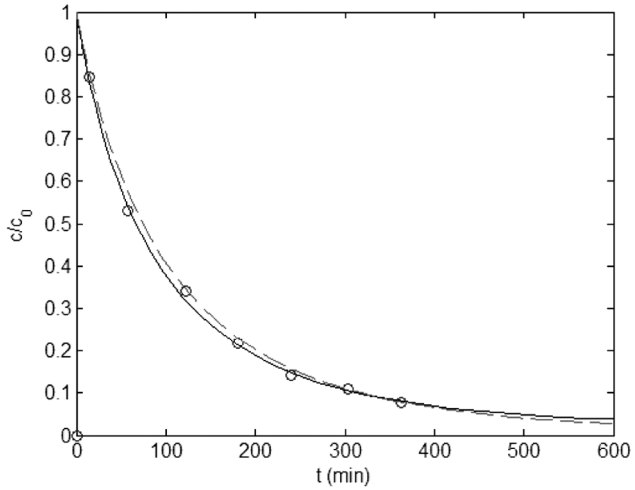


**Fig. 2**. Batch affinity adsorption of β-galactosidase onto anti-β-galactosidase immobilized on porous silica. Operating conditions according to Table 1. (o) Experimental data [17]. (—) MOL solution of the bach affinity transport mode in a MATLAB platform. (- - -) Lumped parameters model solution.

using the numeric method of lines MOL in a MATLAB (v.7.1) platform [36], according to the block diagram shown in Figure 1.

The code STAF was used as the main program. The system parameters, the MATLAB integrator ode15s [37-39] and the printing instructions are incorporated in the main program. The integrator ode 15s (which implements back difference formulas) computes the solution over each time interval. This integrator will, in general, call the function STAF_ODE many times during the calculation of the solution by numerical integration.

The system of ordinary differential equations which approximates the PDEs of the model is programmed in subroutine STAF_ODE. This function calls the functions dss004 and dss044 to calculate the first and second spatial derivatives $\partial c_f/\partial r$ and $\partial^2 c_f/\partial r^2$, using five-point, fourth order finite difference approximations. All the codes were incorporated in MATLAB programs that were run in a PC.

## Results and Discussion

The solution to the transport model for batch affinity adsorption of β-alactosidase onto anti-β-galactosidase immobilized on porous silica was solved using the numerical method of lines (MOL) in a MATLAB platform. This solution was compared with the analytical solution of the lumped parameters batch affinity model and, with the experimental data. The results are shown in Figure 2.

The simulated run using MOL fitted well to the experimental data when the base parameter values were used, except for the kinetic parameter that was set to the relatively high value of $k_1$= 0.0235 mL/mg-s, since in the model this is not a lumped parameter. This result suggests that the mass-transfer process is only controlled by pore diffusion and film resistance.

The simulation run with the analytic solution also fitted well to the experimental data using a kinetic parameter value of $k_{11}$= 0.00022 mL/mg-s. It is well known that lumped parameters approach is more useful when mass-transfer resistances are low. In behalf of these results the MOL approach was used to further conduct the analysis.

The MOL solution of the transport model was employed to describe in more detailed form the affinity chromatography process, e.g. detailing the protein dimensionless concentration profiles in the adsorbent-pore liquid, $c_f/c_0$ , and in the adsorbed phase, $q_f/q_m$ , as function of the dimensionless radio length, $R$, and the real time, $t$ (Fig. 3).

A very sharp protein-concentration profile in the adsorbent-pore liquid is obtained initially (Fig. 3a), suggesting a considerable mass transfer resistance within the adsorbent pores. As the adsorption proceeds the concentrations profiles become less sharp until a very low equilibrium profile is reached after 400 min approximately. The protein concentration profile in the adsorbed phase (Fig. 3b) shows a rapid increase from an initial zero value to an equilibrium value. This profile is more uniform than adsorbent-pore liquid profile and reaches higher values, due the favorable Langmuir equilibrium of the system. Most of the adsorption phenomena is carry out on the external volume of the particle ($R > 0.5$) owing the high mass-transfer resistance within the adsorbent pores.

In order to study how the transport model solution is able to account for variations of operating characteristics with system parameters, a parametric analysis was performed by overlaying batch affinity curves from several computer simulations, in which one parameter was changed while the others were kept constant at the basic set of values shown in Table 1.

The effect of bead diameter and initial protein concentration is reported. The process parameter of most interest is the bead diameter. The bead diameter was changed using ±40 and ±20% variations of the $d_p$ = 150 μm base value. The corresponding curves are shown in Figure 4. A sharper curve and

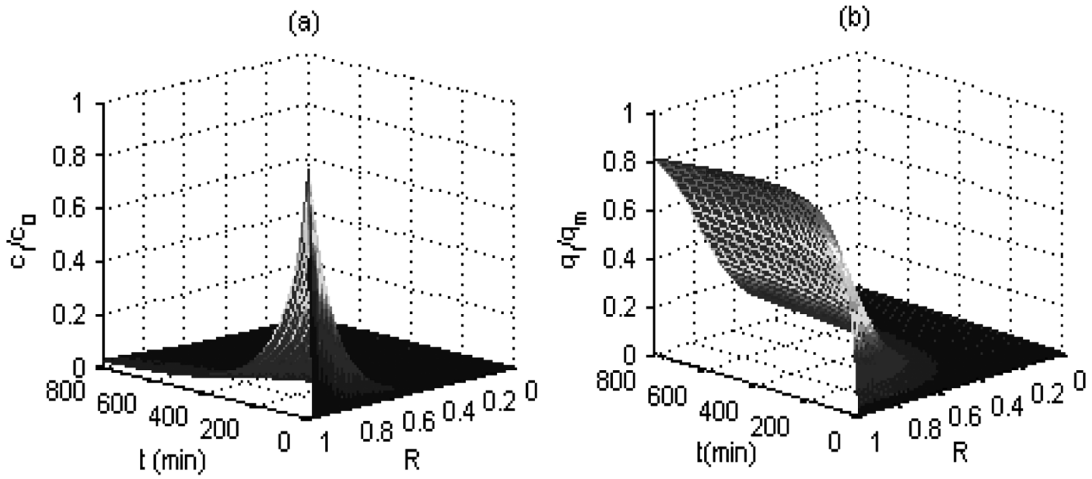


**Fig. 3.** Concentration profiles of β-galactosidase. Operating conditions according to Table 1. (a) Protein dimensionless concentration profiles in the adsorbent-pore liquid. (b) Protein dimensionless concentration profiles in the adsorbed phase.

consequently greater process productivity is obtained as the bead diameter decreases (Fig. 4a). The adsorption rate increases markedly, since the diffusion time is decreased due to the shorter diffusion path. At the same time the area/volume ratio for a single particle ($3/r_m$) increases, giving an increased mass transfer area between the surrounding liquid phase and the bead. Both factors contribute to the increase in total adsorption rate. A shorter diffusion path also produces less pronounced protein concentration profiles in the adsorbed phase (Fig. 4b) and practically no effect on the protein-concentration profile in the adsorbent-pore liquid (Fig. 4c and Fig. 4d).

Upstream perturbations can initiate changes in process concentrations that are important for study. The initial protein concentration was changed using ±40 and ±20% variations of the $c_o$ = 0.0158 mg/mL base value. The corresponding curves are shown in Figure 5. A greater proportional degree of adsorption at equilibrium is achieved at lower protein initial concentration as can be seen in the horizontal portion of the curves of the Figure 5a. It must be noted that protein concentration is normalized. When plotted on this basis, the initial rates of adsorption are slightly affected by protein concentration. However, if absolute amounts of adsorption are being considered, greater degrees and rates of adsorption occur at higher concentrations.

The protein concentration in the adsorbed phase (Fig. 5b) and the protein-concentration the adsorbent-pore liquid (Fig. 5c and Fig. 5d) show this behavior in a more detailed way. The utilization of the adsorption capacity of the matrix is greater at higher solute concentration as these conditions favor a greater extent of adsorption at equilibrium.

**Table 1.** Base case data used in simulation studies of batch affinity adsorption of β-galactosidase onto anti-β-galactosidase immobilized on porous silica [17].

| Variable | Value |
|---|---|
| Initial batch protein concentration | $c_o$ = 0.0158 mg/mL |
| Maximum adsorption capacity | $q_{ma}$ = 2.2 mg/mL |
| Particle porosity | $\varepsilon_i$ = 0.5 |
| Maximum adsorption capacity of solid gel | $q_m$ = 4.4 mg/mL |
| Equilibrium desorption constant | $K_d$ = 0.00022 mg/mL |
| Lumped adsorption kinetic constant | $k_{ll}$ = 0.0055 ml/mg-s |
| Specific adsorption rate constant | $k_l$ = 0.0235 ml/mg-s |
| Particle radius | $r_m$ = 75 μm |
| Film mass-transfer coefficient | $k_f = 5.84 \times 10^{-6}$ m/s |
| Intraparticle protein diffusivity | $D_i = 6.9 \times 10^{-12}$ m²/s |
| Initial batch liquid volume | $V$ = 100 mL |
| Initial adsorbent volume | $v$ = 1.5 mL |
| Ratio of liquid volume to batch system volume | $\varepsilon_b$ = 100/101.5 |

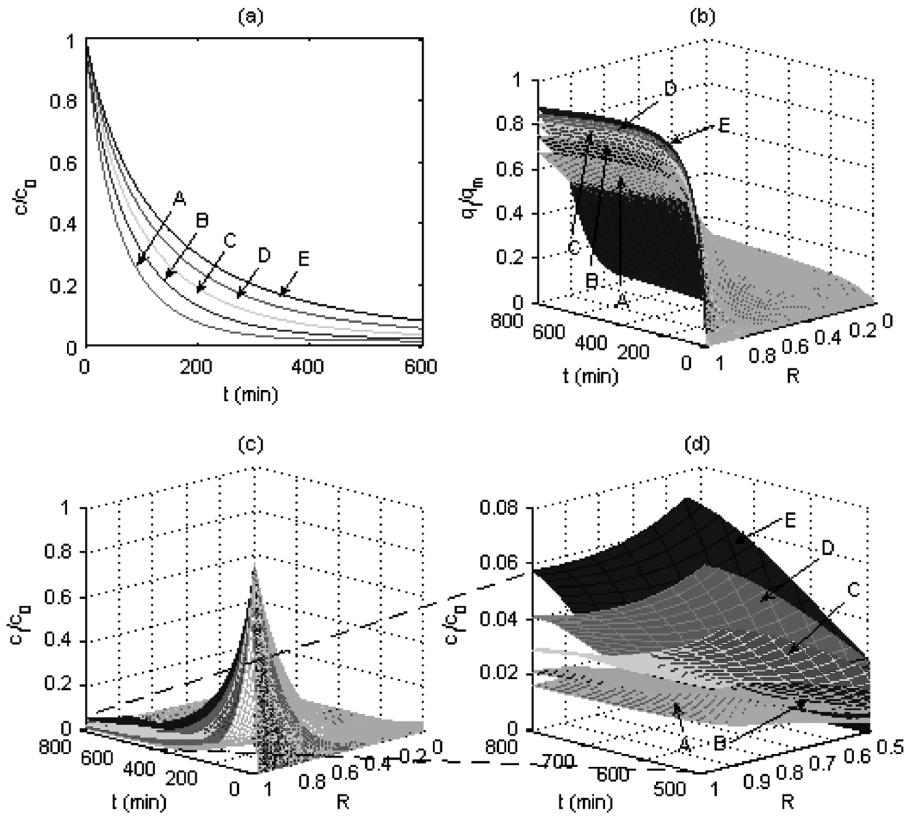


**Fig. 4.** Influence of the bead diameter on batch affinity. Operating conditions according to Table 1. (a) Bulk dimensionless protein concentration. (b) Protein dimensionless concentration profiles in the adsorbed phase. (c) Protein dimensionless concentration profiles in the adsorbent-pore liquid. (d) Zoom area of protein dimensionless concentration profiles in the adsorbent-pore liquid. (A) -40%, (B) -20%, (C) $d_p$ = 150 μm, (D) + 20%, (E) + 40%.

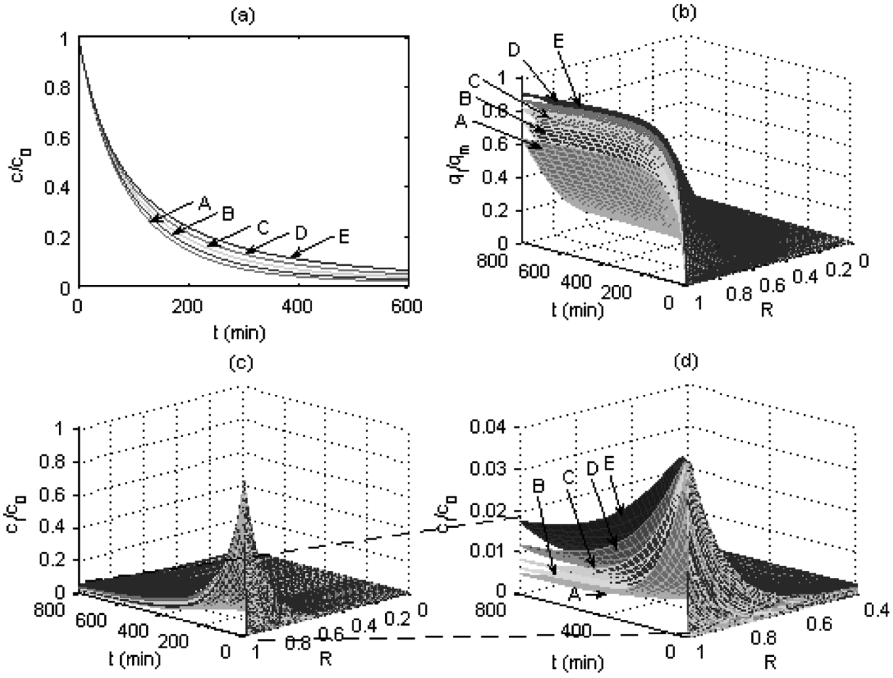


**Fig. 5.** Influence of the inicial protein concentration on the batch affinity behavior. Operating conditions according to Table 1. (a) Bulk dimensionless protein concentration. (b) Protein dimensionless concentration profiles in the adsorbed phase. (c) Protein dimensionless concentration profiles in the adsorbent-pore liquid. (d) Zoom area of protein dimensionless concentration profiles in the adsorbent-pore liquid. (A) -40%, (B) -20%, (C) $c_o$ = 0.0158 mg/mL, (D) + 20%, (E) + 40%.

## Conclusions

The performance of the adsorption of β-galactosidase onto anti-β-galactosidase immobilized on porous silica, in a batch affinity chromatography, was successfully described with a three-resistances model. Programming the model solution was relatively simple using MOL in a MATLAB platform. This solution was compared with the analytical solution of the lumped parameters model. In the simulation studies, the best fitting of the experimental data was obtained with the numerical method of lines solution. The parametric analysis conducted helps to show the influence of both operation and system parameters on the affinity process. In the bead-size parametric study, a sharper adsorption curve and consequently a greater operating throughput was obtained as the bead diameter decreases. The initial concentration study showed that greater degrees and rates of adsorption occur at higher concentrations. The dynamic responses obtained are in concordance with theoretical predictions and show that the transport model can be used as a framework to provide a general description of almost all practical systems, when the appropriate basic experimental parameters and numerical solution are used. The MOL solution of the transport model permitted an accurate prediction of the batch affinity performance and a better understanding of the fundamental mechanisms responsible for the separation. A similar analysis for multicomponent systems should be done to extend the model to more practical situations, since other components in a complex mixture may influence the adsorption properties of the protein.

## Symbols

$c_o$ initial protein concentration, mg/mL
$c$ protein concentration in the bulk liquid, mg/mL
$c_i$ protein concentration in the fluid of the adsorbent pores, mg/mL
$d_P$ adsorbent particle diameter, m
$D_i$ intraparticle protein diffusivity (free molecular diffusivity/pore tortuosity), m²/s
$k_1$ specific adsorption rate constant, mL/mg.s
$k_{11}$ lumped adsorption rate constant, mL/mg.s
$k_{-1}$ specific desorption rate constant, $s^{-1}$
$k_f$ external film mass-transfer coefficient, m/s
$K_d$ equilibrium desorption constant, $k_{-1}/k_1$
$P$ protein molecule
$PS$ protein-active site complex
$q_i$ protein concentration in the adsorbed phase of the adsorbent particles, mg/mL
$q_m$ maximum equilibrium concentration, mg/mL of solid volume of adsorbent
$q_{ma}$ maximum equilibrium concentration, mg/mL of settled volume of adsorbent
$r$ radial distance in the adsorbent particle, m
$r_m$ radius of adsorbent particle, μm
$R$ dimensional radius
$S$ active site
$t$ time, s
$V_S$ batch system volume, mL
$V$ liquid volume, mL
$v$ adsorbent volumen, mL

### Greek letters

$\varepsilon_b$ ratio of liquid external to adsorbent to system volume
$\varepsilon_i$ pore void fraction in the adsorbent particle

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