ADSORPTION KINETIC AND MASS TRANSFER IN AFFINITY CHROMATOGRAPHY

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INTRODUCTION

Affinity chromatography is a powerful technique for the purification of biological macromolecules such a proteins and is a good example of a highly selective separation method[1]. Purification operation includes adsorption, washing, elution and regeneration steps. In the adsorption stage of the process, liquid containing the compound to be purified is then contacted with the adsorbent and provided that adsorption is sufficiently specific, then only the required protein will be adsorbed.

The optimization and scale-up of such affinity separation procedures requires that the equilibrium and mass transfer characteristics are fully understood[2].

A number of discrete steps involve the adsorption of a protein from the bulk solution to a particle of adsorbent. These steps, all of which contribute resistance to mass transfer, include transfer from bulk liquid to the outer surface of the particle (film diffusion resistance), movement by diffusion into the pores of the particle (pore diffusion) and the actual chemical interaction at the binding site (surface reaction resistance) [2].

The goal of this work is to know the concentration inside pores, which can be different of the solution if there are mass transfer resistances and, hence it is possible that the adsorption equilibrium can be changed.

A modified model, from the theory developed by Mao et al[3], has been used to describe the adsorption behavior of proteins in a finite bath. In this model, adsorption process together with external and internal mass transfer have been considered.

The mass transfer rate of the adsorbate from the bulk fluid to the internal particle surface can be expressed as two steps process. First the adsorbate diffuses through the boundary layer and after diffuses through the pore fluid, which is stagnat, and finally is adsorbed or interacted on the surface.

Both mass transfer processes may be described by a linear force approximation, and so as the overall mass transfer process. Then the mass transfer rate is given by:

$$N = K_f(c - c^*) = K_i(c^* - c_i) = K_e(c - c_i) \quad (1)$$

where N is the mass flux of the adsorbate into the particle, K_f is the liquid film mass transfer coefficient, K_i is the apparent pore fluid mass transfer coefficient, and K_e is the overall effective liquid phase mass transfer coefficient. c^* is the intermediate concentration of the adsorbate in the liquid phase at the external surface of the particles, and c_i is the intermediate concentration of the adsorbate in the liquid phase at the internal surface of the particles.

The rate of change in the concentration of the adsorbate in the solid phase then must be equal to the rate of mass transfer, hence:

$$\frac{dq}{dt} = aK_f(c - c^*) = aK_i(c^* - c_i) \quad (2)$$

where the term $a=(3/R_0)$ is the external surface area per unit volume of the adsorbent particles and R_0 is the radius of the particle.

The of rate of change of adsorbate concentration can be written:

$$\frac{dq}{dt} = aK_e(c - c_i) \quad (3)$$

and

$$\frac{1}{K_e} = \frac{1}{K_f} + \frac{1}{K_i} \quad (4)$$

Equation 10 clearly shows that the overall resistance to the mass transfer in the sum of the resistance in the liquid film and the resistance in the pore fluid.

Surface interaction. The interaction between the adsorbate and the immobilized ligand at the internal particle surface can be described by the second-order reversible equation:

$$\frac{dq}{dt} = k_1\left[(q_m - q)c_i - K_d'q\right] \quad (5)$$

Eliminating c_i, c, and its derivate, the following form of the concentration equation can be written as:

$$-\left(\frac{1}{M} + \frac{1}{k_1}\right)\frac{dc}{dt} = (c - x_1)(c - x_2) \quad (6)$$

where

$$M = \frac{A}{R_v q_m - c_t + c} \quad (7) \text{ and } A = aK_eR_v \quad (8)$$

x_1 and x_2 are the roots of quadratic equation:

$$c^2 - Bc - K_d'c_t = 0 \quad (9)$$

where

$$B=c_t - R_vc_{sm} - K'_d \quad (10)$$

At the present case due to the matrix is constituted by porous spheres of Sepharose 4B with an average diameter of 90 μm, a specific surface of $5m^2/ml$, from wich only 8 cm^2 correspond to the external surface, therefore there is the possibility that some part of enzyme is retained into the matrix and not really adsorbed, with which the adsorption equilibrium, K'_d in equation 5, must be related to the free enzyme inside of spheres, which can be different to the solution concentration if there is internal or external mass transfer resistence. This effect can be studied through the partition coefficient, K_P, which can be calculated by using the model developed by Taylor and Swaisgood4

The ***partition coefficient*** is defined as the ratio between the concentration of enzyme inside the pores, phase α inside of pores, and the concentration of enzyme in solution, phase β outside of pores. Accordingly reversible adsorption equilibrium is

established between the enzyme adsorbed and that not adsorbed in the phase α, and is described by:

$$E^{\alpha} + L \rightleftharpoons EL \quad (11)$$

The equilibrium dissociation constant K_d' was defined:

$$K'_d = \frac{c_e^{\alpha} c_l}{c_{el}^{\alpha}} \quad (12)$$

Taking into account that

$$c_{tl} = c_l + c_{el} \quad (13)$$

$$c_{te}^{\alpha} = c_{el}^{\alpha} + c_e^{\alpha} \quad (14)$$

$$K_P = \frac{c_e^{\alpha}}{c_e^{\beta}} \quad (15)$$

By substituting into equation 12 and making some rearrangements, the following equation is obtained:

$$\frac{c_{te}^{\alpha}}{c_e^{\beta}} - K_P = \frac{c_{tl}}{K'_d + K_P c_e^{\beta}} \quad (16)$$

RESULTS

Several experiments were performed to obtain values of partition coefficients and dessorption equilibrium constants inside particles, for Asparaginase in Sepharose 4B activated with CNBr, hexamethylenediamine and L(+)chlorosuccinamic acid, for each pH value, (7.5; 8.0; 8.6) and temperature (298; 300 and 302K) and are given in Table I.

Table I
Partition Coefficients and Desorption Equilibrium Constants inside particles.

	pH=7.5			pH=8.0			pH=8.6		
T(K)	***298***	***300***	***302***	***298***	***300***	***302***	***298***	***300***	***302***
K_P	0.448	0.416	0.351	0.469	0.454	0.399	1.39	1.15	0.759
K'_d (kg/m^3)	0.243	0.270	0.340	0.161	0.190	0.250	0.139	0.154	0.201

From this table can be observed that, when pH was increased the partition coefficient, K_P, increased due to that the amount of enzyme retained inside of particle increases.

In other hand, when temperature increased, desorption also increased and hence K'_d increases, and the amount of enzyme in solution, c_e^{β}, also increased, decresing the value of K_P.

The values of K'_d determined previously were introduced in the model, eq(5) and together with eq.(6) were used to determine the kinetic and mass transfer coefficients.

The predicted concentration-time profiles were compared with experimental data and the result are show in Figure 1 (a, b, c). In these figures, the points are experimental data and the lines are the model prediction.

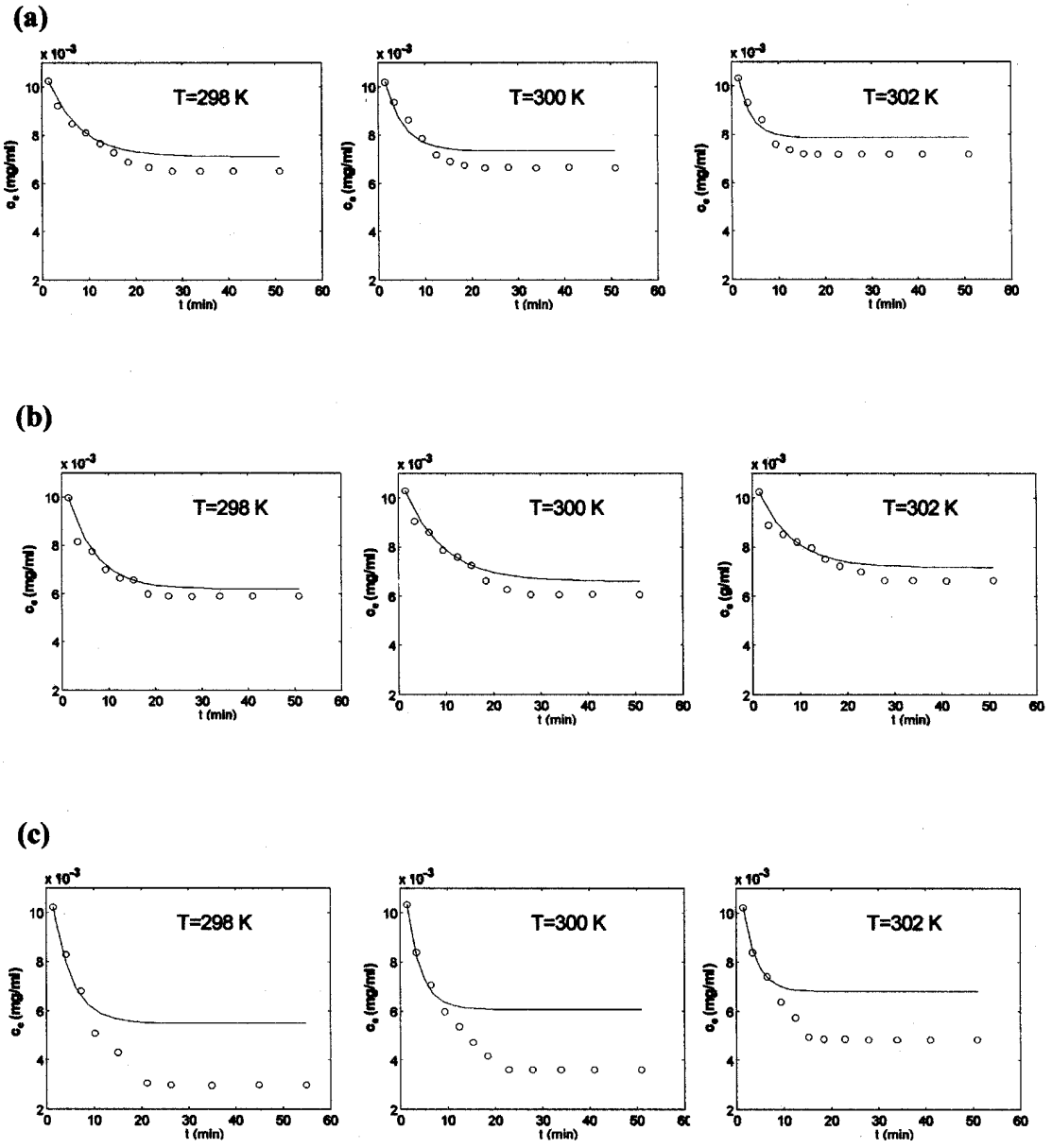

Figure 1. Theoretical and Experimental Concentration Curves for the Adsorption of Asparaginase to activated Sepharose 4B-hexamethylenediamine-L(+)chlorosuccinamic acid, I=0.05M NaCl ,(a) pH=7.5, (b)pH=8.0, (c)pH=8.6.

This profiles were correlated by a computer programme (MATLAB) which evaluates K_i and k_1 according to eq.6, and are given in TableII.

Table II Forward Surface Interaction Rate Constants, Apparent Pore Fluid Mass Transfer Coefficients and Diffusion Coefficients

	pH=7.5			*pH=8.0*			*pH=8.6*		
T(K)	**298**	**300**	**302**	**298**	**300**	**302**	**298**	**300**	**302**
$K_i \cdot 10^6$ (m/min)	0.09	0.15	0.32	0.11	0.20	0.48	2.83	4.50	6.86
$k_1 \cdot 10^4$ (mL/mg s)	4.33	5.72	6.80	3.94	5.27	5.94	3.78	4.88	5.62
$D_e \cdot 10^{12}$ (m^2/s)*	0.07	0.12	0.25	0.09	0.16	0.37	2.20	3.51	4.14

From this table it can be observed that a change of pH from 7.5 to 8.6 increases diffusion coefficient due to electrostatic effects.

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NOTATION

a	external surface area per unit volume of adsorbent particles, m.
c	adsorbate concentration in the liquid phase, $mg \cdot mL^{-1}$.
c^{α}_{el}	concentration of enzyme adsorbed in α phase, $mg \cdot mL^{-1}$
c^{α}_{te}	concentration of total enzyme in α phase, $mg \cdot mL^{-1}$
c^*	intermediate adsorbate concentration in the liquid phase an external surface of particles, $mg \cdot mL^{-1}$
c_0	initial adsorbate concentration in the liquid phase, $mg \cdot mL^{-1}$
c_e^{α}	concentration of enzyme in phase α, $mg \cdot mL^{-1}$
c_e^{β}	concentration of enzyme in phase β, $mg \cdot mL^{-1}$.
c_i	intermediate adsorbate concentration in the liquid phase at internal surface of particles, $mg \cdot mL^{-1}$.
c_l	concentration of ligand, $mg \cdot mL^{-1}$.
c_t	equivalent adsorbate concentration when total amount of the adsorbate in the system was assumed in the liquid phase, $mg \cdot mL^{-1}$.
c_{tl}	concentration of total ligand attached to the particles $mg \cdot mL^{-1}$.
D_e	diffusion coefficient, $m^2 \cdot s^{-1}$.
K'_a	enzyme-ligand adsorption equilibrium constant in phase α, $mL \cdot mg^{-1}$.
K'_d	enzyme-ligand desorption equilibrium constant in phase α, $mg \cdot mL^{-1}$.
k_1	forward surface interaction rate constant, $mL \cdot mg^{-1} \cdot s^{-1}$.
K_e	overall effective liquid phase mass transfer coefficient, $m \cdot s^{-1}$.
K_f	liquid side film mass transfer coefficient, $m \cdot s^{-1}$.
K_i	apparent pore fluid mass transfer coefficient, $m \cdot s^{-1}$.
K_P	partition coefficient(dimensionless).
N	mass flux, $m \cdot mg \cdot mL^{-1} \cdot s$
q	adsorbate concentration on the solid phase, $mg \cdot mL^{-1}$
R_v	volumen ratio of solid phase to liquid phase(dimensionless).
t	time