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Modeling column regeneration effects on ion-exchange chromatography

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Abstract

The effect of in-place regeneration on equilibrium and kinetic characteristics of the adsorption of bovine serum albumin to a DEAE–cellulose anion exchanger has been determined. Regeneration with sodium hydroxide and time of exposure showed no effect on equilibrium behavior. Breakthrough curves were measured for protein adsorption on fixed-bed columns and analyzed by a simple model to determine the relevant rate constants for the adsorption process. It was found that forward adsorption rate constant decreased exponentially with the chemical treatment exposure time. The implications of the results on the design and optimization of ion-exchange chromatographic processes are discussed. © 1997 Elsevier Science B.V.

Keywords: Column regeneration; Adsorption isotherms; Kinetic studies; Computer simulation; Ion-exchange chromatography; Albumin; Proteins

1. Introduction

Ion-exchange adsorbents have found widespread use in the purification of proteins, both in the laboratory and in the production plant. This widespread use is due to their versatility, relative cheapness and their acceptance by the regulatory authorities in processes for the production of pharmaceutical proteins [1]. An important step in the implementation of such processes requires comprehensive attention to good manufacturing practices (GMP), good laboratory practices (GLP) and other guidelines specified by the regulatory agencies. To fulfill some of these regulations the clean-in-place (CIP) procedures became part of the master method for chromatographic column operations [2], in which sodium hydroxide solutions are commonly used for chromatographic system sanitation and cleaning [3]. Frequency of column cleaning will be determined partly by the quality of the feed, which in turn is dependent of the stage of the process, in the early steps the frequency of replacing media will be higher and this parameter must be defined [4].

A factor often overlooked in the development of a chromatographic process is the impact of the CIP treatments on the performance of the chromatographic columns. It has been shown that these types of treatments cause a capacity loss of the adsorbents

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[5]. Several works in predicting the performance of ion-exchange adsorbents, take into account diverse operational and system parameters [6–8], but little consideration is usually given to the extent to which chemical treatments affect either adsorption equilibrium or kinetics. It is clear that such data are necessary for reliable modeling and scale up of these adsorption processes.

This paper presents an approach to predict the performance of ion-exchange columns under reuse conditions, combining simple experiments and theory. The approach requires knowledge of certain parameters that describe the adsorption process. These parameters were measured using equilibrium and fixed-bed adsorption experiments in which bovine serum albumin (BSA) was adsorbed onto DEAE Matrex Cellofine A-200 anion-exchange gel (DEAE–cellulose) exposed over time intervals to a solution of 0.5 *M* sodium hydroxide. Equilibrium and fixed-bed adsorption studies were conducted. The effect of the chemical treatment exposure time on Langmuir isotherm parameters was determined from the resulting equilibrium data. A simple model from Arnold and Blanch was used to describe the breakthrough curve from a fixed bed of adsorbent subject to a constant protein feed [9]. Interaction rate constants for protein adsorption at each time interval of chemical treatment were estimated by comparing experimental breakthrough curves with those generated by the model. The results were used to develop the adsorbent decay model that is able to predict the performance of ion-exchange adsorbents under this type of chemical treatment.

2. Experimental

2.1. Materials

Albumin was purchased from Sigma. The adsorbent used was DEAE Matrex Cellofine A-200 anion-exchange gel (DEAE–cellulose beads) with a particle diameter between 45 to 105 μm purchased from Amicon. For void volume determinations blue dextran (M_r 2 000 000) was purchased from Sigma. All other reagents were analytical grade or higher. Protein solutions were buffered with 10 m*M* of sodium phosphate, pH 7.5. All the experiments were carried out at room temperature (25±3°C).

2.2. Adsorbent preparation and chemical treatment

Known volumes of adsorbent were prepared by allowing a suspension of the adsorbent to settle in a graduated cylinder overnight. The operating systems were prepared by adjusting the buffer volume to the settled adsorbent to obtain 50:50 (v/v) suspensions. All the adsorbent systems were subjected to chemical treatment in a chromatographic column, by exposure to solutions of 0.5 *M* sodium hydroxide for the time intervals under study, without column flow. The chemical treatment solution was loaded to the adsorbent bed to ensure that the equilibrating buffer was completely removed, and to form a 7 cm hydraulic head of solution. Once the treatment was over, the gel was washed with 10 volumes of deionized water, and re-equilibrated with a 0.1 *M* hydrochloric acid solution. The adsorbent from the column was then used for batch or fixed-bed experiments.

2.3. Adsorption isotherms

Adsorption equilibrium studies were carried out in a stirred batch system shown schematically in Fig. 1. A typical experiment consisted of the addition of 1 ml of a 50:50 (v/v) suspension of the adsorbent, to 50 ml of 10 m*M* of sodium phosphate pH 7.5 buffer in the adsorption vessel, and step-wise additions of 50 μl pulses of a 120 mg/ml BSA solution from time equal to zero. Soluble phase protein concentration in the batch system was continuously monitored by recycling the liquid phase through a 20 μm porosity net filter, and through a continuous flow Bio-Rad UV spectrophotometer, using a flow-rate of 1 ml/min. This adsorption step was carried out until no appreciable change was observed in the chart recorder connected to the spectrophotometer, this reading was taken as an equilibrium point. Successive 50 μl pulses were added to the same system to obtain higher equilibrium points, until adsorbent saturation was reached. The amount of protein adsorbed at each point was calculated by mass balances and the values used to determine the adsorption isotherm.

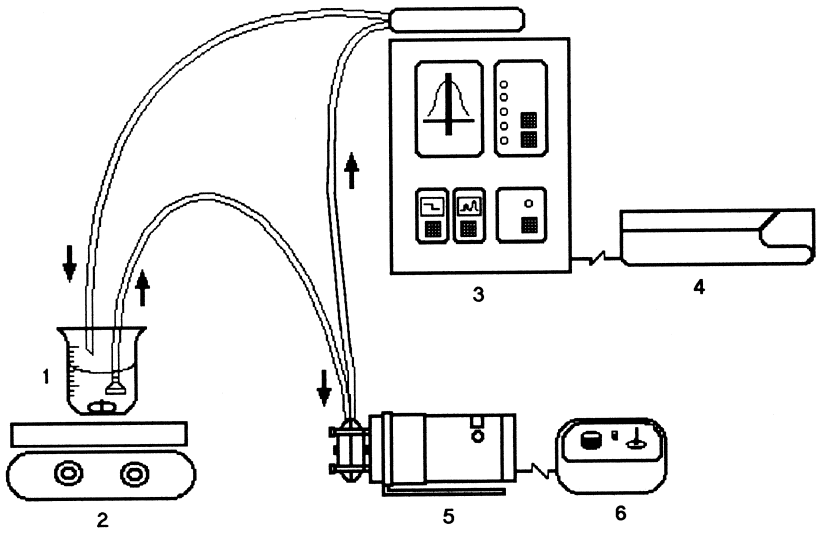

Fig. 1. Apparatus for batch stirred tank experiments. (1) Batch adsorption vessel, (2) stirred magnetic plate, (3) spectrophotometer 280 nm, (4) chart recorder, (5) peristaltic pump and (6) speed control.

At the end of each batch experiment the loaded adsorbent was placed in a chromatographic column and the adsorbed protein eluted with 0.5 *M* sodium chloride in 10 m*M* sodium phosphate pH 7.5 buffer. After the elution step, the adsorbent was washed with deionized water and immediately subjected to chemical treatment with 0.5 *M* solution of sodium hydroxide. Adsorption isotherms were determined at 0, 30, 70, 94, 122, 186 and 273 days of chemical treatment exposure time.

2.4. Fixed-bed adsorption studies

Breakthrough curves were determined by continuously loading protein to the packed bed until the protein outlet concentration c was equal to the protein inlet concentration, c_0.

All column experiments were performed with 2 ml (settled volume) of DEAE Matrex Cellofine A-200 anion-exchange gel packed in a chromatography column, 1 cm diameter. The experiments were carried out at a volumetric flow-rate of 1.0 ml/min in a Bio-Rad chromatographic Econo System. Protein was applied to the packed beds at a concentration of 1 mg/ml (c_0) and the UV absorbance at the outlet stream was recorded at 280 nm. Data was plotted as normalized concentration c/c_0, of the outlet stream against the time of operation. Time zero was taken as the point at which the adsorbate solution first entered the bed. The void volume of the bed was estimated by a pulse of blue dextran under no adsorption conditions using a 1.0 *M* sodium chloride solution.

At the end of each fixed-bed adsorption experiment the adsorbed protein was eluted with 0.5 *M* sodium chloride in 10 m*M* sodium phosphate pH 7.5 buffer. Once the protein was eluted, the packed bed was washed with deionized water. To continue the fixed-bed adsorption studies, the gel was then subjected to the chemical treatment as described above. Breakthrough curves were determined at 0, 39, 55, 76, 109, 140, 207 and 266 days of chemical treatment exposure time.

2.5. Flow-rate variation

To investigate the adsorbent behavior under a lower flow-rate, breakthrough curves were determined for an untreated adsorbent and for an adsorbent with 600 days of alkaline treatment, using a flow-rate of 0.3 ml/min under the same experimental conditions.

2.6. Titration curves

Titration curves were obtained for the untreated adsorbent and for an adsorbent with 600 days of alkaline treatment. An adsorbent sample of 1 ml of a 50:50 (v/v) suspension was placed in 50 ml of a 0.5 *M* sodium chloride solution. Titration was conducted with a 0.05 *M* hydrochloric acid solution.

2.7. Fixed-bed model

To describe the complex interactions between proteins and ion-exchangers a simplified model is often used [1,10]. The model here considers a second-order reversible adsorption, where the protein is assumed to interact with the adsorbent by a monovalent interaction and characteristic constant binding energy.

$$P + S \leftrightarrow PS \tag{1}$$

where P is the protein in solution and S is the adsorption site.

The rate of adsorption for this type of interaction is given by

$$\frac{\partial q}{\partial t} = k_1 c(q_m - q) - k_{-1} q \tag{2}$$

where c and q are the protein concentration in the liquid and adsorbent, respectively, and q_m represents the maximum adsorption capacity of the adsorbent. The rate constants k_1 and k_{-1} are lumped parameters that reflect the contributions of mass transport and binding kinetics as well. At equilibrium, Eq. (2) reduces to the Langmuir isotherm model.

$$q^* = \frac{q_m c^*}{K_d + c^*} \tag{3}$$

where * denotes equilibrium concentrations and $K_d = k_{-1}/k_1$ is the dissociation constant.

Protein adsorption processes are typically carried out in fixed beds. To model the behavior of ion-exchange systems the adsorption model for fixed-bed was also used. The model is based on an isothermal sorption of a single solute in plug flow through a packed bed of uniform spherical particles, having a radius r_a, and a uniform cross-sectional area A of length L and a void fraction ε. Liquid flows through the bed at a superficial velocity v, and initially the column is devoid of solute. At time zero the inlet concentration of solute in the mobile phase to the column is changed to c_0.

It has been shown that when axial dispersion is negligible, column mass balance can be expressed as [9]:

$$\varepsilon \frac{\partial c}{\partial t} = -v \frac{\partial c}{\partial z} - (1-\varepsilon)\frac{\partial q}{\partial t} \tag{4}$$

The analytical solution to Eqs. (2) and (4) was first obtained by Thomas for frontal analysis on an ion-exchange column [11], and may be expressed as follows [12]:

$$\chi(t,L) = \frac{J\left(\frac{n}{r}, n\Gamma\right)}{J\left(\frac{n}{r}, n\Gamma\right) + \left[1 - J\left(n, \frac{n\Gamma}{r}\right)\right]\exp\left[\left(1 - \frac{1}{r}\right)(n - n\Gamma)\right]} \tag{5}$$

where:

$$\chi(t,L) = \frac{c}{c_0} \tag{6}$$

$$r = 1 + \frac{c_0}{K_d} \tag{7}$$

$$n = q_m^s k_1 L \frac{A}{F} \tag{8}$$

$$\Gamma = \left(\frac{K_d + c_0}{q_m^s}\right)\left(\frac{Ft}{AL} - \varepsilon\right) \tag{9}$$

and q_m^s is the adsorbent capacity referred to the settled volume of adsorbent. J is a two parameter function of α and β [13]. According to Thomas [11], when the product of α and β is greater than 36, $J(\alpha,\beta)$ can be calculated more easily, within 1% accuracy, by the following equation:

$$J(\alpha,\beta) = \frac{1}{2}\left\{1 - erf(\sqrt{\alpha} - \sqrt{\beta}) + \frac{\exp\left[-(\sqrt{\alpha} - \sqrt{\beta})^2\right]}{\sqrt{\pi}\left[\sqrt{\beta} + (\alpha\beta)^{\frac{1}{4}}\right]}\right\} \tag{10}$$

2.8. Computer simulations

Computer programs were used to simulate breakthrough curves. All computer programs were run

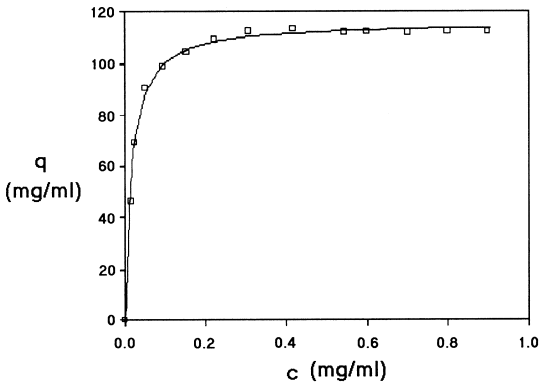

Fig. 2. Equilibrium adsorption isotherm for the adsorption of BSA in 10 m*M* sodium phosphate buffer, pH 7.5 at 25±3°C to DEAE Matrex Cellofine (——— represents the best-fit Langmuir isotherm).

using MathCad version 4.0, in a 486 personal computer.

3. Results and discussion

3.1. Adsorption equilibrium studies

The isotherm for the adsorption of BSA to DEAE Matrex Cellofine A-200, without chemical treatment, in 10 m*M* of sodium phosphate buffer, pH 7.5 is shown in Fig. 2. Equilibrium data was fitted to the Langmuir model by non linear least-squares regression analysis. The solid lines in the figure are the Langmuir isotherms that best fit the data.

The adsorption isotherms for 30, 70, 94, 122, 186 and 273 days of adsorbent chemical treatment were in practice equal to the ones obtained with the untreated adsorbent. The characteristic parameters values were 115±3 mg/ml for maximum adsorption capacity q_m and 0.0150±0.003 mg/ml for the dissociation constant K_d (Table 1).

The equilibrium isotherm for the adsorption system BSA–DEAE Matrex Cellofine A-200 appears to be insensitive to the chemical treatment in the range of exposure time studied, although a relatively high sodium hydroxide concentration and temperature were used in the chemical treatment. This result might not be the same for smaller protein molecules, with larger diffusion paths, than the ones for BSA. These paths may be altered during the chemical treatment of the adsorbent and, at least the adsorbent protein capacity modified.

3.2. Fixed-bed adsorption studies

The breakthrough curves obtained experimentally, at different adsorbent exposure times to 0.5 *M* sodium hydroxide solution, for the fixed-bed adsorption of BSA to DEAE Matrex Cellofine A-200 in 10 m*M* of sodium phosphate buffer pH 7, are shown in Fig. 3. The equilibrium capacity of the column for BSA was slightly greater than that predicted from adsorption isotherms. The best fit for the early portion of the curves (that of most interest) was obtained with a q_m value of 120 mg/ml, the K_d value of 0.015 mg/ml from batch experiments and an assumed k_1 value. A void bed volume of 0.33 determined with blue dextran, was used in these simulations. The lumped forward rate constant k_1 was estimated by comparing experimental breakthrough curves with those generated by the model.

In general, good agreement was observed between experiments and theory, particularly in the early portion of the curve. However, the Thomas solution is particularly useful only when high mass transfer rates are present (new adsorbent), and if precise results need to be obtained a more sophisticated model should be used [1,14,15].

It was found that the k_1 value decreases with the regeneration time, according to the following exponential model:

Table 1
Langmuir isotherm parameters for BSA adsorption on DEAE Matrex Cellofine at different exposure times to alkaline regeneration treatment

Parameter	Regeneration treatment exposure time (days)						
	0	30	70	94	122	186	273
q_m (mg/ml)	114	116	115	112	114	118	112
K_d (mg/ml)	0.018	0.015	0.018	0.012	0.012	0.015	0.018

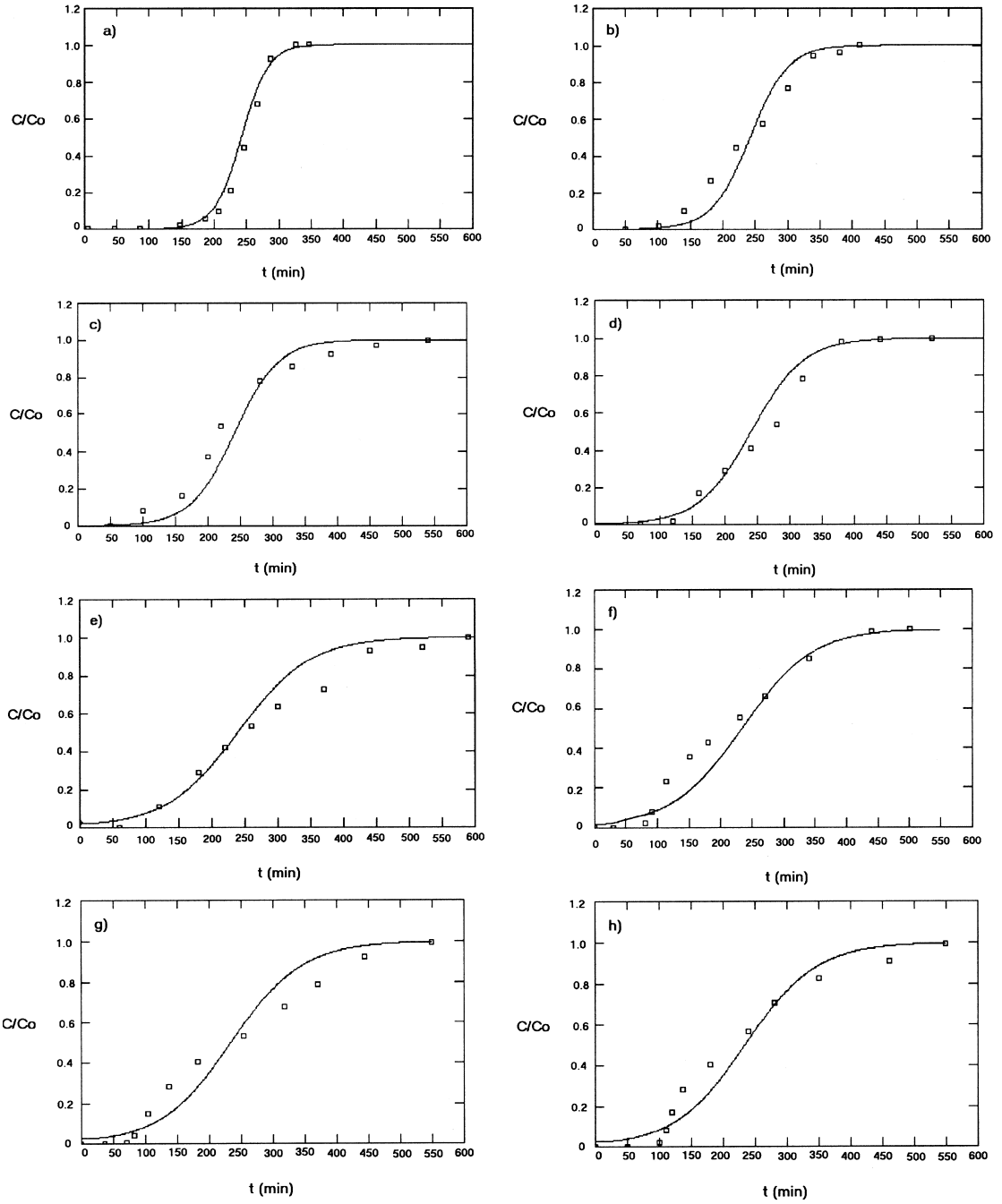

Fig. 3. Breakthrough curves for the fixed-bed adsorption of BSA in 10 m*M* sodium phosphate buffer, pH 7.5 at 25±3°C to DEAE Matrex Cellofine. The column diameter 1.0 cm, adsorbent volume 2 ml, inlet protein concentration 1 mg/ml and flow-rate 1.0 ml/min were kept constant in each experiment. Each curve was determined at different exposure time of the adsorbent to sodium hydroxide treatment. (a) 0 days, (b) 39 days, (c) 55 days, (d) 76 days, (e) 109 days, (f) 140 days, (g) 207 days and (h) 266 days (——— represents the best-fit solution to the fixed-bed model).

$$k_1 = 0.0003 + 0.0006\exp(-0.025T) \quad (11)$$

where T represents the exposure chemical treatment time in days and k_1 has units of ml/mg s. Due this effect, column operation capacity (column capacity for outlet concentration equal 10% of c_0) also decreases with treatment time.

3.3. Flow-rate variation

The breakthrough curves obtained experimentally at a flow-rate of 0.3 ml/min for the untreated adsorbent and for the adsorbent with 600 days of alkaline treatment are shown in Fig. 4. The continuous curve was obtained using a q_m value of 120 mg/ml, a $K_d$value of 0.015 mg/ml and a k_1 value of 0.00022 ml/mg s. The experimental values suggest that at this lower flow-rate, chemically untreated and treated gels presented the same behavior. However, it appears that a dispersion effect diminished the k_1 value.

3.4. Titration curves

Titration curves for the untreated adsorbent and for an adsorbent with 600 days of alkaline treatment are shown in Fig. 5. These curves almost overlap over the entire pH range, suggesting that the ionic character of the DEAE groups remains unchanged under the reported experimental procedure.

According to the equilibrium, fixed-bed and titration results, the chemical treatment affects the adsorption process by altering only the adsorption kinetics. The alkaline treatment very probably altered the chemical structure of the DEAE Cellufine and a consequence of this modification is evidenced by an alteration of the sorption kinetics. This effect can only be detected when the adsorption behavior is affected by the residence time in the column.

Whilst accepting its limitations, the Langmuir isotherm and the kinetic model employed in our study are, simple and useful tools that can be used to help in the prediction of the performance of adsorption systems. Eqs. (5) and (11) integrate the fixed-bed model for the column under chemical treatment. This type of model can be used in process development and design. In optimization studies for replacement time of adsorbents, the fixed-bed model can be used to generate expressions for the variation of column operation capacity with treatment time. In this study the resulting expression for the column operation capacity variation is given by,

$$Q = 52 + 45\exp(-0.014T) \quad (12)$$

About half operation capacity remains unaltered during the chemical treatment exposure time studied.

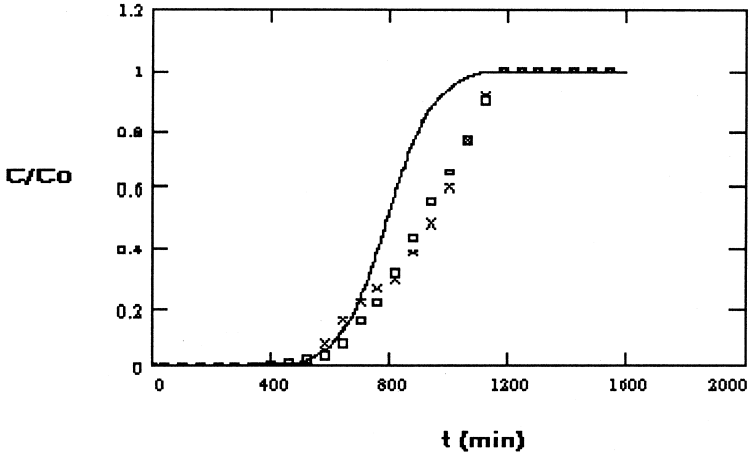

Fig. 4. Breakthrough curves for the fixed-bed adsorption of BSA in 10 m*M* sodium phosphate buffer, pH 7.5 at 25±3°C to DEAE Matrex Cellofine. The column diameter 1.0 cm, adsorbent volume 2 ml, inlet protein concentration 1.0 mg/ml and flow-rate 0.3 ml/min were kept constant in each experiment. Each curve was determined at different exposure time of the adsorbent to sodium hydroxide treatment. (□) 0 days, (×) 600 days (——— represents the best-fit solution to the fixed-bed model).

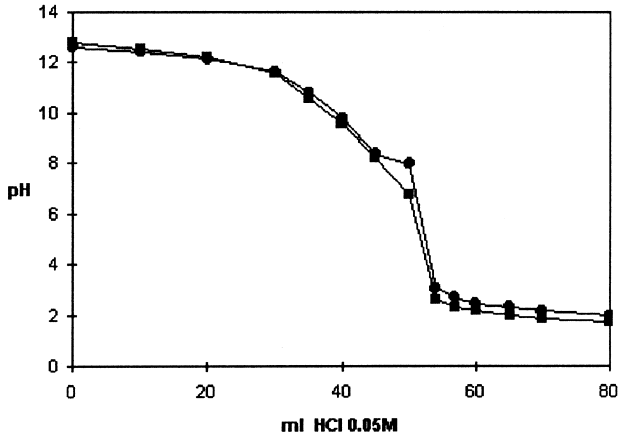

Fig. 5. Titration curves of DEAE Matrex Cellofine. Conditions: 1 ml of adsorbent in 50 ml 0.5 *M* sodium chloride solution. Each curve was determined at different exposure time of the adsorbent to sodium hydroxide treatment. (●) 0 days, (■) 600 days.

4. Conclusions

It has been shown that the alkaline adsorbent regeneration processes can have important effects on fixed-bed adsorption. For DEAE–cellulose, column chemical treatment affects the adsorption process primarily through its effects on adsorption kinetics. In other type of adsorbents equilibrium characteristics might also be affected by chemical treatments [16,17]. The type of chemical treatment for an adsorbent is specific for each process and, as a general rule the effects of these treatments should be investigated for each case individually at an early stage of the process development.

The simple model described in this study can be used as a means to predict the performance of several adsorption processes, including ion-exchange, dye–ligand affinity and immobilized metal affinity chromatography, under regeneration conditions. It can also be useful in the selection and optimization of separation sequences. Much work is needed on the behavior of adsorbents under reuse conditions, to optimize the use of the ones available and to determine research directions for producing new ones.

5. List of symbols

A	cross-sectional column area
c	solute concentration in the bulk phase, mg/ml
c_0	solute concentration in the bulk phase at column inlet, mg/ml
c^*	solute concentration in the bulk phase at equilibrium, mg/ml
F	volumetric flow-rate through packed bed, ml/min
k_1	lumped forward adsorption rate constant, ml/mg min
k_{-1}	lumped reverse adsorption rate constant, 1/min
K_d	dissociation constant, mg/ml
L	length of fixed bed, cm
n	dimensionless number of transfer units for overall process
P	molecule of protein
PS	complex between protein and adsorption site
q	average adsorbate concentration, mg/ml adsorbent
q^*	concentration at equilibrium, mg/ml
q_m	maximum binding capacity of adsorbent, mg/ml
q_m^s	maximum binding capacity of adsorbent, mg/ml adsorbent settled volume
Q	operating adsorption capacity, mg/ml
r	dimensionless separation factor
S	adsorption site
t	time, min
T	chemical treatment exposure time, days

V volume of the fixed bed, ml
z axial distance along the fixed bed, cm

Greeks

$\chi(t,L)$ dimensionless solute concentration
ε void fraction of the fixed bed
Γ dimensionless effluent volume
υ superficial velocity, cm/min

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