

# Mathematical Modeling of Elution Curves for a Protein Mixture in Ion Exchange Chromatography and for the Optimal Selection of Operational Conditions

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**Abstract:** Elution curves in ionic exchange chromatography (IEC) for a three-protein mixture ( $\alpha$ -lactalbumin, ovalbumin, and  $\beta$ -lactoglobulin), carried out under different flow rates and ionic strength conditions, were simulated using two different mathematical models. These models were the Plate Model and the more fundamentally based Rate Model. Relatively low protein concentrations were used to avoid protein–protein interactions. Simulated elution curves were compared with experimental data not used for parameter identification. Deviation between experimental data and the simulated curves using the Plate Model was less than 0.0189 (absorbance units); a slightly higher deviation [0.0252 (absorbance units)] was obtained when the Rate Model was used. A cost function was built that included the effect of the different production stages, namely fermentation, purification, and concentration. These considered the effect on the performance of IEC; yield, purity, concentration and the time needed to accomplish the separation. Operational conditions in the IEC such as flow rate, ionic strength gradient and the operational time can be selected using this model in order to find the minimum cost for the protein production process depending on the characteristics of the final product desired such as purity and yield. This cost function was successfully used for the selection of the operational conditions as well as the fraction of the product to be collected (peak cutting) in IEC. It can be used for protein products with different characteristics and qualities, such as purity and yield, by choosing the appropriate parameters. © 2006 Wiley Periodicals, Inc.

**Keywords:** chromatography; mathematical models; simulation; optimization

## INTRODUCTION

Ionic exchange chromatography (IEC) is probably the most powerful and used method for protein purification. The purification of a protein mixture is accomplished by several chromatographic steps. Modern optimized procedures will typically consist of two chromatographic separation stages, first an IEC followed by a hydrophobic interaction

chromatography (Asenjo and Andrews, 2004). In IEC protein adsorption depends not only on composition and concentration of the mixture but also on operation conditions such as flow rate, ionic strength gradient, sample load, physical properties of the adsorbent matrix, and column dimensions. Mixture composition is usually determined in the production stage (fermentation, sometimes cell disruption and recovery) and thus for a given adsorbent matrix operational conditions such as flow rate and ionic strength gradient have to be chosen in order to improve a function able to represent the performance of the process. Maximization of this performance function can be carried out mathematically if a model able to simulate IEC carried out under different operational conditions is available. Mathematical models for describing a chromatographic separation can be classified depending on the simplifying assumptions considered in its derivation. Models such as the Plate Model can be used for predicting the retention time and the elution curve. More complex models are those based on thermodynamic and transport phenomena that take place in the chromatographic separation process; these models are termed Rate Models.

## The Plate Model

The Plate Model is based on the plate theory. Briefly, the model assumes that the chromatographic column is formed by a number of plates ( $N_p$ ) each of them having the same ratio between the stationary phase volume and the volume of the mobile phase ( $H$ ). For a defined column geometry and if the adsorption kinetics is known the problem is reduced to solve the system of  $N_p$  ordinary differential equations (ODE) shown in Table I. In order to solve this ODE system the ionic strength at each plate ( $i = 1..N_p$ ) has to be computed as a function of time. Table I shows the formula for computing this variable in the case that a constant ionic strength gradient is applied for protein elution (Yamamoto et al., 1983b).

At low protein concentration the adsorption kinetics is computed from the value of the distribution coefficient ( $K$ )

**Table I.** Equations of the plate model.Protein at each plate ( $i = 1, \dots, N_p$ )

$$\frac{dC_i}{d\theta} = \frac{N_p(C_{i-1} - C_i) - C_i H \frac{dK(C_i, I)}{dI} \frac{dI}{d\theta}}{1 + H \left[ K(C_i, I) + C_i \frac{d}{dC_i} K(C_i, I) \right]}$$

$$\begin{aligned} \theta = -\frac{V_m}{V_0} & \quad C_0 = C_1 = \dots = C_{N_p} = 0 \\ -\frac{V_m}{V_0} < \theta \leq 0 & \quad C_0 = 1 \\ \theta > 0 & \quad C_0 = 0 \end{aligned}$$

Ionic strength at each plate ( $i = 1, \dots, N_p$ )

$$\begin{aligned} I_i &= I_0 & \text{for } \theta \leq (1 + HK_{\text{salt}}) \frac{i}{N_p} \\ I_i &= I_0 + G \left[ \theta - (1 + HK_{\text{salt}}) \frac{i}{N_p} \right] & \text{for } \theta > (1 + HK_{\text{salt}}) \frac{i}{N_p} \end{aligned}$$

that depends on the ionic strength of the mobile phase ( $I$ ). The following relationship has been proposed for relating the concentration of the adsorbed protein,  $C^*$ , and that of the protein in solution,  $C$ , (Yamamoto et al., 1983a):

$$C^* = K(I)C; \quad K(I) = (A \cdot I^B + K_{\text{crit}}) \quad (1)$$

Protein displacement in IEC is due to changes in the ionic strength of the mobile phase and thus the distribution coefficient and the number of plates cannot be computed from the first and second normalized central moment of the elution curve. However, because during the traveling of the protein through most parts of the column the protein zone is subject to an ionic strength near to the one at which this emerges from the column ( $I_{\text{max}}$ ) the following relationship has been presented for computing the number of plates (Yamamoto et al., 1983b):

$$N_p = \frac{L}{2D_z/v + d_p^2 HK_{\text{Imax}}^2 v / [30D_{\text{crit}} K_{\text{crit}} (1 + HK_{\text{Imax}})^2]} \quad (2)$$

In relationship (2)  $K_{\text{Imax}}$  is computed from relationship (1) using  $I$  equal to  $I_{\text{max}}$ . Under the assumption that parameters  $K_{\text{crit}}$  and  $D_{\text{crit}}$  do not change with the ionic strength they can be computed from the first and second central moment of elution curves obtained using different flow rates.

## The Rate Model

In the more fundamentally based Rate Model the dimensionless elution curves are obtained from the solution of the following partial differential equation:

$$\frac{\partial c_b}{\partial \tau} = -\frac{\partial c_b}{\partial z} + \frac{1}{\text{Pe}_L} \frac{\partial^2 c_b}{\partial z^2} - \xi_i (c_b - c_{p,r=1}) \quad (3)$$

subject to the initial and boundary conditions given by:

$$\begin{aligned} \tau = 0 \quad c_b &= c_b(0, z) \\ z = 0 \quad \frac{\partial c_b}{\partial z} &= \text{Pe}_L \left[ c_b(0, \tau) - \frac{C_f(\tau)}{C_0} \right] \\ z = L \quad \frac{\partial c_b}{\partial z} &= 0 \end{aligned} \quad (4)$$

In order to solve the partial differential equation in (3) the dimensionless concentration profile for each component in

the liquid phase contained inside the particles,  $c_p$ , has to be computed. These concentration profiles are obtained from the solution of the following partial differential equation:

$$\frac{\partial}{\partial \tau} (\varepsilon_p c_p + (1 - \varepsilon_p) c_p^*) = \eta \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial c_p}{\partial r} \right) \quad (5)$$

subject to the initial and boundary conditions given by:

$$\begin{aligned} \tau = 0 \quad c_p &= c_p(0, r, z) \\ r = 0 \quad \frac{\partial c_p}{\partial r} &= 0 \\ r = 1 \quad \frac{\partial c_p}{\partial r} &= \text{Bi} [c_b(z, \tau) - c_p(r = 1, z, \tau)] \end{aligned} \quad (6)$$

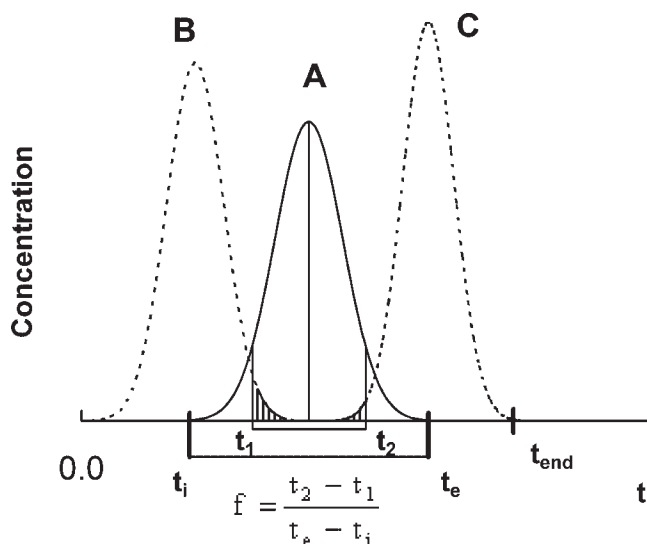
In relationship (4),  $C_f(\tau)$ , is the time dependant feeding concentration (for a protein,  $C_f(\tau)$  will be different from zero while the sample is loaded into the column; for the displacer, the feeding concentration is often a function of time). Dimensionless variables and parameters in relationship (3)–(6) are shown in Table II. Since all mass transfer phenomena are taken into account in partial differential Equations 3 and 5 Rate Models can be used for testing different chromatographic conditions (Gu, 1995) and also of simulating the more complex way of operating a chromatographic process, the simulated moving bed (Lazo, 1999).

## Evaluation of Performance

Operational conditions in IEC such as flow rate and ionic strength gradient are taken into account in both mathematical models and thus predicted elution curves depend on them. However, the quality of the product obtained in IEC is also dependent on external operational conditions such as the flow rate as well as the size of the fraction of the protein product collected as shown in Figure 1 (also called “peak cutting”).

**Table II.** Dimensionless variables and parameters of the rate model.

|  |   |
|--|---|
| Concentration of the mobile phase                          | $c_b = \frac{C_b}{C_0}$                                       |
| Concentration of the liquid inside the adsorbent particles | $c_p = \frac{C_p}{C_0}$                                       |
| Concentration of the adsorbed protein                      | $c_p^* = \frac{C_p^*}{C_0}$                                   |
| Dimensionless time   | $\tau = \frac{t}{L}$  |
| Dimensionless position in the column                       | $z = \frac{z}{L}$   |
| Dimensionless position in the particle                     | $r = \frac{r}{R_p}$   |
| Peclet number  | $\text{Pe}_L = \frac{vL}{D_z}$                                |
| Biot number  | $\text{Bi} = \frac{kR_p}{\varepsilon_p D_p}$                  |
|  | $\eta = \frac{\varepsilon_p D_p L}{R_p^2 v}$                  |
|  | $\xi = \frac{3\text{Bi}\eta(1-\varepsilon_b)}{\varepsilon_b}$ |



**Figure 1.** Elution curves of a mixture of proteins. A is the target protein product and  $f$  the fraction of peak A collected.

From the scheme presented in Figure 1, if the target protein is protein A, the outlet flow can be collected from  $t = t_i$  until  $t = t_e$ , the period during which the concentration of A in the outlet flow becomes important. However, during this time part of the contaminants are also eluted. A way to minimize the contaminant content in the collected volume is by decreasing the collecting interval considering, for example, the time elapsed between  $t_1$  and  $t_2$  (Fig. 1).

In order to define a performance function for an IEC that can be used for choosing operational conditions for the separation of a given protein mixture the following parameters should be taken into account:

- (a) *Concentration* of the target protein,  $x_A$  that is given by:

$$x_A = \frac{\int_{t_1}^{t_2} C_A \cdot F dt}{\int_{t_1}^{t_2} F dt} = \frac{\int_{t_1}^{t_2} C_A dt}{\int_{t_1}^{t_2} dt} \quad (7)$$

As shown in Figure 1 concentration  $x_A$  depends on the collecting time and on the resolution of the purification stage fixed by the flow rate and the ionic strength gradient. Costs involved in the subsequent concentration processes (ultrafiltration, lyophilization) are related to the value of  $x_A$ .

- (b) *Purity* of protein A is defined as the ratio between the mass of protein A and that of all the proteins in the collected volume:

$$\text{Purity of the } i \text{ component} = \frac{x_i}{\sum_{j=1}^m x_j} \quad (8)$$

Purity not only allows establishment of the pertinence of the IEC as a purification stage but it can also be used for estimating costs involved in the forward purification steps.

- (c) *Yield* is defined as the ratio between the mass of the target protein in the collected volume and the mass of the same protein loaded into the column:

$$\text{yield}_A = \frac{\int_{t_1}^{t_2} C_A \cdot F dt}{C_{A0} V_0} \quad (9)$$

The yield of an IEC depends on the collecting time and it can be used to estimate costs of the production stages (fermentation).

- (d) *Process time* is defined as the time at which all the proteins in the mixture loaded into the column are eluted and after which the column can be prepared for the treatment of a new load. Process time can be used as an estimation of the costs involved in the IEC stage.

In this study Plate and Rate Models are used for simulating elution curves of a three-protein mixture in IEC carried out under different operational conditions (flow rates and ionic strength gradients). Parameters in the models are estimated from experimental data. Predictions of the models are compared with experimental data not used for parameter identification. In the experiment Q Sepharose FF is used as the adsorbent matrix. A cost function for the protein production process is proposed and flow rate, ionic strength gradient, and collection time are selected in order to minimize the cost function for different types of protein products.

## MATERIALS AND METHODS

### Solution of the Models

Computer programs for solving mathematical models were created in Matlab 2000. The ODE system in the Plate Model was solved using the Matlab ODE solver based on Runge–Kutta formulas.

For the Rate Model, the numerical method of lines was used in order to obtain, through space discretization of the partial differential equation system, an ODE system. Partial differential equations for concentrations of the different components in the mobile phase and those of the mobile phase contained inside the adsorbent particles were discretized using finite elements and orthogonal collocation methods (Lazo, 1999), respectively. The resulting ODE system was solved using ODE solvers provided by Matlab.

### Elution Curves

A mixture of three proteins (Sigma-Aldrich, St. Louis, MO) was used:  $\alpha$ -lactalbumin (0.2 mg/mL), ovalbumin (0.2 mg/mL), and  $\beta$ -lactoglobulin (0.15 mg/mL) in buffer Tris (pH 8). Experiments were carried out in an FPLC System (Pharmacia Biotech, Uppsala, Switzerland). The adsorbent matrix was Q Sepharose FF (Pharmacia Biotech) packed into a 5/5 column HR (length, 5 cm; diameter, 0.5 cm; Pharmacia

**Table III.** Characteristics of the ion exchange chromatography system used in the experimental runs.

| Adsorbent                | Q Sepharose FF       |
|--------------------------|----------------------|
| $d_p$ (cm)               | $9.3 \times 10^{-3}$ |
| $d_c$ (cm)               | 0.5                  |
| $L$ (cm)                 | 5                    |
| $V_t$ (cm <sup>3</sup> ) | 0.98                 |
| $V_0$ (cm <sup>3</sup> ) | 0.79                 |
| $V_m$ (cm <sup>3</sup> ) | 0.1                  |
| $\epsilon_b$ (—)         | 0.8                  |
| $\epsilon_p$ (—)         | 0.5                  |
| pH                       | 8                    |
| $T$ (°C)                 | 25                   |
| $I_0$ [M]                | 0.001                |

Biotech). Ionic strength was estimated as the conductivity of the solution. Elution curves were obtained from measurements of the absorbance (280 nm) (absorbance detector UV-MII, Pharmacia Biotech) of the outlet flow as a function of time. In all cases 100  $\mu$ L of the protein solution were injected into the column. Table III shows the characteristics of the IEC system used in the experimental runs.

In order to transform absorbance ( $A_{280}$ ) measurements into concentration values calibration curves were built for each protein using solutions of known concentration.

### Pulse Experiment for Estimating Parameters $K_{crit}$ and $D_{crit}$

For computing these parameters the method of moments ( $\mu_1$ , first statistical moment;  $\mu_2$ , second central moment) was applied to elution curves obtained keeping constant the ionic strength of the mobile phase at 0.5 M NaCl. Flow rates of 0.5, 0.7, 1.0, 1.2, and 1.5 mL/min were used. The following relationships were used (Yamamoto et al., 1988):

$$\mu_1 = \frac{1}{v} (1 + HK_{crit})$$

$$\frac{\mu_2}{2L/v} = \left( \frac{D_c}{v} [1 + HK_{crit}]^2 \frac{1}{v} + \frac{HK_{crit}d_p^2}{60D_{crit}} \right) \quad (10)$$

Values of  $K_{crit}$  and  $D_{crit}$ , computed from experimental data using relationships in (10) for each protein, are given in Table IV.

### Distribution Coefficient for the Proteins, $K$

Elution curves for each protein in the mixture were obtained using a constant flow rate ( $F = 1$  mL/min) and different ionic strength gradients,  $g$  ( $g$  equal to 0.055, 0.067, 0.083, 0.108, 0.125, and 0.167 M/mL; NaCl in Tris-HCl 20 mM; pH 8). By

**Table IV.** Parameters in the adsorption kinetics [relationship (1)] on Q sepharose FF for the different proteins.

| Protein                | $A$                  | $B$   | $K_{crit}$ | $D_{crit} \times 10^7$ |
|------------------------|----------------------|-------|------------|------------------------|
| $\alpha$ -lactalbumin  | $3.3 \times 10^{-3}$ | −4.62 | 0.816      | 4.8                    |
| Ovalbumin              | $2.2 \times 10^{-5}$ | −8.55 | 0.597      | 1.4                    |
| $\beta$ -lactoglobulin | $8.1 \times 10^{-4}$ | −7.81 | 1.020      | 1.2                    |

using the ionic strength at which the maximum concentration was obtained,  $I_{max}$ , parameters  $A$  and  $B$  in relationship (1) were obtained from (Yamamoto et al., 1988):

$$g(V_t - V_0) = \frac{I_{max}^{B+1}}{A(B+1)} \quad (11)$$

Computed values of parameters  $A$  and  $B$ , for each protein, are shown in Table IV.

### Distribution Coefficient for the Salt, $K'$

Since the outlet salt concentration begins to increase after a dimensionless time,  $\theta$  ( $\theta = t \cdot F/V_0$ ), greater than  $1 + H \cdot K'$  has elapsed, this time was used for estimating the value of  $K'$ . The value of this parameter was calculated from ionic strength curves obtained with different ionic strength gradients ( $g$  equal to 0.055, 0.108, and 0.167 M/mL) and a flow rate equal to 1 mL/min. For the chromatographic system used here  $K'$  was equal to 3.76.

### Mass Transfer Coefficient

Mass transfer coefficient,  $k$ , for the protein in the stagnant liquid inside the adsorbent particle was computed from the following correlation (Simpson, 1994):

$$Sh = \frac{k d_p}{D_{AB}} = 2 + 1.45 Re^{1/2} Sc^{1/3} \quad (12)$$

The diffusion coefficient of protein A in the liquid phase B,  $D_{AB}$  in m<sup>2</sup>/s, was computed from (Skidmore et al., 1990):

$$D_{AB} = 9.4 \times 10^{-15} \frac{T}{\mu M_A^{1/3}} \quad (13)$$

in which  $M_A$  is the molecular weight of the protein ( $M_{\alpha}$ -lactalbumin = 17.4 KDa;  $M_{ovalbumin}$  = 48 KDa;  $M_{\beta}$ -lactoglobulin = 26 KDa).

## RESULTS AND DISCUSSION

Elution curves of the three-protein mixture in IEC were experimentally recorded for two values of the ionic strength gradient ( $g$ ) and different flow rates ( $F$ ). A low concentration of the protein mixture ( $\alpha$ -lactalbumin and ovalbumin 0.2 mg/mL;  $\beta$ -lactoglobulin 0.15 mg/mL) was used in the experiments in order to avoid possible interaction effects. For the comparison between the simulated elution curves (obtained as concentration profiles) and the experimental curves (obtained as absorbance curves) concentrations were transformed into absorbance values by using the calibration curves built for each protein ( $C_{\alpha}$ -lactalbumin =  $0.18 \cdot A_{280}$ ;  $C_{ovalbumin}$  =  $0.423 \cdot A_{280}$ ;  $C_{\beta}$ -lactoglobulin =  $0.447 \cdot A_{280}$ ).

Values for the purity and retention times obtained from the IEC elution curves simulated using the Plate and Rate Models are shown in Tables V and VI, respectively. For the computing of purity and retention time it was assumed that the eluted volume was collected while the

**Table V.** Results of the simulations for the separation of a three-protein mixture in IEC using the Plate Model for different flow rates,  $F$ , and ionic strength gradients,  $g$ .

| Run | $F$ (mL/min) | $g$ (M/mL) | Purity (%) |       |       | Retention time (min) |       |       | Deviation |
|-----|--------------|------------|------------|-------|-------|----------------------|-------|-------|-----------|
|     |              |            | $P_1$      | $P_2$ | $P_3$ | $P_1$                | $P_2$ | $P_3$ |           |
| 1-a | 0.3          | 0.100      | 54.4       | 49.4  | 80.6  | 12.2                 | 13.3  | 16.5  | 0.0149    |
| 1-b | 0.7          | 0.100      | 50.9       | 42.6  | 54.8  | 5.3                  | 5.8   | 7.1   | 0.0093    |
| 1-c | 1.0          | 0.100      | 50.3       | 39.8  | 47.1  | 3.7                  | 4.1   | 5.0   | 0.0109    |
| 2-a | 0.3          | 0.055      | 63.6       | 54.0  | 84.6  | 17.4                 | 19.8  | 24.9  | 0.0189    |
| 2-b | 0.5          | 0.055      | 59.9       | 48.1  | 71.3  | 10.5                 | 11.9  | 15.0  | 0.0053    |
| 2-c | 1.0          | 0.055      | 55.2       | 42.0  | 51.9  | 5.2                  | 6.0   | 7.5   | 0.0064    |

$P_1$ ,  $\alpha$ -lactoalbumin;  $P_2$ , ovalbumin;  $P_3$ ,  $\beta$ -lactoglobulin.

protein concentration remained higher than 0.001 mg/mL (time elapsed between  $t_i$  and  $t_e$  in Figure 1). Comparisons between experimental and simulated elution curves and the ionic strength profile computed using the Plate Model, for the different flow rates and ionic strength gradients of 0.055 and 0.1 M/mL are shown in Figures 2 and 3, respectively. The deviation between the experimental and simulated elution curves is presented in Table V. Maximum value for the deviation was 0.0189 (absorbance units).

Results in Figures 2 and 3 and Table V indicate that the Plate Model can be used for simulating the elution curve of a protein mixture in IEC. It is important to note that all parameters in the model such as those needed for computing the number of plates and the distribution coefficients for each protein, were obtained from independent experiments.

In order to simulate elution curves in IEC using the Rate Model values for the dimensionless numbers  $Pe_L$  and  $Bi$  for each protein have to be known.  $Pe_L$  was computed based on the assumption that the ratio between the diffusion coefficient and velocity of the mobile phase through the packed column,  $D_z/v$ , is almost constant and equal to the adsorbent particle diameter ( $d_p$ ) (Yamamoto et al., 1983a). Under this assumption  $Pe$  is a constant equal to 540. Relationships for computing  $Bi$  and  $\eta$  (Table II) involve the unknown value of the diffusion coefficient inside the adsorbent particle,  $D_p$ . It was assumed that values for  $D_p$ , for each protein were those

estimated for  $D_{crit}$  (Table IV). Under this assumption  $Bi$  numbers were higher than 1 implying that the internal diffusion has to be considered. Values for  $\eta$  were estimated for each protein from the experimental results. These values of  $\eta$  were the ones used in the simulations. Hence the elution curves of the Rate Model were adjusted to the experimental results using  $\eta$  as a protein specific parameter (which eventually results in  $D_p$  as a variable parameter). The comparison of the experimental and simulated elution curves and ionic strength profile computed using the Rate Model are shown in Figures 4 and 5. In these simulations the adsorption kinetics shown in relationship (1) and parameters in Table IV were used. Table VI shows the deviation between simulated and computed values; the maximum deviation was 0.0252 (absorbance units). From the comparison of the results presented in Tables V and VI the average prediction deviation obtained with the Rate Model was slightly higher than that obtained using the Plate Model.

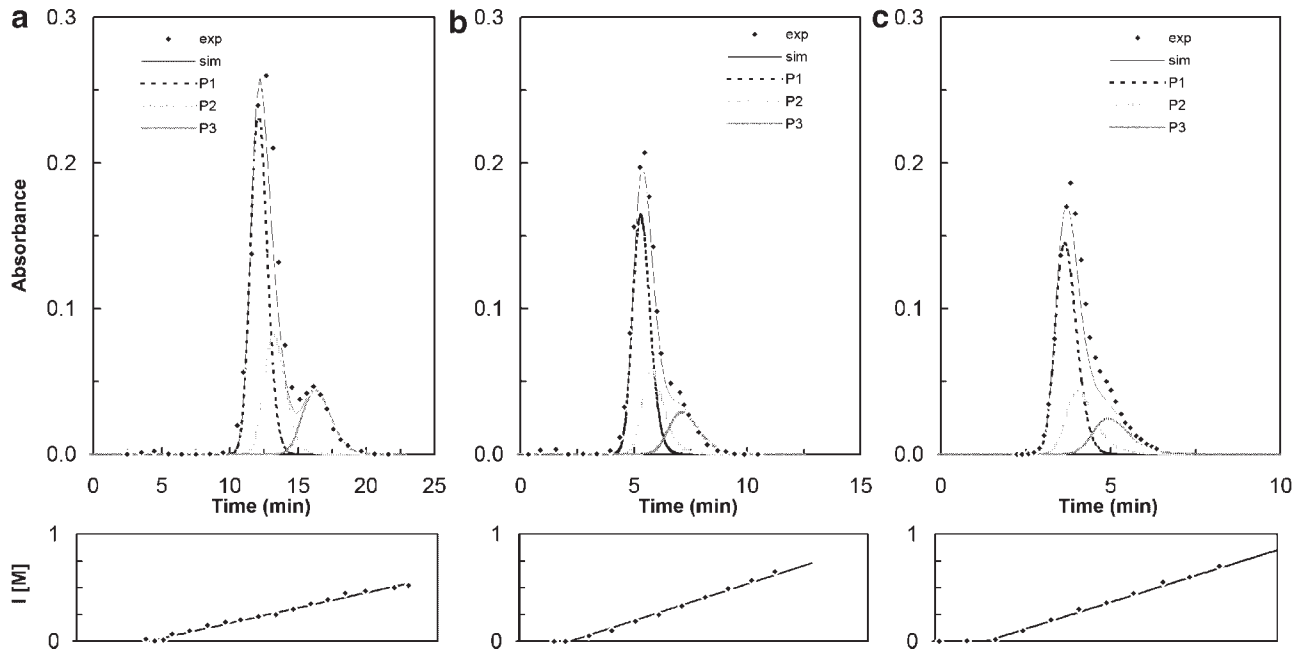
The Rate Model has several advantages over the Plate Model, the most important being that it can be extended for simulating elution curves of more concentrated protein mixtures, where protein interaction effects could be significant and more complex relationships for the adsorption kinetics must be used. Nevertheless, for the case under study the Plate Model is easier to implement computationally and also has a lower CPU demand due to the small size of the ODE system that has to be solved. While in the Plate Model

**Table VI.** Results of the simulations for the separation of a three-protein mixture in IEC using the Rate Model for different flow rates,  $F$ , and ionic strength gradients,  $g$ .

| Run | $F$ (mL/min) | $g$ (M/mL) | Purity (%) |       |       | Retention time (min) |       |       | Deviation |
|-----|--------------|------------|------------|-------|-------|----------------------|-------|-------|-----------|
|     |              |            | $P_1$      | $P_2$ | $P_3$ | $P_1$                | $P_2$ | $P_3$ |           |
| 1-a | 0.3          | 0.100      | 64.3       | 63.1  | 78.7  | 12.5                 | 13.8  | 16.6  | 0.0192    |
| 1-b | 0.7          | 0.100      | 55.5       | 55.5  | 59.6  | 5.3                  | 5.9   | 7.1   | 0.0111    |
| 1-c | 1.0          | 0.100      | 53.0       | 52.2  | 74.3  | 3.7                  | 4.1   | 5.0   | 0.0152    |
| 2-a | 0.3          | 0.055      | 78.3       | 47.5  | 67.9  | 17.0                 | 19.6  | 24.4  | 0.0252    |
| 2-b | 0.5          | 0.055      | 65.3       | 55.2  | 58.0  | 10.2                 | 11.8  | 14.6  | 0.0110    |
| 2-c | 1.0          | 0.055      | 56.8       | 48.5  | 60.4  | 5.0                  | 5.9   | 7.3   | 0.0059    |

$P_1$ ,  $\alpha$ -lactoalbumin;  $P_2$ , ovalbumin;  $P_3$ ,  $\beta$ -lactoglobulin.



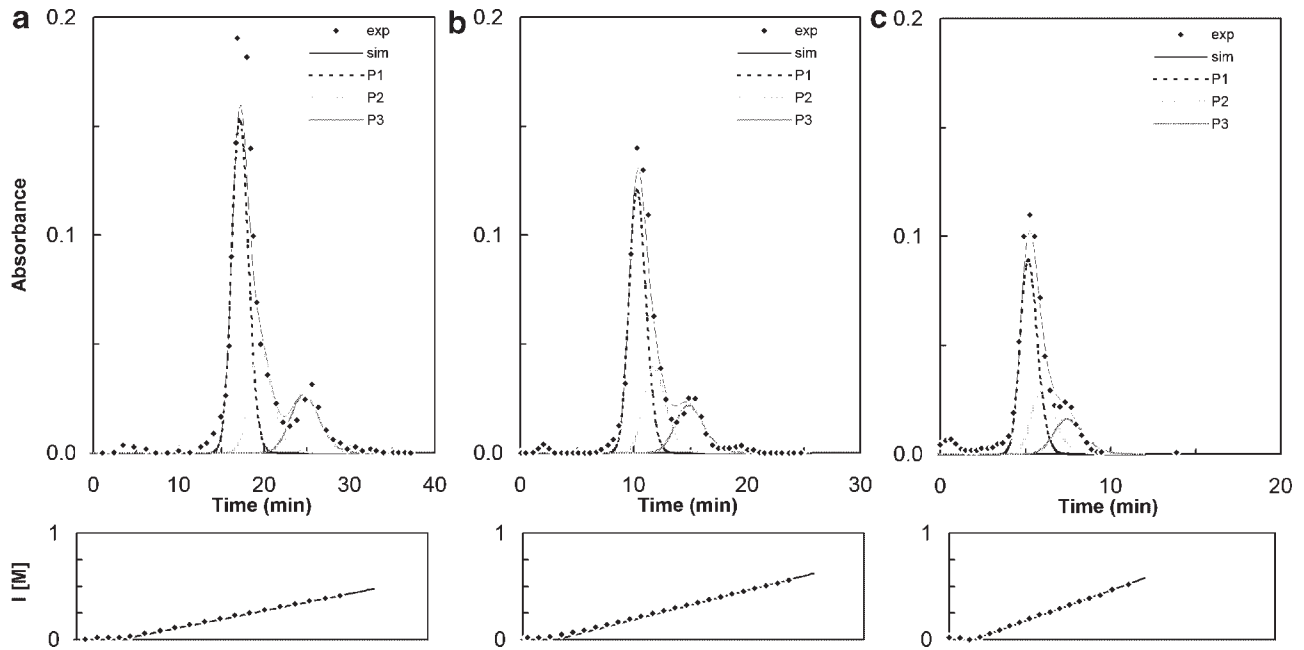


**Figure 2.** Experimental and simulated elution curves of the three-protein mixture using the Plate Model ( $P_1$ ,  $\alpha$ -lactalbumin;  $P_2$ , ovalbumin;  $P_3$ ,  $\beta$ -lactoglobulin) for an ionic strength gradient of 0.055 M/mL and different flow rates. **a:** 0.3 mL/min  $N_{P1} = 18$ ;  $N_{P2} = 6$ ;  $N_{P3} = 6$  **(b)** 0.7 mL/min  $N_{P1} = 11$ ;  $N_{P2} = 4$ ;  $N_{P3} = 4$  **(c)** 1 mL/min  $N_{P1} = 6$ ;  $N_{P2} = 2$ ;  $N_{P3} = 2$ .

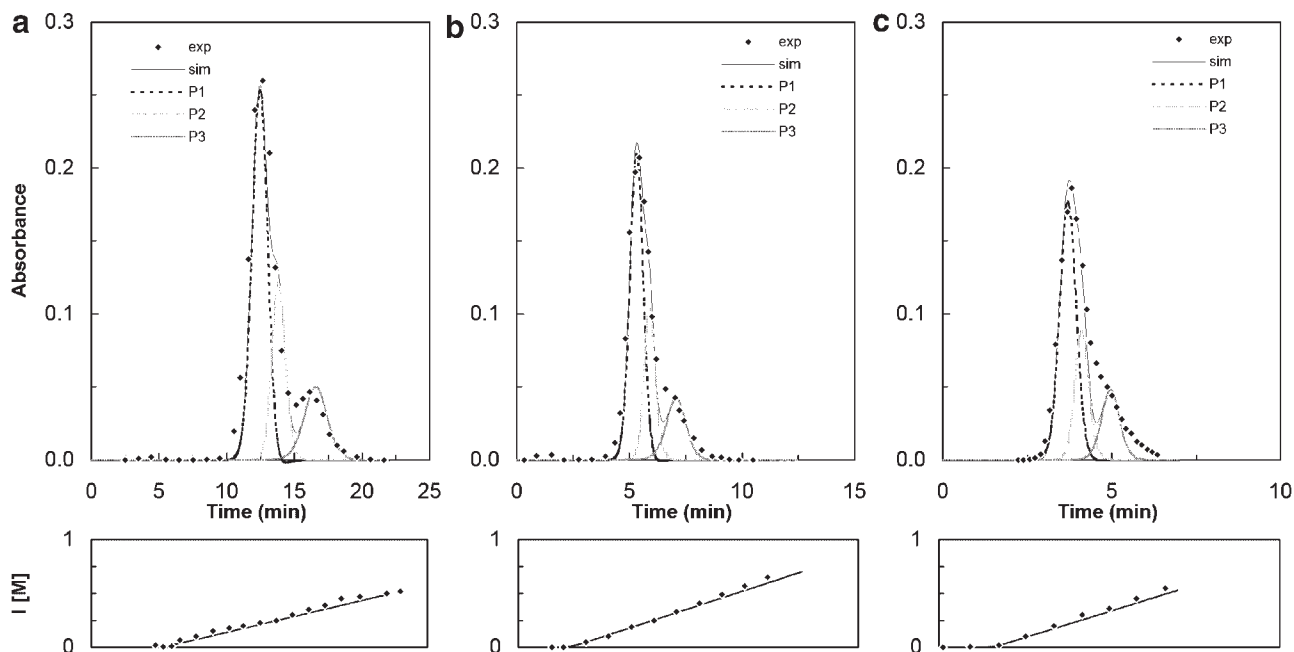
$[N_{P1} + N_{P2} + N_{P3}]$  ODE were solved the number of ODE in the Rate Model was  $N_1 \cdot N_2 \cdot [\text{number of proteins in the mixture} + 1]$ , with  $N_1$  the number of nodes in the axial position in the column and  $N_2$  the number of nodes inside the adsorbent particle. In this work values for  $N_1$  and  $N_2$  were 20 and 2, respectively.

### Effect of the Parameter Values on the Results Predicted by the Plate Model

The capability of the Plate Model for simulating elution curves in IEC depends on the value of parameters that have to be estimated from experimental data. These parameters are



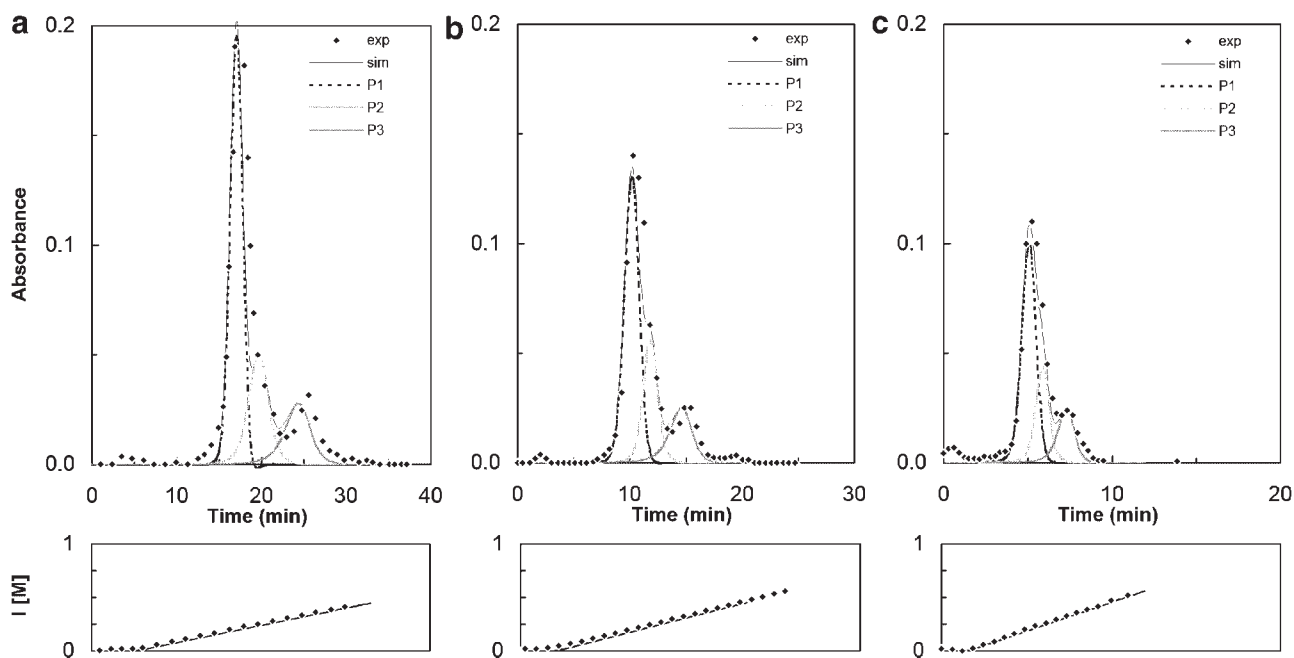
**Figure 3.** Experimental and simulated elution curves of the three-protein mixture using the Plate Model ( $P_1$ ,  $\alpha$ -lactalbumin;  $P_2$ , ovalbumin;  $P_3$ ,  $\beta$ -lactoglobulin) obtained for an ionic strength gradient of 0.1 M/mL and different flow rates. **a:** 0.3 mL/min  $N_{P1} = 26$ ;  $N_{P2} = 10$ ;  $N_{P3} = 9$  **(b)** 0.5 mL/min  $N_{P1} = 12$ ;  $N_{P2} = 5$ ;  $N_{P3} = 4$  **(c)** 1 mL/min  $N_{P1} = 9$ ;  $N_{P2} = 3$ ;  $N_{P3} = 3$ .



**Figure 4.** Experimental and simulated elution curves of the three-protein mixture using the Rate Model ( $P_1$ ,  $\alpha$ -lactalbumin;  $P_2$ , ovalbumin;  $P_3$ ,  $\beta$ -lactoglobulin) obtained for an ionic strength gradient of 0.055 M/mL and different flow rates. **a:** 0.3 mL/min  $\eta_{P1} = 10$ ;  $\eta_{P2} = 9.5$ ;  $\eta_{P3} = 4.5$  **(b)** 0.7 mL/min  $\eta_{P1} = 6$ ;  $\eta_{P2} = 6$ ;  $\eta_{P3} = 3$  **(c)** 1 mL/min  $\eta_{P1} = 4$ ;  $\eta_{P2} = 4$ ;  $\eta_{P3} = 4$ .

those in the adsorption kinetic relationship (A and B) and the values for  $K_{crit}$  and  $D_{crit}$ . In order to test the effect that a deviated value could have on the predictions given by the Plate Model, simulations were carried out using parameters having  $\pm 10\%$  error. The number of plates, purity and retention times for each protein computed using the deviated parameters are shown in Table VII for the case in which the

flow rate and ionic strength gradient were equal to 0.3 mL/min and 0.1 M/mL, respectively. These results show that positively deviated values of parameters A and B do not affect the value for the number of plates, although negative deviations increase the number of plates for  $\alpha$ -lactalbumin. The use of positively deviated values of  $K_{crit}$  and  $D_{crit}$  increases the number of plates while negative deviations



**Figure 5.** Experimental and simulated elution curves of the three- protein mixture using the Rate Model ( $P_1$ ,  $\alpha$ -lactalbumin;  $P_2$ , ovalbumin;  $P_3$ ,  $\beta$ -lactoglobulin) obtained for an ionic strength gradient of 0.1 M/mL and different flow rates. **a:** 0.3 mL/min  $\eta_{P1} = 14$ ;  $\eta_{P2} = 3.5$ ;  $\eta_{P3} = 4.5$  **(b)** 0.5 mL/min  $\eta_{P1} = 5.5$ ;  $\eta_{P2} = 4.5$ ;  $\eta_{P3} = 3.5$  **(c)** 1 mL/min  $\eta_{P1} = 3$ ;  $\eta_{P2} = 2.5$ ;  $\eta_{P3} = 3.5$ .

**Table VII.** Effect of the parameter values in the plate model on the number of plates,  $N_p$ , purity and retention time computed using a flow rate of 0.3 mL/min and an ionic strength gradient of 0.1 M/mL.

| Parameter  | Deviation | $N_p$ |       |       | Purity (%) |       |       | Retention time (min) |       |       | Deviation |
|------------|-----------|-------|-------|-------|------------|-------|-------|----------------------|-------|-------|-----------|
|            |           | $P_1$ | $P_2$ | $P_3$ | $P_1$      | $P_2$ | $P_3$ | $P_1$                | $P_2$ | $P_3$ |           |
| A          | +         | 26    | 10    | 9     | 54.5       | 49.4  | 80.6  | 12.3                 | 13.4  | 16.5  | 0.0149    |
|            |           | 26    | 10    | 9     | 54.0       | 49.2  | 81.3  | 12.4                 | 13.5  | 16.6  | 0.0229    |
|            |           | 26    | 10    | 9     | 55.0       | 50.0  | 81.6  | 13.3                 | 14.5  | 17.6  | 0.0729    |
|            |           | 28    | 11    | 9     | 54.2       | 50.2  | 81.7  | 12.3                 | 13.4  | 16.5  | 0.0171    |
| $K_{crit}$ |           | 29    | 11    | 10    | 55.6       | 49.3  | 84.4  | 12.3                 | 13.4  | 16.5  | 0.0166    |
| $D_{crit}$ |           | 27    | 10    | 9     | 56.5       | 48.6  | 79.9  | 12.2                 | 13.3  | 16.3  | 0.0133    |
| A          | −         | 28    | 10    | 9     | 55.0       | 47.9  | 79.1  | 11.2                 | 12.2  | 15.2  | 0.0654    |
| B          |           | 25    | 9     | 8     | 57.3       | 45.8  | 79.2  | 12.3                 | 13.4  | 16.5  | 0.0165    |
| $K_{crit}$ |           | 24    | 9     | 8     | 57.6       | 44.8  | 78.9  | 12.3                 | 13.4  | 16.5  | 0.0154    |
| $D_{crit}$ |           |       |       |       |            |       |       |                      |       |       |           |

$P_1$ ,  $\alpha$ -lactalbumin;  $P_2$ , ovalbumin;  $P_3$ ,  $\beta$ -lactoglobulin.

result in a decrease in the number of plates for almost all of the proteins in the mixture. Retention time is quite sensitive to the value of  $B$ , parameter that defines the effect of the ionic strength on the distribution coefficient. These results and the simulation results presented in Figures 2 and 3 show that the methodologies used for parameter identification provide good estimates.

### Selection of the Operational Conditions in IEC and Cost Function

Results in Tables V and VI show that flow rate and the ionic strength gradient affect the purity and retention time of the different proteins in the mixture. A higher purity is obtained by using a small ionic strength gradient for a given flow rate. However, when a small value of the ionic strength gradient is chosen the peak width increases, the maximum protein concentration decreases and the retention time increases. Since IEC is in many cases one step in the protein production process its output will affect other steps. The best way of relating how the results obtained in IEC (process time, concentration of the target protein in the collected volume and purity and yield of the target protein) affect other stages in the production process is through a cost function since in many cases the value of the product is fixed by the market and thus the main way to increase the profit is through the reduction of the processing costs. A cost function for a protein production process and how the chromatography performance affects it, similar to that proposed previously (Huenupí et al., 1999) for a protein extraction process, can be defined as follows:

$$\text{Cost} = a_1 \frac{B_1}{\text{Yield}} + a_2 \left( 1 - \frac{\text{Purity}}{B_2} \right) + a_3 \frac{B_3}{C_A} + a_4 \frac{t_{\text{end}}}{B_4} \quad (14)$$

The first term in Equation 14 takes into account costs involved in the fermentation in such a way that as the IEC's yield decreases more protein mixture will be needed to fulfill the required production level. The second term

represents costs involved in further purification steps for instance hydrophobic interaction chromatography (Asenjo and Andrews, 2004). As the purity of the product eluted from the IEC increases these costs decrease and they become equal to zero in the case where the separation is accomplished in this stage only. Costs related to concentration processes such as dialysis, ultrafiltration, or freeze-drying, are inversely related to the concentration of the product obtained in the IEC which corresponds to the third term in Equation 14. The last term in Equation 14 takes into account the costs of the IEC determined by the processing time. A longer processing time may result in higher resolution but this will determine the use of a larger unit or more than one unit in parallel thus increasing the cost for the specified production level. Values of coefficients  $a_1$ ,  $a_2$ ,  $a_3$ , and  $a_4$  in relationship (14) give the relative weights to the different terms in the cost function; the sum of these coefficients is constrained to be 1. Parameters  $B_1$  to  $B_4$  in relationship (14) are introduced in order to scale the different variables. Values for these parameters will depend on the system geometry and the range of operational conditions that can be used in a given system. Relationship (14) states that costs of the different stages, given by the different terms are linearly related to the variables. However, scaling indexes similar to those used for equipment scale-up (exponents in the different terms) can be introduced in order to build a more rigorous model (Huenupí et al., 1999).

The cost function in relationship (14) was evaluated considering the case in which the target protein is ovalbumin, a protein whose retention time was found to be between those of the other two proteins in the mixture, as a way to consider the worst case in a given protein purification process. It was assumed that flow rate and ionic strength gradient are constrained to take values between 0.3 and 1.0 mL/min, and 0.055 and 0.105 M/mL, respectively. From the scheme shown in Figure 1 the fraction ( $f$ ) of the peak collected is given by:

$$f = \frac{t_2 - t_1}{t_e - t_i} \quad (15)$$



For the range of operational conditions tested values for parameters  $B_1$ ,  $B_2$ ,  $B_3$ , and  $B_4$ , in relationship (14) were chosen so that each term could reach a maximum value of 1. Hence  $B_1$ ,  $B_2$ ,  $B_3$ , and  $B_4$  were 0.72, 77%, 0.0345 g/l and 28.54 min, respectively.

Values of coefficients  $a_1$ ,  $a_2$ ,  $a_3$ , and  $a_4$  depend on the characteristics of the target protein such as the required final purity and its synthesis during the fermentation. Costs involved in a fermentation process for protein synthesis can be assumed to represent between 30 and 70% of total production cost (Huenupri et al., 1999). Purification costs ( $a_2 + a_4$ ) can represent between 10 and 50% of a protein production process. Costs for the concentration stages ( $a_3$ ) can be considered lower than those involved in the purification stages (between 10 and 30%).

### Minimum Cost Operation, Selection of Peak Size

Simulations were carried out using different values of the flow rate and ionic strength gradients and the cost function was evaluated for different values of  $f$  [fraction of product peak collected (Eq. 15)]. Three different combinations for the  $a_i$  ( $i = 1, \dots, 4$ ) coefficients were considered in order to simulate conditions for the production of different types of target proteins. Table VIII shows the flow rate, ionic strength gradient and the value for  $f$  found, for which the minimum value of each of the cost functions was obtained.

In case 1 the operational conditions for IEC in a process in which costs for the fermentation process, subsequent purification steps, and IEC contribute in the same degree to the total production costs ( $a_1 = a_2 = a_4 = 0.30$ ) are presented. This could be the case of an enzyme required with a low purity and for which purification is carried out in order to eliminate contaminants that decrease its activity, for instance an industrial enzyme. For this case flow rate, ionic strength

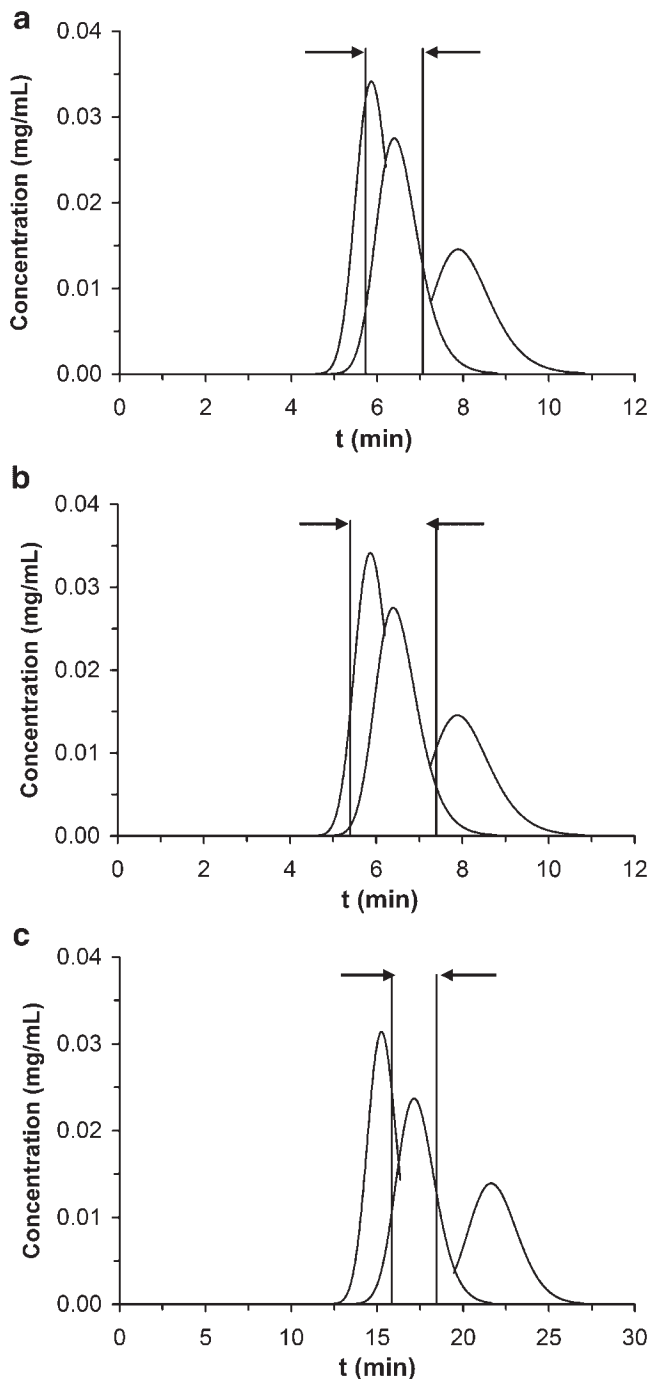
gradient and fraction collected were equal to 0.6 L/min, 0.105 M/mL and 0.5, respectively. As expected the final purity is relatively low, 53.1%, and a high yield is obtained.

Case 2 (Table VIII), shows the operational conditions in the IEC for the production of a target protein having very high fermentation costs ( $a_1 = 0.55$ ). Subsequent purification stages ( $a_2 = 0.2$ ) and those involved in the IEC ( $a_4 = 0.2$ ) are of the same magnitude and lower than in the previous

**Table VIII.** Operational conditions, protein yield and purity in a chromatography separation for minimum production cost based on the cost function given by Equation 14 and different relative weights ( $a_i$ ) for the different production stages.

|                 | Case 1                              | Case 2 | Case 3 |
|-----------------|-------------------------------------|--------|--------|
| $a_1$           | 0.30                                | 0.55   | 0.30   |
| $a_2$           | 0.30                                | 0.20   | 0.50   |
| $a_3$           | 0.10                                | 0.05   | 0.05   |
| $a_4$           | 0.30                                | 0.20   | 0.15   |
| Min cost        | 0.593                               | 0.651  | 0.510  |
|                 | Operational conditions <sup>a</sup> |        |        |
| $F$ (mL/min)    | 0.6                                 | 0.6    | 0.3    |
| $g$ (M/mL)      | 0.105                               | 0.105  | 0.065  |
| $f$ (—)         | 0.50                                | 0.75   | 0.45   |
|                 | Results                             |        |        |
| Yield           | 0.8195                              | 0.9455 | 0.7605 |
| Purity (%)      | 53.10                               | 47.32  | 73.67  |
| $C_A$ (mg/mL)   | 0.0260                              | 0.0200 | 0.0243 |
| $t_{end}$ (min) | 9.82                                | 9.82   | 25.34  |

<sup>a</sup>operational conditions were constrained to take values of 0.3–1.0 mL/min for  $F$ , 0.055–0.105 M/mL for  $g$  and 0.0–1.0 for  $f$  (with increments of 0.1, 0.005, and 0.05, respectively).



**Figure 6.** Elution curves of the mixture of proteins in a chromatography separation for the minimum production cost given in Table VIII. Between the arrows the fraction of protein product collected for (a) case 1 (b) case 2 (c) case 3. The three cases correspond to protein products with different characteristics as described in the text.

case. This could be the case of an intracellular target protein with low substrate into target protein yield. In this case costs for cell disruption and separation from cell debris are assumed to be included in those for the fermentation. Results indicate that during the IEC a higher fraction of the volume should be collected ( $f = 0.75$ ). This results in an even lower purity and higher yield than in the previous case.

IEC operational conditions for the production of a target protein required with a high final purity, for instance a pharmaceutical product, are shown in Case 3 (Table VIII). Costs involved in the subsequent purification stages are set as 50% of the total production costs ( $a_2 = 0.5$ ). Operational conditions in the IEC stage for this case are those of a product with a high purity (73.67%). In order to achieve the high purity IEC must be carried out at the lowest flow rate (0.3 mL/min) and with a small ionic strength gradient (0.065 M/mL). As fermentation costs are relatively low collection of the eluted protein corresponds to a small fraction of total elution time ( $f = 0.45$ ) in order to obtain a high purity protein fraction. This results in a higher purity and lower yield than in the two previous cases and a longer processing time which results in a higher resolution. The actual fractions collected in all cases are clearly shown in Figure 6.

## CONCLUSIONS

The elution curve of a protein mixture in IEC can be simulated using either the Plate or Rate Models. Deviations obtained with both models are of the same magnitude although results predicted by the Plate Model are better for the protein mixture used in this work (0.0189), in addition to much lower computational CPU costs. Although the Rate Model is more fundamentally based it needs many more parameters that have to be estimated from theoretical correlations not specific for the system under study. Moreover, since values for the diffusion coefficient inside the adsorbent particle were not available one dimensionless number ( $\eta$ ) in the Rate Model was estimated. On the other hand, predictions given by the Plate Model depend on parameters that are estimated from experiments specifically designed for the system under study. Relatively low protein concentrations were used to avoid protein–protein interactions. At higher protein concentrations the equilibrium relationships would have to be modified and in many cases the protein peaks would become less symmetrical.

Simulation of IEC for protein purification can be used as a tool for choosing operational conditions such as flow rate, ionic strength gradient and the externally fixed operational condition that in this work was termed the collecting time (fraction collected,  $f$ ). In order to do this a performance function for IEC has to be defined. However, since a purification stage such as IEC is integrated into the protein production process its performance is affected by previous and possibly subsequent processing and purification stages. In this work a cost function for the whole protein production process that can be used for the selection of the operational conditions as well as the fraction of the product to be collected (peak cutting) in IEC was built and tested. This function can be used for protein products with different characteristics and qualities such as purity and yield by choosing the appropriate parameters.

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