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Review

Mathematical Modeling of Protein Chromatograms

The most used mathematical models with a phenomenological basis for simulating chromatographic curves of proteins in size exclusion chromatography, ion exchange chromatography, affinity chromatography, and hydrophobic interaction chromatography are reviewed. The plate model (PM) and the general rate model (GRM) are briefly described, followed by various applications of these models to the different chromatographic strategies. Based on these examples it is concluded that the GRM is the most complete and informative model, despite it needs several parameters that have to be estimated from theoretical correlations nonspecific for proteins. Additionally, values for the effective pore diffusion coefficient are not generally available. Appropriate calibration leads in most cases to predictions that compare favorably with experimental data. The possibility to predict chromatographic curves under different operational conditions similar to those used at industrial scale by applying mathematical models is still a challenge because it could contribute to the reduction of costs involved in suitable purification processes. In addition, new proteins are continually designed and for each case different conditions are needed.

Keywords: Chromatography, General plate model, Plate model, Process modeling, Protein chromatograms

Received: June 01, 2011; *revised:* August 01, 2011; *accepted:* August 01, 2011

DOI: 10.1002/ceat.201100282

1 Introduction

Most biotechnology industry products require a purification process after product recuperation in order to achieve a proper degree of purification [1]. The necessary purity level depends on the use of the final product, thus therapeutic products require high levels of purity (usually 99.9 % or higher) when compared to products intended for industrial use, such as enzymes. In the case of highly purified products, the process involves a number of purification stages, usually chromatographic steps. The design of the sequence of purification stages represents a compromise between the desired degree of purity and the resolution and activity obtained [2]. Each stage uses a physicochemical property which is distributed differently among the target product and its contaminants. In this way, this physicochemical property defines the chromatographic stage: size and shape in the case of size exclusion chromatography (SEC) [3], charge in ion exchange chromatography (IEX) [4], a specific property in affinity chromatography (AC) [5–7], and hydrophobicity in hydrophobic interaction chromatography (HIC) [8–11].

There are several approaches for modeling elution curves in chromatography, however, two of them have been more commonly used: the theoretical plate model (PM) [12] and the general rate model (GRM) [13]. Both models have been applied to describe the breakthrough curves in frontal analysis [14–18] and to represent isocratic and gradient elution curves of a pulsed injection [19, 20]. Simulations have been applied to study phenomenological aspects of the process and extended to optimize the separation of binary protein mixtures and of monomer/aggregated protein species. Both approaches consider that adsorption is given by an adsorption isotherm, whose mathematical form and parameters may vary depending on process conditions and model constraints. Most of the models based on fundamental principles use the Langmuir isotherm for describing protein interaction with the adsorbent. Estimation of mass transfer parameters is strongly affected by the type of isotherm, and then the description of adsorption is a key factor in model accuracy and usefulness. The different approaches used to describe macromolecular retention and elution in SEC, HIC, IEX, and AC are presented below.

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1.1 Affinity Chromatography (AC)

AC is widely recognized as the protein purification method with the highest selectivity. It has been used to purify monoclonal antibodies on immobilized protein A columns [5], human tissue plasminogen from blood plasma by immobilized lysine [6], and ATP-dependent kinases and NAD^+-dependent dehydrogenases by immobilized 5′-AMP [7], to name just a few. In the case of proteins without a proper affinity partner, alternative technologies include the recombinant fusion of a tag with a highly specific binding partner, such as S-transferase tag [21, 22], calmodulin-binding peptide tag [23–25], streptavidin tag [26, 27], FLAG peptide tag [28, 29], and polyhistidine tag [28, 30, 31]. Recent mathematical modeling related to AC includes a two-zone model (TZM) used for describing the rate of solute uptake by the stationary phase of a sorption-type chromatography [32] and a support vector machine regression model to predict the retention of peptides in immobilized metal-affinity chromatography [33].

1.2 Ion Exchange Chromatography (IEX)

IEX is widely used in purification process [34–38], usually as an early stage followed by HIC [1]. Purification of a mixture of proteins by means of IEX is based on the electrostatic interaction between proteins and a charged stationary phase. For instance, when the stationary phase is positively charged (anion exchangers), negatively charged proteins bind reversibly to the matrix. Nearly neutral or positively charged proteins establish weaker interactions with the matrix and pass through the column. In most cases, elution is achieved with an increasing gradient of salt concentration, even when the use of a pH gradient is also possible [4]. The mathematical modeling of IEX has been tackled using various approaches. The retention time in IEX columns has been predicted using semi-empirical models based on the properties of the protein and the ligand on the stationary phase, such as the steric mass-action model (SMA) [39], the available area model [40, 41], or the stoichiometric displacement model [42–44]. Quantitative structure-property relationship (QSPR) models based on structural descriptors have been applied to take into account changes in the charge distribution on the protein surface produced by changes in the pH of the mobile phase [45, 46]. In addition, models based on electrostatic calculations and van der Waals forces [47], Gibbs free energy [48], and, more recently, molecular dynamics [49] have been developed.

1.3 Hydrophobic Interaction Chromatography (HIC)

In HIC, high concentrations of a cosmotropic salt promote the reversible binding of proteins to the hydrophobic ligand attached to the stationary phase support. Protein adsorption occurs as a result of the interaction between hydrophobic zones on the protein surface and the hydrophobic ligands [8]. Elution is achieved when the ionic strength of the mobile phase is reduced using a linear or a stepwise procedure [9–11]. Protein behavior in HIC depends on the type of salt and its initial concentrations [10, 50], characteristics of the chromatographic matrix [51], and hydrophobicity of the protein [52, 53]. Great efforts have been devoted to predict the retention time of proteins in HIC based on estimations of their hydrophobicity. In fact, models based on structural information [53, 54], amino acid sequence [55, 56], statistical description of the protein surface [57, 58], thermodynamic models [59], molecular docking simulations [60], and QSPR models based on a number of protein properties [45, 46] have been proposed. In addition, models that consider operational conditions have been divided in (i) thermodynamic models which take into account temperature and salt concentration [61], (ii) statistical models based on operational parameters such as ionic strength, surface tension, and ligand density [62], and (iii) models based on the ammonium sulfate concentration in the elution gradient [63].

1.4 Size Exclusion Chromatography (SEC)

SEC is a nonadsorption chromatography that separates proteins solely on the basis of molecular size and shape. Separation is achieved using a porous matrix to which the molecules, for steric reasons, have different degrees of accessibility. Then smaller molecules have greater access and larger molecules are excluded from the matrix. Hence, proteins are eluted from the SEC column in decreasing order of size [3]. SEC is also called gel filtration (GF) or gel permeation chromatography.

2 Theoretical Plate Model (PM)

Application of the plate theory to chromatography was introduced by Martin and Synge in 1941. The theory based on the *theoretical plate* concept was developed for substances with linear distribution isotherms [64]. The theoretical PM considers that the chromatographic column is divided in a number of theoretical stages in equilibrium with each other called theoretical plates (N_p), each of them having the same ratio between the stationary phase volume and the volume of the mobile phase (H). The model supposes that the protein concentration in the stationary phase is in equilibrium with that in the mobile phase, and that the time required to achieve equilibrium is negligible. Also, it is assumed that (i) the flow is constant and there is no mixing among the plates, (ii) the distribution coefficient for the salt (K_{salt}) is not affected by the presence of protein, and (iii) the number of theoretical plates for protein and salt are constants.

According to the plate model, the protein concentration in each plate (C_{bi}) is given by the following equation [12]:

$$\frac{dC_{bi}}{d\tau} = \frac{N_p(C_{bi-1} - C_{bi}) - C_{bi}\,H\dfrac{dK(C_{bi}I_i)}{dI}\dfrac{dI_i}{d\tau}}{1 + H\left[K(C_{bi}I_i) + C_{bi}\dfrac{dK(C_{bi}I_i)}{dC_{bi}}\right]} \qquad (1)$$

$\forall\, i = 1, ..N_p$

where τ is the dimensionless time, K is the distribution coefficient for the protein, and I_i is the ionic strength of the mobile phase at plate i.

When protein elution is promoted by an ionic strength gradient with constant steepness, the ionic strength at plate i as a function of time is given by the following expression [55]:

$$I_i = \begin{cases} > I_0, & \tau \le (1 + HK_{salt})\dfrac{i}{N_p} \\ I_0 + G\left[\tau - (1 + HK_{salt})\dfrac{i}{N_p}\right], & \tau > (1 + HK_{salt})\dfrac{i}{N_p} \end{cases}$$
$$\forall\, i = 1,\ldots, N_p \tag{2}$$

where G is the steepness ionic strength gradient and I_0 is the initial ionic strength.

Even though the model is relatively simple with few parameters, it does not take into account the interactions between proteins that occur during adsorption. Some proteins follow the Langmuir isotherm when interacting with weak ion-exchangers. At low protein concentration, the adsorption kinetics is computed from the value of a distribution coefficient $K(I)$ that depends only on the ionic strength (I). Yamamoto et al. proposed the following equation for the concentration of the adsorbed protein (C^*_{bi}) [12, 65]:

$$C^*_{bi} = K(I)C_{bi} = (AI^B + K_{crit})C_{bi} \tag{3}$$

where K_{crit} is the critical distribution coefficient which is estimated from the first moment of elution curves carried out at different flow rates keeping constant the ionic strength of the mobile phase [34]. Parameters A and B for a given protein in Eq. (3) are estimated from elution curves carried out with different gradients keeping the flow rate constant [34].

An isotherm such as that in Eq. (3) does not take into account interactions among proteins in a mixture. Thus, although elution profiles of the proteins in a mixture can be predicted, they are independently calculated.

The set of N_p ordinary differential equations (ODE) described by Eq. (1) is solved considering the following initial and sample conditions:

$$\begin{aligned} &C_{bo} = \ldots = C_{bNp} = 0 && \tau = -\frac{V_m}{V_R} \\ &C_{bo} = 1 && -\frac{V_m}{V_R} < \tau \le 0 \\ &C_{bo} = 0 && \tau > 0 \end{aligned} \tag{4}$$

where C_{bo} is the protein concentration in the feeding, V_m is the sample volume, and V_R is the retention volume.

When the protein is subject to an ionic strength along the column near to the one at which it is eluted (I_{max}), the following equation has been proposed [12] in order to calculate N_p:

$$N_p = \frac{L}{\dfrac{2D_L}{v} + \dfrac{d_p^2 HK_{lmax}^2 v}{30D_{crit}K_{crit}(1 + HK_{Imax})^2}} \tag{5}$$

where L is the length of the column, D_L is the axial dispersion coefficient, v is the velocity of the mobile phase, d_p is the particle diameter, K_{lmax} is the distribution coefficient calculated for $I = I_{max}$, and D_{crit} is the critical diffusivity.

It is important to note that the model given by Eqs. (1)–(5) is not a general but a proposed one, which has been used by several researchers. Particular applications of PM will be described below.

2.1 PM for IEX

The PM has been used by several authors to describe and simulate chromatographic separation processes in IEX. Shene et al. [66] simulated the chromatographic separation of three proteins (α-lactalbumin, ovalbumin, and β-lactoglobulin) on Q-Sepharose. Simulations were carried out with the parameters in Eq. (3) (also shown in Tab. 1), which were estimated from experimental data. The elution curve of the low-concentration (<0.2 mg mL^{-1}) protein mixture in IEX was successfully predicted by the PM. This work demonstrated that the prediction capability of the PM is dependent on the values of parameter K_{crit} that influences N_p for a given protein; on the other hand, the retention time is quite sensitive to the value of B.

Table 1. Parameters of the adsorption kinetics used for simulating IEC on Q-Sepharose FF by the PM [66].

Protein	A	B	K_{crit}
α-Lactalbumin	$3.3 \cdot 10^{-3}$	–4.62	0.816
Ovalbumin	$2.2 \cdot 10^{-5}$	–8.55	0.597
β-Lactoglobulin	$8.1 \cdot 10^{-5}$	–7.81	1.020

2.2 PM for HIC

In the case of HIC, Hahn et al. [67] applied the PM to adjust the breakthrough curves of several globular proteins using different stationary phases in order to estimate the dispersion parameters and to compare the dynamic binding capacity (DBC) of different HIC sorbents. The breakthrough curve was considered as the integral of a Gaussian curve, and N_p was calculated from a normalized response curve, as indicated in Eq. (6). Here, the numerator corresponds to the time at 50 % relative response, and the denominator is the standard deviation.

$$N_p = \frac{t_{0.5}^2}{(t_{0.5} - t_{0.15})^2} \tag{6}$$

N_p was calculated from the breakthrough curves, and the molecular diffusivity in free solution (D_m) was estimated from the plot of reduced height equivalent theoretical plates (h) versus dimensionless velocity (ReSc), given by Eqs. (7) and (8), respectively. The results are summarized in Tab. 2.

$$h = \frac{2D_L}{d_p v} + \frac{\varepsilon v}{1 + k'} \cdot \left(\frac{k'}{1 + k'}\right)^2 \cdot \left(\frac{1}{3\varepsilon_p k_p}\right) \tag{7}$$

$$\mathrm{ReSc} = \frac{v d_p}{D_m} \tag{8}$$

Table 2. Mass transfer parameters derived from the different models used in chromatographic techniques.

Protein	D_p [10^{-8} cm^{2}s^{-1}]	D_m [10^{-6} cm^{2}s^{-1}]	Remarks
Ion exchange chromatography			
α-Lactalbumin	49.3 [65] 18.4–385.4[a] [66]	1.1 [73]	Q-Sepharose FF [66, 73]
Ovalbumin	13.5–385.4[a] [66]	n. a.[b]	Q-Sepharose FF [66]
BSA	26.8	0.7	Q-Sepharose FF [73]
β-Lactoglobulin	16.9–367.1[a] [66]	n. a.	Q-Sepharose FF [66]
Conalbumin	23.9	0.6	Q-Sepharose FF [73]
Hydrophobic interaction chromatography			
Lysozyme	7.3 [67] 9.0 [20] 0.36 [78]	1.1 [67] 1.1 [69] 1.2 [17]	Toyopearl Phenyl 35 µm [67] Toyopearl 650S [20] Phenyl Sepharose FF [69] Phenyl 650M [78] Different media [17]
α-Lactalbumin	0.7 [67] 7.0 [20]	1.1 [17, 67]	Toyopearl Phenyl 35 µm [67] Toyopearl 650S [20] Different media [17]
Ovalbumin	1.2 [20]	0.8 [93]	Sepharose HP [20]
BSA	4.5 [67] 0.8 [20]	0.7 [67] 0.6 [93]	Toyopearl Phenyl Sepharose HP [20]
Lactoglobulin	4.4 [67]	0.7 [67]	Toyopearl Phenyl 35 µm [67]
Immunoglobulin G	0.5 [67]	0.5 [67]	Toyopearl Phenyl 35 µm [67]
RNA	6.6 [20]	1.2 [93]	Toyopearl 650S [20]
Lectin	0.3 [78]	n. a.	Phenyl 650M [78]
Affinity chromatography			
IgG1	19.9	0.5	HiTrap rProtein A FF [72]
IgG2a	19.9	0.5	HiTrap rProtein A FF [72]
IgG2b	19.9	0.5	Trap rProtein A FF [72]
BSA	6.86	0.7	Blue Sepharose CL-6B (Cibacron Blue F-3GA covalently attached to Sepharose CL-6B) [72]
Rabbit hemoglobin	6.94	0.7	Used Blue Sepharose CL-6B [64]
BSA	5.6	0.7	Cibacron Blue 3GA modified Sepharose CL-6B [94]
Bovine hemoglobin	11	0.8	Cibacron Blue 3GA modified Sepharose CL-6B [94]
Lysozyme	21.7	1.1	Affi-Gel 4.5 µm·cm^{-3} Cibracron Blue F-3GA [92]

[a] Estimated from the dimensionless value ($\eta = \varepsilon_p D_p L/(R_p^2 v)$) [66].
[b] n.a. = not available.

Eq. (7) is a simplification of the van Deemter equation [68] which considers that the contribution from mobile phase diffusion is negligible and surface diffusion is not dominant under the experimental conditions used by Hahn et al. [67]. Here, k_p is the pore diffusion mass transfer coefficient, defined by Eq. (9):

$$k_p = \frac{10 D_p}{d_p} \tag{9}$$

where D_p is the pore diffusion coefficient. The authors proved that pore diffusion controlled the mass transfer when ReSc ranges between 10^2 and 10^3, and h fluctuates between 10 and 700 in agreement with LeVan [68].

Additionally, PM has been used to describe and simulate gradient elution curves of proteins in HIC [69]. By using a linear isotherm, it was possible to determine N_p for different feeding rates and to estimate the diffusivity of lysozyme (see Tab. 2). N_p was inversely proportional to the feeding rate. The author concluded that the feeding rate indirectly affects protein retention in HIC, while elution gradient steepness and sample volume influence retention time, peak height, and resolution. In the simulations, a larger gradient steepness resulted in less dispersion, whereas a larger sample volume increased dispersion.

3 General Rate Model (GRM)

The GRM for modeling and simulating chromatographic separations was described by Gu [13]. This mathematical model offers several advantages because it is based on fundamental principles. It is able to predict phenomena of interaction between proteins as well as the effect of different process conditions and adsorbent properties.

For each protein in the mixture and also for the displacer compound (salt, protons, etc.), the

model consists of a set of two governing partial differential equations (PDE) that describe the movement of the proteins and displacer through the bed and inside the adsorbent particles. In addition, a differential equation describing the adsorption kinetics for each protein is also part of the GRM. The mechanism through which the proteins bind the adsorbent particles and are afterwards eluted defines the relationships between adsorption/desorption constant rates and concentration of the displacer compound.

The GRM is generally formulated and solved considering several assumptions and conditions: the column is isothermal, radial dispersion is negligible in the bed (no concentration gradients in radial direction); adsorbent particles are spheres of constant radii (R_p); the porosities of the bed (ε_b) and adsorbent particles (ε_p) are constant, at the column inlet protein dispersion flux equals the input mass flux, and at the column outlet protein dispersion flux is null; distribution of the different compounds inside the adsorbent particles is symmetric, and at the surface of adsorbent particles diffusional flux to/from the inside of the particle equals the mass transfer flux from/to the mobile phase; before the sample is loaded, the system does not contain protein, at this time the bed has been equilibrated with a buffer having specific properties.

Mass balances applied to each of the components in the fluid phase in a differential element of column length give rise to PDE (i = 1,...N+1; N represents the number of proteins in the mixture and N+1 corresponds to the displacer compound) given by:

$$-D_{Li}\frac{\partial^2 C_{bi}}{\partial Z^2}+\nu\frac{\partial C_{bi}}{\partial Z}+\frac{\partial C_{bi}}{\partial t}+\frac{3k_i(1-\varepsilon_b)}{\varepsilon_b R_P}(C_{bi}-C_{pi,R=R_p})=0 \tag{10}$$

In PDE (Eq. (10)), the first and second terms represent the transport of protein by axial dispersion and convective flow, respectively. The last term in Eq. (10) is the mass transfer flux from the mobile phase to the adsorbent particle interface.

Inside the adsorbent particles, proteins are either in solution (C_{Pi}) or adsorbed onto the particle surface (C_{Pi}^*). PDE for the proteins inside the adsorbent particle is:

$$(1-\varepsilon_P)\frac{\partial C_{Pi}^*}{\partial t}+\varepsilon_P\frac{\partial C_{Pi}}{\partial t}-\varepsilon_P D_{Pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{Pi}}{\partial R}\right)\right]=0 \tag{11}$$

Protein accumulation inside the adsorbent particles is due to any differences between component diffusion through the stagnant fluid phase (third term in Eq. (11)) and its adsorption rate (first term in Eq. (11)). Some authors do not consider the first term in Eq. (11) for the displacer compound under the assumption that this compound does not interact with the adsorbent.

Adsorption of a protein onto the surface of solid particles is a reversible process. The desorption reaction is assumed to be of first order in the concentration of the adsorbed protein. The adsorption reaction depends on the protein concentration in the stagnant phase and also on the concentration of sites available for the adsorption. Thus, it follows:

$$\frac{\partial C_{Pi}^*}{\partial t}=k_{ai}C_{Pi}\left(C^\infty-\sum_{j=1}^{N}C_{Pj}^*\right)-k_{di}C_{Pi}^* \tag{12}$$

In the differential equation (12) rate constants for the adsorption and desorption kinetics are given by k_a and k_d, respectively. C^∞ is the adsorbent saturation capacity, a property of the adsorbent that depends on the protein properties. In a mixture formed by proteins of similar sizes and structures, the term in brackets is valid. However, upon binding, a protein may interact with a number of sites. In addition, binding of the protein may sterically shield or block a number of sites. Zhang and Sun [70] used two parameters for modeling these effects: the characteristic number of binding sites and a steric factor.

When the saturation capacities are the same for all components (C^∞) and the adsorption/desorption rates are relatively large compared to the mass transfer rates, then instant adsorption/desorption equilibrium can be assumed. When both sides of Eq. (12) can be set to zero, the Langmuir isotherm is obtained:

$$C_{pi}^*=\frac{a_i C_{pi}}{1+\sum_{j=1}^{N}b_j C_{pj}} \tag{13}$$

The Langmuir parameters b_j and a_i are equal to the ratio k_{aj}/k_{dj} for protein j and b_iC^∞, respectively. Adsorption and desorption constants (and thus the Langmuir parameters a_i and b_i) are not constant in HIC, IEC, or AC, but they depend on the concentration of the displacer compound.

The relationship proposed by Melander et al. [50] that relates the retention factor k' and the concentration of the displacer has been used in the simulation of HIC [71], AC [72], and IEX [77]:

$$\log_{10}k'=\alpha'-\beta\log_{10}C_{N+1}+\gamma C_{N+1} \tag{14}$$

where β and γ are the electrostatic and hydrophobic interaction parameters, respectively, and α' is a constant involving characteristic system parameters. These three parameters have to be determined experimentally for each protein. The retention factor and Langmuir parameter b are related through $k'=\phi C^\infty\cdot b$, in which ϕ is the ratio between the particle skeleton volume and the volume of the mobile phase including the particle macropores inside the column [74]. Thus, it follows for the Langmuir parameter b:

$$\log_{10}b=\log_{10}\left(\frac{k_a}{k_d}\right)=\alpha-\beta\log_{10}C_{N+1}+\gamma C_{N+1} \tag{15}$$

In Eq. (15), the term ϕC^∞ was lumped into a parameter α ($=\alpha'\log_{10}(\phi C^\infty)$). In the HIC simulation β was set to zero [71] because the process is driven by the hydrophobic interaction effect while for IEX γ was set to zero [73].

In the simulation of AC it was assumed that the properties of the mobile phase (C_{N+1}, representing the pH and ionic strength) affect the desorption rate constant in Eq. (12) [72]; this effect was modeled through simple relationships. For the elution with changes in the pH of the mobile phase it follows:

$$k_{di}=\alpha_i' e^{\beta_i'\left(C_0/C_{N+1,max}-C_{N+1}/C_{N+1,max}\right)} \tag{16}$$

For elution induced by changes in the ionic strength of the mobile phase, the following relationship was used:

$$k_{di} = a_i' e^{\beta_i'\left(C_{N+1}/C_{N+1,max} - C_0/C_{N+1,max}\right)} \quad (17)$$

Parameters a_i' and β_i' for each protein depend on the protein-adsorbent affinity, and C_0 is the initial ionic strength or pH of the mobile phase. The relationships proposed for the desorption rate constant permit its increase once the ionic strength or pH in the mobile phase reaches the value at which protein affinity to the adsorbent is reduced.

The use of dimensionless variables simplifies the model solution and also reduces the number of parameters grouping them into more significant terms, enabling their comparison under different operational conditions. By using the dimensionless variables $c_{bi} = C_{bi}/C_{oi}$, $c_{pi}^* = C_{Pi}^*/C_{oi}$, $\tau = tv/L$, $r = R/R_p$, and $z = Z/L$, the PDEs and the differential equation in the GRM are given by:

$$-\frac{1}{Pe_{Li}}\frac{\partial^2 c_{bi}}{\partial z^2} + \frac{\partial c_{bi}}{\partial z} + \frac{\partial c_{bi}}{\partial \tau} + \xi_i(c_{bi} - c_{pi,r=1}) = 0 \quad (18)$$

$$\frac{\partial}{\partial \tau}\left[(1-\varepsilon_p)c_{pi}^* + \varepsilon_p c_{pi}\right] - \eta_i\left[\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_{pi}}{\partial r}\right)\right] = 0 \quad (19)$$

$$\frac{\partial c_{pi}^*}{\partial \tau} = Da_i^a c_{pi}\left(c_i - \sum_{j=1}^{N}\frac{C_{oj}}{C_{oi}}c_{pi}^*) - Da_i^d c_{pi}^*\right) \quad (20)$$

In these equations, the following dimensionless numbers appear: Peclet number Pe_{Li} ($= vL/D_{Li}$), Biot number Bi_i ($= k_i R_p/(\varepsilon_p D_{pi})$), $\eta_i = \varepsilon_p D_{pi} L/(R_p{}^2 v)$, $\xi = 3Bi_i\eta_i(1-\varepsilon_b)/\varepsilon_b$, and Damköhler numbers for adsorption $D_{ai}{}^a$ ($= Lk_{ai}/v$) and desorption $D_{ai}{}^d$ ($= Lk_{di}/v$). The dimensionless initial and boundary conditions are given by:

$$\begin{aligned}
&\text{I.C.1}: c_{bi}(0,z) = 0 \quad \text{for } i = 1...N \text{ and } c_{bN+1}(0,z) = c_0 \\
&\qquad\qquad \text{at } 0 \le z \le 1 \\
&\text{I.C.2}: c_{pi}(0,z,r) = 0 \quad \text{for } i = 1...N \text{ and } c_{pN+1}(0,z,r) = c_0 \\
&\qquad\qquad \text{at } 0 \le z \le 1; 0 \le r \le 1 \\
&\text{I.C.3}: c_{pi}^*(0,z,r) = 0 \quad \text{for } i = 1...N \text{ at } 0 \le z \le 1; 0 \le r \le 1 \\
&\text{B.C.1.1}: \frac{\partial c_{bi}}{\partial z} = Pe_{Li}\left[c_{bi}(\tau,0) - \frac{C_{Fi}(\tau)}{C_{0i}}\right] \text{ at } z = 0 \\
&\text{B.C.1.2}: \frac{\partial c_{bi}}{\partial z} = 0 \qquad \text{at } z = 1 \\
&\text{B.C.2.1}: \frac{\partial c_{pi}}{\partial r} = 0 \qquad \text{at } r = 0 \text{ for } 0 \le z \le 1 \\
&\text{B.C.2.2}: \frac{\partial c_{pi}}{\partial r} = Bi_i\left[c_{bi}(\tau,z) - c_{pi}(\tau,z,1)\right] \\
&\qquad\qquad \text{at } r = 1 \text{ for } 0 \le z \le 1
\end{aligned} \quad (21)$$

Here, c_0 is the initial dimensionless concentration of the displacer compound. In the model, $t = 0$ corresponds to the moment at which the sample starts to enter the column. Under the assumption that the volume space between the sample loop and the column inlet is negligible, and if the dimensionless time it takes to pump the sample is τ_{imp}, the concentration of protein i at the column inlet is:

$$\frac{C_{Fi}(\tau)}{C_{0i}} = \begin{cases} 1 & 0 \le \tau \le \tau_{imp} \\ 0 & \tau > \tau_{imp} \end{cases} \quad (22)$$

For the displacer compound:

$$\frac{C_{FN+1}(\tau)}{C_{0i}} = \begin{cases} c_0 \ \tau \le \tau_{imp} \\ c_0 \pm \dfrac{\Delta c_m}{\Delta \tau}(\tau - \tau_{imp}) \ \tau > \tau_{imp} \end{cases} \quad (23)$$

where $\Delta c_m/\Delta\tau$ is the gradient steepness in terms of dimensionless variables. The positive sign in Eq. (23) applies in chromatographic separations in which elution is carried out through increases of the displacer concentration in the mobile phase while the negative sign is used when elution is carried out through decreases in its concentrations.

Equations in the GRM are solved numerically. The finite element (with quadratic elements) and the orthogonal collocation methods are often used to discretize the partial bulk-phase and particle-phase differential equations, respectively. This approach generates a large set ($m_z{\cdot}(N{+}1){+}2m_r m_z{\cdot}(N{+}1)$ where m_z is the number of nodes in axial position in the bed and m_r is the number of nodes in radial position inside the adsorbent particles) of differential equations. Even though simulation of chromatographic curves could be used for the search of parameters in the GRM that cannot be determined experimentally, the size of the differential equation system limits its use when it is coupled with optimization algorithms. However, recently, a genetic algorithm was applied for the search of some parameters in the model [72], and a fast and accurate solver for the GRM has been proposed by von Lieres and Andersson [75], where the model equations are spatially discretized with finite volumes and the weighted essentially nonoscillatory (WENO) method is used. In general, GRM of a strongly nonlinear two-component system is solved within few seconds.

Additionally, the GRM has the ability to model and simulate protein behavior in simulated moving bed chromatography (SMBC) with very good results at small scale [76] and at miniplant scale [77]. Moreover, Cramer and co-workers [78] have used the GRM coupled with the kinetic form of the steric mass action isotherm for the determination of the parameters of the physical model, which are used in a hybrid model framework for the optimization of preparative IEX. By applying this strategy, the computational time required for simulation and optimization was significantly reduced.

Recently, Vicente et al. [79] demonstrated that the GRM is capable of quantitatively describe bovine serum albumin (BSA) binding at DEAE and elution for protein titers from dilute conditions up to overloaded conditions and a broad range of salt concentrations using surface plasmon resonance (SPR) spectroscopy as a tool for analyzing protein binding and elution. Particular applications of the GRM will be described below.

3.1 GRM for SEC

Only few applications of the GRM for modeling of SEC have been reported in the literature. Li and co-workers [80] used a GRM for the scale-up predictions of preparative SEC columns for BSA, myoglobin, and ovalbumin. The physical parameters

investigated in the performance of SEC were the dimensionless numbers Pe, Bi, and η, particle radius (R_p), effective pore diffusion coefficient (D_p), and pore tortuosity (t_{tor}). The results confirmed a very accurate scale-up prediction of retention times and peak shapes of eluted myoglobin and ovalbumin.

On the other hand, GRM was successfully applied to model the elution curves and retention times of α-lactalbumin and β-lactoglobulin in SEC [81]. In this study, the influences of Pe_L, Bi, η, and injected sample volume were evaluated. The results indicated that simulations are more sensitive to the dimensionless numbers Pe_L and η than to Bi.

3.2 GRM for IEX

In the case of IEX, the use of the GRM has been successful with several applications in breakthrough curves and pulse injection.

3.2.1 Breakthrough Curves

Kaczmarski and co-workers [14] evaluated the mass transfer kinetics of BSA in IEX under nonlinear conditions using the GRM. Specifically, they studied the influences of axial dispersion, resistance to mass transfer from the bulk of the mobile phase to the surface of the packing particles, and intra-particle mass transfer resistances on the breakthrough curves of BSA. The comparison of the obtained results with the GRM and the simple transport-dispersive model as well as the lumped pore diffusion model demonstrated that the use of an oversimplified model (transport-dispersive model) for the analysis of chromatographic data can lead to erroneous interpretations of the experimental data and to misunderstandings of the fundamentals of the processes involved [14].

In the case of a polyclonal IgG mixture (considering two pseudovariants), Forret and co-workers [15] used the GRM to simulate three breakthrough curves at different ionic strengths (I = 0.07 M, 0.12 M, and 0.17 M at 0.11 cm s^{-1}). The results indicate that the model with the parameter values regressed by gradient elution experiments was able to predict the curves with good approximation. Melter et al. [16] characterized the retention behavior of three monoclonal antibodies (MAb) on a weak cation exchanger (Fractogel EMD COO-(S)). The profiles of breakthrough experiments under highly nonlinear conditions were well predicted by the GRM. In this case, the effective pore diffusion coefficient (D_p) was the only fitted parameter (see Tab. 2).

3.2.2 Pulsed Injection

Nilsson [19] used three models (reaction-dispersive, transport-dispersive, and GRM) for evaluating if protein separation by IEX is controlled by mass transfer. Three different prepacked columns with different bead sizes were used for the separation of IgG and BSA. The results indicated that the performance of different columns is best described by different model structures, because different behaviors are rate-limiting. Specifically, diffusive effects demonstrate that the effective pore diffusion coefficients (D_p) are much higher than expected compared with the data in literature, indicating that there are other forces dominating the mass transport in the beads.

Chan et al. [82] compared two models: a simple equilibrium-dispersive (ED) model and the GRM for purification of alcohol dehydrogenase and myoglobin. The results proved that, while the ED model is a good approximation in most cases, in others the more detailed GRM is required.

Additionally, in the simulation of IEX for diluted protein samples (α-lactalbumin 0.2 mg mL^{-1}, ovalbumin 0.2 mg mL^{-1}, and β-lactoglobulin 0.15 mg mL^{-1}) the elution curves computed with the GRM were adjusted to the experimental data using the dimensionless number η as a protein-specific parameter [66]. At the tested low protein concentration, predictions of the GRM were comparable to those predicted by the PM. The GRM has several advantages over the PM, 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. Simulation of elution curves of high protein concentrations in IEX has been accomplished by using the GRM; 37.5 mg of total protein was injected into 1 mL column [73]. The model was able to predict the retention time and the shape of the eluted protein peak for different operating conditions (flow rate 0.3–1.0 mL min^{-1}); ionic strength gradient 0.0133–0.0233 M mL^{-1} (M/CV)). For the evaluated process conditions the axial dispersion was negligible ($Pe_L > 300$) and the mass transfer process was controlled by intra-particle diffusion ($Bi > 10$).

Elution curves of a polyclonal IgG mixture on a strong cation exchanger were simulated with the GRM [15]. The IgG mixture was simplified by considering two pseudovariants. The adsorption isotherm for the salt was written as a linear isotherm (Tab. 3) and for the adsorption isotherms of the different antibodies a competitive multicomponent Langmuir isotherm was used (Tab. 3). In general terms, the model based on two pseudovariants was adequate for modeling the adsorption process carried out under different operational conditions (interstitial velocitiy 0.02–0.31 cm s^{-1}; gradient length 75–150 (CVs); injected mass of the mixture 38–119 mg).

The GRM has also been applied by Melter et al. [16] for predicting the elution curves of three monoclonal antibodies (F1, F2, and F3) on a weak cation exchanger (Fractogel EMD COO-(S)). The model was able to predict isocratic and linear gradient runs under different conditions (flow rate and amount injected). Even for very long gradient runs (CVs) the GRM prediction matched the experimental elution profiles well. However, the authors consider that correlations for describing the effect of the separation conditions (salt and solute concentration) on the diffusion rate must be included to improve the predictions.

Due to its theoretical basis the GRM needs several parameters. Most of them can be estimated from theoretical and empirical relationships that, although not specific for the systems under study, provide satisfactory predictions. Perhaps the most representative parameter of a given system is the effective pore diffusion coefficient (D_p). In this review, a summary of mass transfer parameters (diffusivity in free solution, D_m, and D_p) is provided in Tab. 2. Additionally, Carta et al. tested different

Table 3. Isotherms for describing protein adsorption used in the rate model.

Isotherm	Equation	Remarks
Linear	$C_p = bC_p^*$	b = equilibrium constant and a function of temperature and salt concentration [84, 85].
Langmuir	$C_p = \frac{C_p^*}{K \cdot \left(C^\infty - C_p^*\right)}$	C^∞ = resin capacity [86, 87].
Competitive Langmuir	$C_{p1} = \frac{C_1^\infty C_{p1}^*}{K\left(C_1^\infty C_2^\infty - C_2^\infty C_1^* - C_1^\infty C_2^*\right)}$	Subscripts 1 and 2 = two different solutes; applied by [86, 89].
Multicomponent competitive Langmuir	$C_{pi}^* = \frac{H_i C_{pi}}{1 + \sum_{j-1}^{n} \frac{H_j}{C_{pj}^\infty C_{pj}}}$	Subscripts j and i = different solutes; H_i = Henry constant for the i^{th} component [15].
Multicomponent Langmuir	$C_{pi}^* = \frac{a_i C_{pi}}{1 + \sum_{j-1}^{n} b_j C_{pj} C_{0j}}$	Subscripts j and i = different solutes; C_0 = initial solute concentration; a_i = isotherm parameter derived from data adjustment; n = number of solutes [86].
Preferential interaction quadratic	$C_{pi}^* = \frac{k'_i \cdot \left(a_i C_{pi} + d_i C_{pi}^2\right)}{1 + \sum_{j=1}^{n} k'_j \cdot \left(e_j C_{pj} + f_j C_{pj}^2\right)}$	k' = capacity factor; n = number of components; a, e, d, f = isotherm parameters derived from data adjustment; applied by [78, 91].
Colloidal energetics	$C_p = \frac{C_p^*}{b} \exp\left[\left(a\sqrt{C_p^*} + \delta\right)\exp\left(\frac{\omega}{C_p^*}\right)\right]$	a, δ, ω = parameters that control smoothness of the transition from the linear region of the isotherm to the plateau; b = equilibrium constant [17].
Steric mass-action (affinity chromatography)	$\frac{C_{pi}^*}{C_{pi}} = K_{a,i}\left[\frac{L_t - \sum_{i=1}^{N}\left(n_i + \sigma_i + K_S C_S^a \sigma_i\right)\cdot C_{pi}^*}{1 + K_S C_s^a}\right]^{n_i}$	L_t = concentration of the sites that interact with the proteins; n = binding sites in the protein; σ = steric factor of the protein; a = salt coefficient; K_S = equilibrium constant for the salt; K_a = equilibrium constant for the ion-exchange process; v = characteristic charge; A = ion-exchange capacity [39, 95].
Steric mass-action (IEC for a single protein)	$\frac{C_{pi}^*}{C_{pi}} = K_a\left[\frac{A - (v + \sigma_a)\cdot C_{pi}^*}{C_S}\right]^v$ $K_a = \left(\frac{C_p^*}{C_p}\right)\left(\frac{C_S}{C_p^*}\right)^v$	

methods to determine the effective pore diffusion coefficients (D_p) [83]. The proved capacity of the GRM for simulating IEX under different conditions has led to software development such as Chromulator-IEX which solves GRM equations numerically using finite elements and orthogonal collocation considering the steric mass-action isotherm (Tab. 3). It features a graphical user interface, visualization of effluent histories, animations showing profile development in the column, and position-time plots.

3.3 GRM for HIC

In the case of HIC, the GRM has been widely used for modeling of both breakthrough curves and pulsed injection.

3.3.1 Breakthrough Curves

The GRM has also been applied for studying and simulating frontal analysis. To et al. [17] used this model to simulate the breakthrough curves of four model proteins on different commercial HIC supports having a phenyl group as hydrophobic ligand. In the model, adsorption equilibrium was described by the colloidal energetics isotherm developed by Oberholzer and Lenhoff [18] given in Tab. 3. Mass transfer parameters were estimated (see Tab. 2) and the authors found that the pore structure affected the protein transport in the HIC system. Besides, they suggested that adsorbed proteins probably retard intra-pore protein diffusion. Finally, some discrepancies between experimental data and predictions were found, possibly due to impurities in the protein solutions.

3.3.2 Pulsed Injection

The GRM has been applied by several authors to describe and simulate isocratic and gradient elution profiles of pulsed-injected proteins in HIC. To et al. [20] studied transport and kinetic parameters of proteins in HIC using isocratic elution by considering a linear isotherm to describe adsorption. The diffusivity of five model proteins in eight commercial phenyl supports were estimated by considering the first two moments of the linear rate model. The model predictions were fitted to experimental isocratic elution data and effective pore diffusion coefficients were estimated (see Tab. 2). The authors concluded that the protein conformational stability affected the accuracy of model predictions. In addition, the authors modified the linear rate model to include protein folding/unfolding kinetics. The results led to the conclusion that the HIC sorbent properties also affect the accuracy of model predictions. Muca et al. [84] used the GRM to investigate the influence of sample solvent on the chromatographic behavior of proteins in HIC. In the dynamic model they considered that the adsorption equilibrium was adequately described by a linear isotherm (see Tab. 3) and used the model to simulate isocratic and gradient elution. The authors concluded that the mass transfer resistances and unfolding kinetics significantly contributed to protein conformational changes. Additionally,

Xiao et al. [85] used a linear isotherm to model isocratic elution curves accounting for conformational changes during an HIC process. In the model, they considered two conformational states, namely, folded and unfolded, and then two conservation equations were obtained and solved numerically. As a model protein, the authors used α-lactalbumin, since its conformational stability is strongly dependent on the presence or absence of calcium. The model was successful in describing the effect of the modulator salt and calcium. Finally, the authors highlighted the importance of considering the effect of mobile phase modifiers on protein stability in HIC.

The GRM has been implemented by considering that adsorption equilibrium is given by a Langmuir isotherm [86]. McCue et al. [87] used this approach to study protein monomer/aggregate separation in HIC. They represented pure monomer adsorption equilibrium by means of a Langmuir isotherm and simulated isocratic elution curves through the GRM. The model successfully predicted the separation of a monomer from aggregated species.

Chan et al. [82] compared two models: a simple equilibrium-dispersive (ED) model and the GRM for purification of alcohol dehydrogenase from a yeast homogenate supernatant in HIC. The results indicated that the GRM provided slightly better predictions. However, due to the difficulty in obtaining the model parameters, the ED model is a better choice for the simulations.

Additionally, the GRM has been solved considering some modifications of the Langmuir isotherm, such as the competitive Langmuir isotherm developed by Katti and Guiochon [88] and Phillips et al. [89] and the multicomponent Langmuir isotherm [86]. The equations of these isotherms are listed in Tab. 3. Jakobsson et al. [90] applied the competitive Langmuir isotherm to represent adsorption of a protein mixture in HIC. They included this expression in the GRM in order to simulate gradient elution curves of a binary protein mixture and to optimize an HIC step for protein purification. McCue et al. [87] used this isotherm to solve the GRM for simulating isocratic curves of a monomer and aggregate protein mixture. Lienqueo et al. [71] included the multicomponent Langmuir isotherm in the GRM to describe and simulate elution curves of protein mixtures in HIC as well as to optimize their separation using salt gradient elution.

The preferential interaction quadratic (PIQ) isotherm (see Tab. 3) has also been included in the GRM to describe adsorption equilibrium. This isotherm was first introduced by Xia et al. [91] and considers the nonlinear effect of salt on protein retention coupled with nonlinear adsorption behavior at high protein concentration. The authors found that this isotherm was adequate to describe nonlinear adsorption of proteins and low-molecular-weight modifiers in HIC. Nagrath et al. [72] used the PIQ isotherm for characterizing HIC systems and to model nonlinear behavior of proteins in HIC with the GRM. Isocratic and gradient elution profiles were simulated and mass transfer parameters (given in Tab. 2) were estimated from the model resolution. The authors concluded that the limiting steps for protein mass transfer were the pore and surface diffusion.

3.4 GRM for Affinity Chromatography (AC)

In the case of AC the application of the GRM is relatively new with only few examples. Elution curves of serum albumin and hen egg white lysozyme solution, which have different affinities to Cibacron Blue F-3GA in a small column (7.9 mL) at 0.1 mL min^{-1}, were used for estimation of parameters in the model. Satisfactory scale-up predictions were obtained for larger columns (98.2 and 501 mL) at different flow rates (1–8 mL min^{-1}) [92].

Asenjo's team studied the elution curves of BSA and rabbit hemoglobin (Hb), which have different affinities to Blue Sepharose, for estimation of parameters in the GRM [72]. The obtained values were used for simulating the elution curve of the two-protein mixture with different salt gradients (5.5 and 10 CV). The Hb peak in the model indicates virtually no displacement in the elution time in contrast to the BSA peak with 8 % displacement. Experimental elution curves of both proteins and also those of the protein mixtures showed a tail which could not be predicted by the model. The authors attributed this behavior to problems with the experimental salt gradient towards the end of the run and some glycosylation heterogeneity in the sample. However, it is also possible that for the system (adsorbent and proteins) the proposed adsorption kinetics given by the relationship in Eq. (17) for k_d in Eq. (12) was not able to describe changes in the interaction of the proteins with the adsorbent, as the ionic strength of the mobile phase increased. Additionally, elution curves of a mixture of mouse IgGs (IgG1, IgG2a, and IgG2b) with different affinities to Protein A, eluted by the decrease of mobile phase pH, were simulated with the GRM and the relationship given in Eq. (16) for k_d. The model with the parameters estimated from single protein elution curves was able to simulate the behavior of the protein mixture elution curves obtained with different pH gradients (10–20 CV), flow rates (1–3 mL min^{-1}), and protein loads (0.0038–0.095 mg mL^{-1} of Protein A). In addition, the model was able to predict the nonlinearity of the pH gradient that occurs experimentally.

4 Conclusions

There are several models to represent and simulate protein behavior in chromatography, however, the most successful are the plate model (PM) and the general rate model (GRM). Both models are appropriate for studying phenomenological aspects related with mass transfer in chromatographic processes. In general terms, the PM is limited to adsorption kinetics that are well described by a linear isotherm, i.e., diluted protein solutions.

On the other hand, the GRM is the most complete model and exhibits a higher versatility since it allows describing adsorption equilibrium through different isotherms and is suitable for modeling and simulating chromatographic processes under protein overload conditions. This versatility is due to its basis on momentum and mass transfer phenomena that take place inside the adsorbent particles and in the mobile phase. Because of this complexity, several parameters are needed for simulating processes carried out under conditions

different to those applied for parameter estimation. The use of theoretical correlations that are not specific for protein (e.g., for computing Pe_L and k) proved to be practical. Efforts should be focused on defining strategies for estimating parameters and transforming this information into correlations. In the present review, it was demonstrated that in several studies the right parameters allow the mathematical models to successfully predict different chromatographic techniques. In spite of the few correlations for estimating mass transfer parameters in chromatographic processes, mathematical models constitute a tool that would enable defining operational conditions reducing the cost associated with trial-and-error experiments. Numerical methods combined with the high computing capability of relatively economic computers allow solving complex models such as GRM within seconds or minutes. Most of the reported studies use model proteins; efforts on describing complex mixtures should be made in order to validate mathematical model predictions.

Predictions through mathematical models could be applied for adsorbent design, protein modifications, such as protein labeling, and scale-up of chromatographic processes. Few works have been carried out on this aspect mainly due to the lack of information related to parameters for both target proteins and contaminants.

Acknowledgment

This review was supported by Fondecyt Grants 1100437 and 1080143, Fondecyt Research Initiation Grant 11080016, and the Institute for Cell Dynamics and Biotechnology (ICDB): A Center for System Biology (project ICM # P05-001-F).

The authors have declared no conflict of interest.

Symbols used

A	[–]	parameter in the adsorption kinetic expression (Eq. 3)
a	[–]	Langmuir parameter in Eq. (13)
a_i	[–]	parameter in preferential interaction quadratic [78, 91]
B	[–]	parameter in the adsorption kinetic expression (Eq. 3)
b	[L mol^{-1}]	Langmuir parameter (equilibrium constant) (Eq. 13)
Bi_i	[–]	Biot number
C^∞	[mol L^{-1}]	adsorbent saturation capacity
C_0	[mol L^{-1}]	initial concentration
c_0	[–]	dimensionless initial concentration of modulator
C_b	[mol L^{-1}]	protein concentration in the mobile phase
C_{b0}	[mol L^{-1}]	initial protein concentration in the mobile phase
C_b^*	[mol L^{-1}]	concentration of the adsorbed protein
c_b	[–]	dimensionless protein concentration in the mobile phase
C_{Fi}	[mol L^{-1}]	concentration of protein i in the feeding
C_{FN+1}	[mol L^{-1}]	concentration of the displacer compound in the feeding
$C_{N+1,max}$	[mol L^{-1}]	maximum pH/ionic strength in the mobile phase
C_p	[mol L^{-1}]	protein concentration in the stagnant liquid inside the particle
c_p	[–]	dimensionless protein concentration in the stagnant liquid inside the particle
C_p^*	[mol L^{-1}]	protein concentration adsorbed to the particle
c_p^*	[–]	dimensionless protein concentration adsorbed to the particle
C_s	[mol L^{-1}]	salt concentration
D_a^a	[–]	adsorption Damköhler number
D_a^d	[–]	desorption Damköhler number
D_{crit}	[m^2 s^{-1}]	critical diffusivity
D_L	[–]	axial dispersion coefficient
D_m	[m^2 s^{-1}]	diffusivity in free solution
d_p	[m]	particle diameter
D_p	[m^2 s^{-1}]	effective pore diffusion coefficient
d_i	[–]	parameter in preferential interaction quadratic [78, 91]
e_j	[–]	parameter in preferential interaction quadratic [78, 91]
f_j	[–]	parameter in preferential interaction quadratic [78, 91]
G	[M mL^{-1}]	salt gradient steepness
H	[–]	volumetric phase ratio
H_i	[–]	parameter in multicomponent competitive Langmuir [15]
h	[–]	reduced height equivalent to a theoretical plate
I	[mol L^{-1}]	ionic strength
I_i	[mol L^{-1}]	ionic strength in the mobile phase at plate i
I_{max}	[mol L^{-1}]	ionic strength at elution
I_0	[mol L^{-1}]	initial ionic strength
$K(C_{bi}I_i)$	[–]	distribution coefficient in Eq. (1)
$K(I)$	[–]	distribution coefficient in Eq. (3)
K	[–]	parameter in competitive Langmuir equation [86, 87, 89]
k'	[–]	retention factor
k_a	[(s M)$^{-1}$]	rate constant for adsorption
K_{crit}	[–]	critical distribution coefficient
k_d	[s^{-1}]	rate constant for desorption
k_i	[m s^{-1}]	mass transfer coefficient for protein i
k_i', k_j'	[–]	parameters in preferential interaction quadratic [78, 91]
K_{Imax}	[–]	distribution coefficient at $I = I_{max}$
k_p	[m s^{-1}]	pore diffusion mass transfer coefficient
K_{salt}	[–]	distribution coefficient of salt
L	[m]	column (bed) length
m_z	[–]	number of nodes in axial position

m_r	[–]	number of nodes in radial position
N_p	[–]	number of theoretical plates
N	[–]	number of components (proteins) in the solution
Pe_L	[–]	Peclet number
Q_s	[mol L^{-1}]	salt concentration adsorbed to the particle
R	[m]	radial position inside the particle
r	[–]	dimensionless radial position in the particle
ReSc	[–]	dimensionless velocity
R_p	[m]	particle radius
t	[s]	time
$t_{0.15}$	[s]	time at 15 % relative response
$t_{0.5}$	[s]	time at 50 % relative response
t_{tor}	[s]	pore tortuosity
v	[m s^{-1}]	velocity of the mobile phase
V_m	[mL]	sample volume
V_R	[mL]	retention volume
Z	[m]	axial position in the column (bed)
z	[–]	dimensionless axial position in the column (bed)
α'	[–]	constant of Melander model [40]
α	[–]	lumped parameter in Eq. (15)
α_i'	[–]	parameter in desorption rate constant relationship (Eq. 16) for protein i
β	[–]	electrostatic interaction parameter
β_i'	[–]	parameter in desorption rate constant relationship (Eq. 16) for protein i
$\Delta c_m/\Delta\tau$	[–]	gradient steepness in terms of dimensionless variables
γ	[–]	hydrophobic interaction parameter
e	[–]	void fraction
ε_b	[–]	column (bed) porosity
e_p	[–]	particle porosity
Θ	[–]	dimensionless time
ϕ	[–]	volume ratio between adsorbent particle skeleton and the mobile phase inside the particle
τ	[–]	dimensionless time
τ_{imp}	[–]	dimensionless time for pumping the sample into the column
η	[–]	dimensionless number
ξ	[–]	dimensionless number

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