A General Rate Model of Ion-Exchange Chromatography for Investigating Ion-Exchange Behavior and Scale-up

Aaron Mehay and Tingyue Gu*
Department of Chemical and Biomolecular Engineering, Ohio University, Athens, USA

Abstract

This work presents a general rate model for ion-exchange in column chromatography with the steric mass-action isotherm that is suitable for ion-exchange systems involving macromolecules such as large proteins. This comprehensive model considers axial dispersion, interfacial film mass transfer between the bulk-fluid phase and the particle phase, and intraparticle diffusion. The model system has been solved numerically using the finite element method and the orthogonal collocation method with a graphical user interface for Windows based personal computers. The effect of pH is modeled by including buffers, acids, and bases as species in the model system, allowing the induced pH gradients resulted from H⁺ or OH⁻ sorption by the ion exchanger to be described in the model. Feed profiles of all the species can be specified individually allowing complicated gradient patterns. The software, known as Chromulator-IEX, may be a useful tool for the investigation of ion-exchange chromatography behavior and its scale-up.

Keywords: Ion-Exchange; Chromatography; Modeling; Scale-up; Gradient; pH transition; Protein purification

Introduction

In the biotechnology industry, downstream processing of a protein product usually takes up the majority of the overall production cost [1]. Because of a low product concentration in the feed and a high purity requirement, a multi-stage process is almost invariably required for a protein product intended for pharmaceutical applications. A typical downstream process consists of four stages, namely removal of insolubles, isolation, purification and polishing [2]. The core of the process is the purification stage. It centers on one or more liquid chromatography (LC) steps. The steps before these LC steps are used to disrupt the cells if the product is intracellular, to remove insolubles (e.g., cell debris), to eliminate the majority of the impurities and to reduce the feed volume in order to maximize the resolution and throughput of the LC steps while reducing costs by allowing the use of a smaller column volume. Among the various forms of LC, ion-exchange chromatography (IEC) is one of the most common because of its good resolution and high capacity. Reverse-phase liquid chromatography (RP-LC) typically achieves a better resolution than IEC. However, RP-LC uses a solvent mobile phase that can lead to denaturation of a protein product. Its loading capacity is also usually much lower than that of IEC.

To dislodge proteins from the ion-exchange resin inside an LC column after loading, a salt solution, often in a gradient feed pattern, can be used to exchange with the sorbed proteins. Another common practice is to use a pH gradient to elute the proteins with or without a salt gradient. Modeling IEC with pH gradients requires the concentration of H⁺ (or OH⁻) to be known throughout the column. This is more complicated than modeling other ions because H⁺ and OH⁻ can combine with each other and with other ions in buffers in addition to binding to the resin. In order to calculate the pH, a charge balance must be performed on all ions in a particular location in the column. When buffers are present, a set of implicit equations must be solved for the H⁺ or OH⁻ concentration. If sorption of H⁺ or OH⁻ is ignored, then the charge balance is not necessary, and the concentrations of coions (not including buffers) do not need to be known. However, induced pH transitions can occur as a result of this sorption. Ghose et al. [3] observed pH dips as large as 1 pH unit in the effluent of a cation-exchange column in response to step changes in NaCl feed, and a similar pH increase in anion exchange. In order to model these phenomena, sorption of H⁺ and OH⁻ on the ion-exchange resin must be considered.

A general rate model for LC consists of a bulk-fluid phase mass transfer partial differential equation (PDE) and a particle phase mass transfer equation coupled with an equation to describe the elute-stationary binding mechanism (e.g., an isotherm). For high performance LC (HPLC), the concentration profiles for eluates in the bulk-fluid phase are very stiff. This makes solving the bulk-fluid phase PDE very demanding numerically. An efficient discretization method is needed for the column length axis. Raghavan and Ruthven used the orthogonal collocation (OC) method for fix-bed adsorption column modeling [4]. This method is efficient for relatively non-stiff systems. However, it cannot be used for stiff systems that require an excessive number of OC points because the OC method is designed to work stably for a few OC points. To overcome this limitation, Yu and Wang used the OC on finite element (OCFE) method [5] for ion-exchange chromatography modeling. Since there is no limitation on the number of finite element, the OCFE method can be used for very stiff concentration profiles. Wang’s group subsequently used this method to simulate an IEC system with a mass-action isotherm in both equilibrium and nonequilibrium forms [6]. None of these models have included the calculation of pH in the presence of a buffer. Frey and coworkers [7] used a lumped pore resistance model to describe protein IEC with adsorbing buffers. This model was used to simulate the elution of bovine serum albumin (BSA) and hemoglobin on the Q-Sepharose FF (strong anion exchanger) gel in the presence of

*Corresponding author: Dr. Tingyue Gu, Professor, Department of Chemical and Biomolecular Engineering, Ohio University, Athens, OH 45701-2979, USA, Tel: 740-593-1499; Fax: 740-593-0873; E-mail: gu@ohio.edu

Received April 16, 2014; Accepted May 03, 2014; Published May 07, 2014

A general rate model was used to model the mass transfer in IEC. This model considers axial dispersion, interfacial film mass-transfer resistance, and intraparticle diffusion in the multicomponent LC system. The dimensionless equations for eluite species i are as listed below for the bulk-fluid phase and the particle phase, respectively [12].

\[
\frac{\partial c_{bi}}{\partial \tau} = \frac{1}{Pe_{Li}} \frac{\partial^2 c_{pi}}{\partial z^2} - \frac{\partial c_{bi}}{\partial z} - \xi_i (c_{bi} - c_{pi,r=1})
\]

\[
(1 - e_p) \frac{\partial q_i}{\partial \tau} + e_p \frac{\partial c_{pi}}{\partial \tau} = \eta_i \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial c_{pi}}{\partial r} \right)
\]

The dimensionless boundary conditions are

\[
\frac{\partial c_{bi}}{\partial z} = 0, \text{ at } z = L
\]

\[
\frac{\partial c_{bi}}{\partial z} = Pe \left[ c_{bi} - c_p(\tau) \right], \text{ at } z = 0
\]

\[
\frac{\partial c_{pi}}{\partial r} = 0, \text{ at } r = 0
\]

\[
\frac{\partial c_{pi}}{\partial r} = Bi_i (c_{bi} - c_{pi,r=1}), \text{ at } r = 1
\]

In Eq. (6), \( c_i^p(\tau) \) is the dimensionless feed concentration of species i as a function of dimensionless time, and its value determines the type of chromatography (e.g., isocratic elution or gradient elution) being employed, or the sample injection shape (e.g., a pulse). Time zero is the time a species first enters the column inlet. The constants used to nondimensionalize the concentrations, \( c_{pi} \), are arbitrary, but their values are usually chosen to be the largest value fed to the column. The characteristic charge could be considered constant under varying pH on the same ion exchanger.

The characteristic charge of BSA to be a linear function of pH and the equilibrium of pH. Conversely, Strong and Frey [8] considered the characteristic charge could be considered constant in response to changes in the mobile phase pH was developed by theoretical model for the changes in SMA parameters of a protein but not adsorption azeotropes in displacement chromatography. A ion-exchange. Their SMA derivations also lead to selectivity reversals, proteins that block the access to salt ions on the ion exchange resin.

Cramer [10] devised a steric mass-action (SMA) isotherm to extend the mass-action isotherm that is commonly used for ion-exchange, providing an extended Langmuir isotherm for some small pores with binding sites inside and thus it had a smaller resistance, and intraparticle diffusion in the multicomponent LC system. The dimensionless equations for eluite species i are as listed below for the bulk-fluid phase and the particle phase, respectively [12].

A general rate model was used to model the mass transfer in IEC.
inherently multicomponent. In general there is no explicit analytical expression for $Q$. Thus, the partial derivatives, $\partial Q/\partial C_j$, which are needed to solve the particle phase PDE after orthogonal collocation discretization [13], become implicit as well. This hampers the numerical solution to the IEC model. One way to avoid this difficulty is to step back to the isotherm precursor, i.e., the rate expression that models the ion-exchange reaction in Eq. (8) as shown below, where $k_{ij}$ is the forward reaction rate constant for the exchange of species $i$ in solution with species $j$ in the sorbed (stationary) phase as shown in Eq. (8), and $k_{ji}$ the backward reaction rate constant for the exchange of species $j$ in the solution with species $i$ in the sorbed phase. The rate constants can be related to the equilibrium constants by the following definition, where $K_i = k_{ij}/k_{ji}$.

The ion-exchange equilibrium isotherm expressed by Eq. (9) is a subset of Eq. (12). When the rate constants are sufficiently large, local equilibrium is achieved such that $\partial Q/\partial t = 0$. Eq. (12) degenerates to Eq. (9). Eq. (10) is satisfied throughout the solution procedure if it is satisfied in the initial condition for the sorbed phase. A dimensionless form of Eq. (12) is

$$\frac{\partial \tilde{Q}_i}{\partial \tau} = \left( D_{ij} C_{i0}^{(ij)} Q_{j0}^{(ij)} - D_{ji} C_{j0}^{(ji)} Q_{i0}^{(ji)} \right)/U_j$$

where $D_{ij}$ and $D_{ji}$ are Damköhler numbers (dimensionless). The Damköhler numbers are related to the rate constants by

$$D_{ij} = C_{i0}^{(ij)} C_{j0}^{(ij)} L_k^{ij} k_{ji}$$

$$D_{ji} = C_{j0}^{(ji)} C_{i0}^{(ji)} L_k^{ji} k_{ij}$$

For a system with $N_s$ species (including the ion that is used to equilibrate the column initially), $N_s(N_s-1)/2$ rate constants or Damköhler numbers can be chosen independently of the $N_s-1$ equilibrium constants. In the nonequilibrium model used by Ernest et al. [6], $k_s = 0$ for all $i, j \neq 1$. The kinetic expression given by Eq. (14) does not suffer from the drawback of species 1 having to be present to be able to present the system to approach equilibrium. It is not generally practical to determine the actual values of the rate constants, but by selecting large values for $D_{ij}$, equilibrium will be achieved, and the simulation results will not be sensitive to changes to these $D_{ij}$ values as long as they are sufficiently large (e.g., 1000). Fixing arbitrary large values for $D_{ij}$ leads to large values for $k_{ij}$. Given $K_i$ values, $k_{ij}$ values and subsequently $D_{ij}$ values can be calculated from Eq. (13) and Eq. (15b), respectively. By doing so, the kinetic model in Eq. (14) reduces to equilibrium isotherm during simulation without the aforementioned implicit isotherm problem that hampers the numerical solution to the model.

Numerical Solution

An analytical solution to a general rate model is only possible if the isotherm is a linear one as demonstrated by Lee et al. using Laplace transfer [14]. The model system consisting of Equations (1), (2) and (14) is highly nonlinear due to the complicated ion-exchange binding mechanism. In this work, it was solved using a numerical solution strategy described as follows. The finite element method is used to discretize the dimensionless column axis $z$ and the OC method is used to discretize the dimensionless particle radius $\tau$ [13]. For a typical IEC system, one or two interior OC points together with a built-in exterior OC point are sufficient to describe the concentration profiles in the particle phase. For large beads in some preparative- and large-scale applications, more OC points are needed as recently demonstrated by Gu et al. in adsorption LC [12]. After the discretization of Equations (1) and (2), the resultant ordinary differential equation (ODE) system will consist of $N_s(2N_s+1)(2N_s+1)$ ODEs if $N_s$ number of finite elements and $N_s$ number of interior OC points are used for a system with $N_s$ species. The ODE system is solved using an efficient public-domain ODE solver known as VODE developed by Brown et al. [15]. The numerical solution in this work was implemented using the C++ computer language with a graphical user interface (GUI) to produce a software program known as Chromator-IEX. The computation time...
on today’s personal computers is typically a matter of seconds. Very stiff systems may take up to several minutes or more (Figure 1).

Results and Discussion

Figure 2 shows an example of the GUI of the software in which the absolute tolerance (atol) for the ODE solver is set to $1 \times 10^{-5}$. The system has four species with species one being the salt ion that is used to elute out three other species isocratically. Species 1 is also used to equilibrate the column before injecting the feed containing species 2, 3 and 4. The pulse feed has duration of 2 in dimensionless time. It does not contain species 1. The results are shown in the simulated chromatogram in Figure 3 exhibiting the effluent concentration profiles of the four species. In Figure 2, the value of 1 in the $c_i$ (subscript $i$ for initial rather than species $i$ here) data entry column indicates that the IEC column is initially equilibrated with the $C_{eq}$ concentration for species 1 (the salt ion), while the three zeroes point out that the IEC column does not contain any amount of species 2, 3 and 4 initially. If “Calculate pH” is checked in Figure 2, pKa and SF (salt fraction) values must be entered.

The software allows individual species’ feed profiles to be specified separately. For example, the isocratic elution case in Figure 2 can be switched to a gradient elution with a salt (species 1) gradient from 0.5 to 1 dimensionless concentration (i.e., half of the maximum in the feed) to 1 with a dimensionless time frame of 0 to 18. Figure 4A shows that the concentration in the feed is 0.5 to 1 dimensionless concentration for species 1 (the salt ion), while the three zeroes point out that the IEC column does not contain any amount of species 2, 3 and 4 initially. If “Calculate pH” is checked in Figure 2, pKa and SF (salt fraction) values must be entered.

"Gradient Elution, last component is displacer” and “Custom Mode of Operation” options are selected, which allow feed profiles to be specified in a new pop-up window. Figure 4B shows that species 1 concentration in the feed is 0.5 to 1 dimensionless concentration for the dimensionless time frame of 0 to 18. Figure 4C specifies that the feed for species 2 is a pulse injection lasting 2 dimensionless time. Species 3 and 4 are specified the same way as that for species 2. Figure 5 shows the simulated chromatogram using the parameters in Figure 4. The column is initially equilibrated with species 1 with a dimensionless concentration of one, which shows up in the effluent profile for the salt at dimensionless time 0. Compared with the isocratic elution outcome in Figure 3, the gradient elution in Figure 5 does not exhibit a drastic improvement, because the gradient turns out to have delayed the speed of peak migration causing the peaks to diffuse more. More elaborate feed patterns can be simulated by using the “Add” button Figure 4C to supply dimensionless concentration and dimensionless time data points piecemeal in order to construct a feed profile of a species.

To illustrate the transient ion-exchange behavior, the software utilizes a built-in movie player to show the bulk-fluid phase concentration profiles of all the species at different axial positions inside the IEC column. Figure 6 is a screenshot of the concentration profiles for the case in Figure 5 at dimensionless time $\tau = 4.5$. The concentrations of the four species at column position $z=1$ (i.e., column exit) match those in Figure 5 at $\tau=4.5$.

In Figure 7A, Ghose et al. [3] experimentally demonstrated the pH spike and pH dip phenomena by feeding three different buffers at the same pH to a Fractogel SO$_3^-$ (M) ion-exchange column. The column was preconditioned with 2 M NaCl and 250 mM sodium citrate buffer at pH 5.5, equilibrated with 25 mM sodium citrate buffer at pH 5.5, and eluted at with 0.5 M NaCl and 25 mM sodium citrate buffer at pH 5.5. They commented that a pH dip below 5.0 or lower could cause significant bioactivity losses for some proteins. Figure 7B shows simulated results using $P_e=500$, $\eta=5$, $B_i=5$ for all species, and $K_{w,H+} = 1$, $\Lambda=95$ equiv/L, $\epsilon_f=0.4$, $\epsilon_s=0.5$. The model in this work successfully demonstrates both the pH spike and the pH dip at the correct elution volume positions compared with the experimental data in Figure 7A. In a separate case, Figure 8 shows that the model fits the experimental data on Dowex 50 ion-exchange resin well. The literature data in Figure 8 are from the work by Dranoff and Lapidus [16].
Conclusions

A general rate model for IEC was presented and solved numerically. The model considered axial dispersion, interfacial film mass-transfer resistance, intraparticle diffusion and the steric mass-action isotherm for ion-exchange. The numerical difficulty caused by the implicit isotherm was avoided by using its precursor kinetic expression and setting the kinetic rate constants to sufficiently large values to achieve equilibrium. The model system coupled with the pH expression to calculate the pH value everywhere inside the column successfully simulated the phenomenon of induced pH transitions in IEC. Such transitions are important in chromatofocusing and in the prevention of protein denaturation due to large pH spikes and dips. The model and software presented in this work should be useful tools to those involved in the scale-up of ion-exchange chromatography. It can also be used in teaching and investigating IEC behavior.

Symbols

\[ \text{Bi} = \text{Biot number for mass transfer, } \frac{kR_p}{\varepsilon_p D_p} \]

\( C_0 \) = Feed concentration of a solute, max \( C_f(t) \) (mol\( \cdot \)L\(^{-1} \))

\( C_b \) = Concentration of a solute in the bulk-fluid phase (mol\( \cdot \)L\(^{-1} \))

\( c_b = C_b/C_0 \)

\( C_f(t) \) = Feed concentration profile of solute (mol\( \cdot \)L\(^{-1} \))

\( c_f(\tau) = C_f(t)/C_0 \)

\( c_i = \) Initial dimensionless concentration everywhere in the column before sample loading (subscript \( i \) means "initial" here)

\( C_p \) = Concentration of a solute in the stagnant-fluid phase inside particle macropores (mol\( \cdot \)L\(^{-1} \) based on particle skeleton volume)

\( c_p = C_p/C_0 \)

\( d \) = Column inner diameter

\( D_a \) = Axial dispersion coefficient (m\(^2\)\( \cdot \)s\(^{-1} \))

\( K = \) Equilibrium constant

\( D_m \) = Intraparticle molecular diffusivity (m\(^2\)\( \cdot \)s\(^{-1} \))

\( D_p \) = Effective diffusivity in particle macropores (m\(^2\)\( \cdot \)s\(^{-1} \))

\( Da = \) Damköhler number

\( k = \) Film mass transfer coefficient (m\( \cdot \)s\(^{-1} \))

\( k_{ij} \) = Rate constant in ion-exchange reaction

\( K_{ij} = \) Dimensionless mass action equilibrium constant referenced to species \( j \), \( k_{ij}/k_{ji} \)

\( L \) = Column length (m)

\( N_c \) = Number of interior collocation points

\( N_e \) = Number of quadratic finite elements

\( N_s \) = Number of species

\( Pe_L = \) Peclet number of axial dispersion, \( vL/D_b \)

\( Q = \) Sorbed concentration of a species on the resin (mol\( \cdot \)L\(^{-1} \) based on particle skeleton)

\( q = \) Dimensionless \( Q \), \( Q/C_0 \)

\( R \) = Radial coordinate for a particle in cylindrical coordinate system (m)

\( R_p \) = Particle radius (m)

\( r = \) Dimensionless radial coordinate, \( R/R_p \)

\( t = \) Time (s)

\( v = \) Interstitial velocity, (m\( \cdot \)s\(^{-1} \))

\( Z \) = Column axial coordinate in cylindrical coordinate system (m)

\( z = Z/L \)

Greek Letters

\( \epsilon \) = bed voidage (bed void fraction)

\( \varepsilon = \) particle porosity

\( \eta = \) dimensionless mass-transfer parameter, \( \varepsilon_iD/L(R_p\eta) \)

\( \Lambda = \) ion exchange capacity in equivalents per unit particle skeleton volume

\( \nu = \) Charge for species

\( \xi = \) dimensionless mass-transfer parameter, \( 3Bi\eta(1-\varepsilon_i)/\varepsilon_b \)

\( \sigma = \) Steric factor

\( \tau = \) dimensionless time, \( vt/L \)

References

