INTRODUCTION
In gradient elution, a modulator is often used in the mobile phase to adjust eluent strength for better results in chromatographic separations. Compared with isocratic elution, the modulator concentration in the mobile phase in gradient elution is increased or decreased continuously with time (Antia and Horvath, 1989). Therefore, gradient elution can be used to separate components which have a wide range of retentivity with no loss of resolution. Gradient elution is able to produce high peak heights in a shorter operation cycle compared with isocratic elution. For these reasons, gradient elution has been widely used in high performance liquid chromatography for analytical purposes. Increasingly, gradient elution is applied to preparative- and large-scale chromatography for the separation of various macromolecules, such as proteins. The wide range of retentivity of proteins makes gradient elution indispensable.

Gradient elution for analytical purposes often involves small and dilute samples. In preparative- and large-scale gradient elution chromatography the column is often overloaded in terms of feed volume or concentrations, or both. Thus, interference effects, axial dispersion and mass transfer resistances such as interfacial film mass transfer and intraparticle diffusion become important. Mathematical modeling and theoretical analysis play an important role in the scale-up process. The majority of the existing theoretical models for gradient elution are designed in linear concentration range with no mass transfer or kinetic resistances. Most scale-up processes for protein purification using gradient elution were carried out empirically (Furusaki et al., 1987). Furthermore, mass transfer resistances can be very significant, especially for macromolecules. Various dispersive effects such as axial dispersion, mass transfer resistances, and slow kinetics often counterbalance the thermodynamic effects of adsorption and desorption which depend on the gradient slope, the initial modulator concentration and the adsorption properties of the eluents and the modulator. The theoretical basis of gradient elution methods in nonlinear chromatography has not been well established (Antia and Horvath, 1989).

Because of the complications involved in the modeling of gradient elution processes, very few existing models considered mass transfer resistances (Furusaki et al., 1987) or the kinetic resistances (Pitts, 1976), although some considered axial dispersion (Pitt, 1976; Furusaki et al., 1987; Antia and Horvath, 1989; Kang and McCoy, 1989). Table 1 is a summary of some existing mathematical models in the literature for gradient elution.

Linear gradient such as linear solvent strength (LSS) (Snyder, 1980) is the most common and simplest gradient method. However, stepwise linear gradient (also known as segmented linear gradient) and nonlinear gradient have the advantage of higher efficiency (Ghrist and Snyder, 1988).

A modulator may affect the retentivities of the eluents in different ways. Two types of interaction between the modulator and the eluents and six common correlations for the retention factor of an eluite versus the concentration of the modulator are summarized in the footnotes of Table 1. In some cases the modulator competes with the eluents directly for binding sites on the stationary phase, such as ion-exchange chromatography (or electrostatic interaction chromatography), hydrophobic interaction chromatography, or reverse phase chromatography (Pitt, 1976; Kopaciewiz et al., 1983; Geng and Regnier, 1984; Regnier and Mazaroff, 1987). Then, the modulator can be treated as a competing component in the...
Table 1. Comparison of mathematical models for gradient elution chromatography

<table>
<thead>
<tr>
<th>Reference</th>
<th>Correlation of $k'$ vs $C_\alpha$</th>
<th>Isotherm</th>
<th>Interaction</th>
<th>Gradient method</th>
<th>Axial dispersion</th>
<th>Film mass transfer</th>
<th>Intraparticle diffusion</th>
<th>Kinetic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitt (1976)</td>
<td>(1)</td>
<td>Linear</td>
<td>(a)</td>
<td>Linear</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Linear</td>
</tr>
<tr>
<td>Jandera and Churateck (1980, 1981)</td>
<td>(1), (2)</td>
<td>Linear</td>
<td>(b)</td>
<td>Linear</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hearn and Grego (1983, 1985)</td>
<td>(1), (2)</td>
<td>Linear</td>
<td>(b)</td>
<td>Linear</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Armstrong and Boehm (1984)</td>
<td>(2)</td>
<td>Linear</td>
<td>(a)</td>
<td>Linear</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Kennedy et al. (1986)</td>
<td>(1)</td>
<td>Linear</td>
<td>(a)</td>
<td>Linear</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Furusaki et al. (1987)</td>
<td>(3)</td>
<td>Linear</td>
<td>(b)</td>
<td>Linear</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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</tr>
<tr>
<td>Yamamoto et al. (1987)</td>
<td>(3)</td>
<td>Linear</td>
<td>(b)</td>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>D'Agostino et al. (1988)</td>
<td>(5)</td>
<td>Linear</td>
<td>(b)</td>
<td>Linear</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ghrist and Snyder (1988)</td>
<td>(2)</td>
<td>Linear</td>
<td>Linear and stepwise</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Markowski and Golikiewicz (1988)</td>
<td>(1), (2)</td>
<td>Linear</td>
<td>(b)</td>
<td>Stepwise</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Martin (1988)</td>
<td>(2)</td>
<td>Linear</td>
<td>(b)</td>
<td>Linear</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Merengo et al. (1988)</td>
<td>(6)</td>
<td>Linear</td>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Antia and Horvath (1989)</td>
<td>(1), (2)</td>
<td>Langmuir</td>
<td>(b)</td>
<td>Linear</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Kang and McCoy (1989)</td>
<td>(3)</td>
<td>Linear</td>
<td>Linear</td>
<td>Linear</td>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>This work</td>
<td>(4)</td>
<td>Langmuir</td>
<td>(b)</td>
<td>Linear</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>2nd order</td>
</tr>
</tbody>
</table>

1) $\log k' = a - \beta \log C_\alpha$; 2) $\log k' = a - \beta C_\alpha$; 3) $\log (k' - a) = - \beta C_\alpha$; 4) $\log k' = a - \beta \log C_\alpha + \gamma C_\alpha$; 5) $\log k' = a_0 + a_1 C_\alpha + a_2 C_\alpha^2 + \cdots$; 6) $k' = a_0 + a_1 C_\alpha + a_2 C_\alpha^2 + \cdots$.

(a) Modulator competes with eluates for binding sites, but does not affect the $k'$ values of the eluates. (b) Modulator affects the $k'$ values of the eluates, but has negligible adsorption on the stationary phase.
multicomponent isotherm together with the eluates. In other cases, the modulator affects the retentivity of an eluite primarily by changing its adsorption equilib-
rium constant.

For smaller molecules, the linear dependency be-
tween log k' vs log C_m, log k' vs C_m, or log (k' − a) vs
C_m, may be anticipated (Table 1). However, for ma-
cromolecules such as polypeptides, the modulator
properties affect the retentivity of an
eluite primarily by changing its adsorption equilib-
rium constant. For smaller molecules, the linear dependency be-
tween log k' vs log C,, log k' vs C,, or log (k' − a) vs
C,, may be anticipated (Table 1). However, for ma-
cromolecules such as polypeptides, the linear depend-
cences are true only over relatively narrow modulator
concentration ranges (Aguilar et al., 1985). Further-
more, the β values (Table 1) can be negative, i.e.
the retention time of the solute increases when the modul-
ator concentration increases. The β and β values for
polypeptides are usually large compared to those for
small molecules such as simple organic acids (Aguilar
et al., 1985). Experimentally, the plots of log k' vs C,
or log C, for polypeptides have been found to be
nonlinear and pass through minima (Hearn and
Grego, 1983; Armstrong and Boehm, 1984; Kennedy
et al., 1986; Melander et al., 1989).

For macromolecules different modulator concen-
trations may result in certain changes such as changes
of conformation and ionization in macromolecules,
changes in the populations of accessible binding sites
(Hearn and Grego, 1983), or changes in interaction
mechanisms (Kennedy et al., 1986; Melander et al.,
1989). For instance, the interaction mechanism is
changed from electrostatic to hydrophobic interaction
with an increased modulator concentration in
ion-exchange chromatography (or electrostatic inter-
action chromatography). A popular eluite-modulator
relationship [correlation (4) in footnote 1 of Table
1] proposed by Melander et al. (1989), is suitable for
both electrostatic and hydrophobic interactions. This
relationship was supported by some thermodynamic
arguments (Melander et al., 1989).

A general model for gradient elution for protein
purification is necessary for the optimal design
of chromatographic systems. In this work a general rate
model has been presented for the study of gradient
elution in multicomponent nonlinear chromatography.
The model considers axial dispersion, film mass trans-
fer, intraparticle diffusion, second-order kinetics, and
uses the eluite-modulator relationship proposed by
Melander et al. (1989). The model is capable of simul-
ating various gradient operations including linear,
nonlinear and stepwise linear operations. An efficient
and robust numerical procedure is used for the solu-
tion to the general rate model.

THEORY AND MODEL

The following basic assumptions are needed for the
general rate model used in this study:

(1) The column is packed with porous adsorbents
which are spherical and uniform in size.
(2) The concentration gradients in the radial direc-
tion of the bed are negligible.
(3) The adsorption and desorption (dissociation)
follow the second-order kinetics.

MODEL FORMULATION

Based on these basic assumptions, the following
governing equations can be formulated from the dif-
ferential mass balances for each component in the
bulk fluid and the particle phases.

\[ -D_{bi} \frac{\partial^2 C_{bi}}{\partial Z^2} + \frac{\partial C_{bi}}{\partial Z} + \frac{\partial C_{bi}}{\partial t} + \frac{3k_i(1 - \epsilon_p)}{\epsilon_p R_p} \times (C_{ni} - C_{pi}) = 0 \]  

\[ (1 - \epsilon_p) \frac{\partial^2 C_{pi}}{\partial t^2} + \frac{\partial C_{pi}}{\partial t} - \epsilon_p D_{pi} \left[ \frac{1}{R^2} \frac{\partial}{\partial R} \left( R^2 \frac{\partial C_{pi}}{\partial R} \right) \right] = 0. \]

The ordinary differential equation below describes the
second-order kinetics.

\[ \frac{\partial C_{pi}}{\partial t} = k_{bi} C_{bi} \left( C_{ei} - \sum_{i=1}^{n} C_{pi} \right) - k_{bi} C_{pi}. \]

The three coupled model equations have the following
initial and boundary conditions:

\[ C_{bi} = C_{bi}(0, Z) \]  

\[ C_{pi} = C_{pi}(0, R, Z) \]  

\[ C_{pi} = C_{pi}(0, R, Z) \]  

\[ Z = 0, \quad \frac{\partial C_{bi}}{\partial Z} = 0 \]  

\[ Z = L, \quad \frac{\partial C_{bi}}{\partial Z} = 0 \]

\[ R = 0, \quad \frac{\partial C_{pi}}{\partial R} = 0 \]

\[ R = R_p, \quad \frac{\partial C_{pi}}{\partial R} = \frac{k_{bi}}{\epsilon_p D_{pi}} (C_{ni} - C_{pi} - \epsilon_p). \]

Equations (1) and (2) are coupled via C_{pi}, which is the
concentration of component i at the surface of a
particle. In eq (2) and (3), C_{pi} is the concentration
of component i in the solid phase of the adsorbents
based on the unit volume of the solid, excluding pores.
Concentrations C_{bi} and C_{pi} are based on the unit
volume of mobile phase fluid.

By introducing the following dimensionless quantities:

\[ c_{bi} = C_{bi}/C_{0i}, \quad c_{pi} = C_{pi}/C_{0i}, \quad c_{pi} = C_{pi}/C_{0i}, \quad r = R/R_p, \quad z = Z/L, \quad \tau = \epsilon_p L \]

\[ P_{ei} = v L/D_{bi}, \quad B_i = k_{bi} R_p/\epsilon_p D_{pi} \]

\[ \eta_i = \epsilon_p D_{pi} L/R^2 v, \quad \zeta_i = 3B_i \eta_i(1 - \epsilon_p)/\epsilon_p \]

\[ D_{pi} = L(k_{pi} C_{pi})/v, \quad D_{pi} = L k_{pi} /v \]

the PDE system can be transformed into the following
dimensionless forms.

\[ \frac{1}{Pe_{ij}} \frac{\partial^2 c_{bi}}{\partial z^2} + \frac{\partial c_{bi}}{\partial t} + \frac{\partial c_{bi}}{\partial t} + \xi_i(c_{bi} - c_{bi,r-1}) = 0 \]  

(11)

\[ (1 - c_p) \frac{\partial c_{pi}}{\partial t} + c_p \frac{\partial c_{pi}}{\partial t} - \eta_i \left[ \frac{1}{2} \frac{\partial^2 c_{pi}}{\partial t^2} \right] = 0 \]  

(12)

\[ \frac{\partial^2 c_{bi}}{\partial z^2} = D_{a'i} c_{pi} \left( c_{i} - \sum_{j=1}^{N_s} C_{oi} c_{pj} \right) - D_{a'i} c_{pi}. \]  

(13)

I.C.

\[ c_{bi} = c_{bi}(0, z) \]  

(14)

\[ t = 0, \quad c_{pi} = c_{pi}(0, r, z) \]  

(15)

\[ c_{pi}^* = c_{pi}^*(0, r, z) \]  

(16)

B.C.

\[ z = 0, \quad \frac{\partial c_{bi}}{\partial z} = P_{e_{ii}}(c_{bi} - C_{f_i}(t)/C_{oi}) \]  

(17)

\[ z = 1, \quad \frac{\partial c_{bi}}{\partial z} = 0 \]  

(18)

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

(19)

\[ r = 1, \quad \frac{\partial c_{pi}}{\partial r} = B_i(c_{pi} - c_{pi,r-1}) \]  

(20)

If the adsorption and desorption rates, or the adsorption and desorption Damkohler numbers, \( D_{a'i} \) and \( D_{a'i}^* \), are sufficiently high, the left-hand side of eq. (13) can be set to zero and eq. (13) then reduces to the common multicomponent Langmuir isotherm if the saturation capacities are the same for all the components, which is the case in this work.

\[ C_{pi} = \frac{a_i C_{pi}^*}{1 + \sum_{j=1}^{N_s} b_j C_{pj}^*}, \text{ i.e. } c_{pi} = \frac{a_i C_{pi}^*}{1 + \sum_{j=1}^{N_s} (b_j C_{pj})} \]  

(21)

where \( b_j = k_{ai}/k_{di} \) and \( a_i = b_i C^m \). It is obvious that \( b_i C_{oi} = D_{a'i}/D_{a'i}^* \).

**Initial and boundary conditions for gradient elution**

The last component (component labeled \( N_s \)) is designated as the modulator in the gradient elution system. The modulator and eluates comply with the following correlation.

\[ \log b_i = \alpha_i - \frac{1}{\beta_i} \log C_{0,N_s} + \gamma_i C_{0,N_s} \]  

(22)

which comes from correlation (4) in footnote 1 in Table 1. The parameters in correlation (4) are usually obtained from retention data in linear chromatography in which \( K' = \phi C^m \). The adsorption equilibrium value \( b_i \) for a component can be used in the nonlinear range of the isotherm if it is assumed that the isotherm is Langmuir type in the concentration concerned (Antia and Horvath, 1989). The value of \( \phi C^m \) can be separated from \( \phi \theta \) and lumped into the \( \alpha \) term in correlation (4) to obtain eq. (22). It has been assumed that eluates do not interfere with each other's correlation parameters, \( a_i, \beta_i \) and \( \gamma_i \). The saturation capacities for all the eluates are the same (\( C^m \)) and they are not affected by the modulator concentration.

This correlation implies that when the modulator concentration is zero, the \( b_i \) values for eluates are infinity which cause irreversible bindings. It is desirable that the column should be presaturated with a small nonzero modulator concentration (denoted \( C_{mo} \)).

Since there are only correlations for equilibrium constants, not kinetic constants, we still can use the kinetic model with eq. (13). The asymptotic limit of the kinetic model is the equilibrium rate model which assumes that there exists an equilibrium for each component between the pore surface and the stagnant fluid phase in the macropores. We only need to set the Damkohler numbers for adsorption and desorption to high values (say, no less than 1000) and keep the ratio \( D_{a'i}/D_{a'i}^* = b_i C_{oi} \) for eluates \( (i = 1, 2, \ldots, N_s - 1) \).

The detailed initial conditions \((t = 0)\) for the gradient elution system are as follows:

for the eluates \((i = 1, 2, \ldots, N_s - 1)\)

\[ c_{bi} = c_{pi} = c_{pi}^* = 0 \]  

(23)

and for the modulator \((i = N_s)\)

\[ c_{bi} = c_{pi} = c_{pi}^* = 0 \]  

\[ c_{pi} = C_{mo}/C_{oi} = C_{mo}, \]  

(24)

\[ C_{mo}^* = 0. \]  

(25)

The dimensionless feed concentration profiles for the boundary conditions at the column inlet [eq. (17)] are for the eluates \((i = 1, 2, \ldots, N_s - 1)\)

\[ C_{f_i}(0)/C_{oi} = \begin{cases} 1 & 0 \leq \tau \leq \tau_{imp} \\ 0 & \tau > \tau_{imp} \end{cases} \]  

(26)

and for the modulator \((i = N_s)\)

\[ C_{f_i}(0)/C_{oi} \begin{cases} = C_{mo}/C_{oi} \quad \tau < \tau_{imp} \\ \geq (or \leq) & \tau \geq \tau_{imp} \end{cases} \]  

(27)

The upper boundary values of the rectangular sample pulse of the eluates are taken as their reference concentration values, \( C_{oi} \). For the modulator, its reference concentration value can take any convenient value, such as the \( C_{mo} \) which is the modulator concentration in the column prior to gradient take-off. The gradient profile of the modulator concentration can take any shape after sample injection, i.e. when \( \tau > \tau_{imp} \). If the feed concentration of the modulator is unchanged even after sample injection, the process becomes isocratic. If the take-off of the modulator concentration after sample injection is of a nonlinear fashion, i.e. \( C_{f_i,N_s}(\tau)/C_{0,N_s} \) vs. \( \tau \) is nonlinear for \( \tau > \tau_{imp} \), the process becomes a nonlinear gradient elution. Stepwise linear gradients are not considered nonlinear gradients.
Solution strategy

The two PDEs, eqs (11) and (12) are discretized with the finite-element and the orthogonal-collocation methods, respectively (Gu et al., 1990; Gu, 1990). Equation (13) is already an ODE and needs no discretization. The model equations, eqs (11)–(13), are then converted to an ODE system.

If \( Ne \) elements and \( N \) interior collocation points are used for the discretization of eqs (11) and (12), there will be \( N_s(2Ne + 1)(2N + 1) \) ODEs in the final ODE system (Gu, 1990). The ODE system is then solved with the IVPAG subroutine from IMSL (1987), which uses Gear’s stiff method for initial value ODE problems.

The use of a kinetic rate model instead of an equilibrium rate model gives a special advantage in dealing with gradient elution with variable \( b_i \) values for the eluites. In the equilibrium rate model (Gu et al., 1990), the multicomponent isotherm [such as eq. (21)] is directly inserted into the particle phase governing equation to eliminate \( c\_p \) in eq. (12). This makes the left-hand side of eq. (12) too complicated for the evaluation of time derivatives since \( a_1 \) and \( b_i \) are also time-dependent variables. The chain rule method used for decoupling the time derivatives of \( c\_p \)s cannot be used for this kind of gradient case. Fortunately, all the complications are not present if a kinetic model is used.

RESULTS AND DISCUSSION

To demonstrate the capability of the computer code based on the general rate model for gradient elution, several cases have been presented in this work. Parameter values used for simulation are listed in Table 2. CPU (central processing unit) times for some cases are also listed in the table. In all cases, the sample size is \( \tau_{\text{imp}} = 0.3 \), and \( \varepsilon_p = \varepsilon_b = 0.4 \). The error tolerance of the ODE solver in the FORTRAN code is \( 10^{-5} \). It is assumed that the adsorption of the modulator onto the stationary phase is negligible. This is done by setting the right-hand side of eq. (13) to zero.

To show the advantage of gradient elution over isocratic elution and the importance of the selection of a gradient method, a four-component system is chosen as an example. Components 1–3 are eluites and component 4 is a modulator. The relationship of the adsorption equilibrium constant of each eluite versus the modulator concentration is plotted in the double logarithmic scale shown in Fig. 1. In this system, separation has to be carried out in the lower modulator concentration range (electrostatic interaction range) since the affinities of the three eluites are too close in the higher modulator concentration range.

Figure 1 shows that the affinities of the three eluites differ widely when the modulator concentration is low. Isocratic elution takes too much time for a complete base-line separation of the three eluites as shown in Fig. 2 which gives the dimensionless effluent history of the four components of the gradient system. Furthermore, the eluite 1 peak and the eluite 2 peak are considerably diluted, especially the eluite 3 peak. To ensure a base-line separation, the modulator concentration in this isocratic elution cannot be increased, otherwise overlapping of the first two peaks will occur.

With a linear gradient of the modulator concentration in the mobile phase, the separation of the three eluites can be drastically improved. Figure 3 shows that a base-line separation of the three eluites can be carried out within a dimensionless time of \( \tau = 8 \) instead of \( \tau = 120 \) in Fig. 2, and the peak heights of the second and third eluites are increased many fold. Although regeneration of the column following a gradient elution takes some time, the cycle time of gradient elution is still much shorter than that of isocratic elution. The bandwidth in terms of dimensionless time of eluite 3 peak in Fig. 3 is \( \Delta \tau = 1 \) and in Fig. 2 \( \Delta \tau = 60 \). The areas of the eluite 3 peak in both cases are the sample size \( \tau_{\text{imp}} = 0.3 \). The ratio of the two bandwidths indicates that the average concentration of eluite 3 peak in Fig. 3 is 60 times higher than in

<table>
<thead>
<tr>
<th>Figure</th>
<th>Species</th>
<th>( Pe_t )</th>
<th>( \eta_t )</th>
<th>( Bl_t )</th>
<th>( C_{ti} )</th>
<th>( C^* )</th>
<th>( Ne )</th>
<th>( N )</th>
</tr>
</thead>
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<tr>
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<td>1 \times 10^{-4}</td>
<td>9-11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>350</td>
<td>4</td>
<td>30</td>
<td>1 \times 10^{-5}</td>
<td>1 \times 10^{-4}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>400</td>
<td>6</td>
<td>20</td>
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<td></td>
</tr>
<tr>
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<td>4</td>
<td>400</td>
<td>100</td>
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<td>2 \times 10^{-4}</td>
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<td>6</td>
<td>20</td>
<td>2 \times 10^{-4}</td>
<td>2 \times 10^{-4}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>400</td>
<td>100</td>
<td>5</td>
<td>10</td>
<td>0</td>
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</table>

*Dimensionless initial modulator concentrations are: \( \tau = 0.027 \); Figs 3–5, 0.015; Fig. 7, 0.217; Fig. 8, 0.25. Gradient profiles \( C_{\tau,N} \) are for Fig. 3, 0.015 + 0.015(\( \tau - \tau_{\text{imp}} \)) for \( \tau \geq \tau_{\text{imp}} \); Fig. 4, 0.015 + 0.007(\( \tau - \tau_{\text{imp}} \)) for \( \tau \geq \tau_{\text{imp}} \); Fig. 5, 0.015 + 0.015(\( \tau - \tau_{\text{imp}} \)) for \( \tau \leq 3.1 \), and \( -0.223 + 0.1(\tau - \tau_{\text{imp}}) \) for \( \tau \geq 3.1 \); Figs 6, 0.25 - 0.05(\( \tau - \tau_{\text{imp}} \)) for \( \tau \geq \tau_{\text{imp}} \). CPU times on SUN 4/390 computer for some simulations are: Fig. 2 (\( Ne = 7 \), 13.36 min; Fig. 3 (\( Ne = 9 \), 24.00 min; Fig. 4 (\( Ne = 9 \), 25.74 min; Fig. 5 (\( Ne = 11 \), 49.89 min.)
Fig. 2. The effluent history and the influent history (dashed line) of the modulator are both shown in Fig. 3 to show the effect of the modulator on the eluites. The gradient serves to reduce the excessive retentivities of the second and especially the third eluite.

It is obvious that the gap between the peaks of eluites 2 and 3 cannot be shortened to reduce the time required for a base-line separation of the three eluites, otherwise the resolution of the first two peaks will suffer. Apparently a nonlinear gradient can improve the situation by producing a sharper increase of the modulator concentration on a later stage which does not affect the resolution of the first two peaks. A quadratic gradient is chosen for the demonstration. The effluent history in Fig. 4 clearly shows that the gap between the second and third peaks is reduced and the separation time is shortened compared to Fig. 3. It is very interesting to note that the peak height sequence in Fig. 4 is reversed compared to Fig. 3 because of the change in gradient method.

In practice, nonlinear gradient imposes a higher demand on the hardware and software of the chromatographic system for accurate delivery of the mobile phase (Jandera et al., 1980). Instead of using nonlinear gradients, stepwise linear gradients are more popular (Ghrist and Snyder, 1988). Stepwise linear gradients can be used to replace nonlinear gradients. Figure 5 has the same conditions as Figs 3 and 4, except the gradient method used in the elution. In Fig. 5 there are two linear gradients. The dashed line in the figure is the gradient profile of the modulator in the mobile phase at the column inlet. Mathematical expressions for the gradient profiles are given
Modeling of gradient elution in multicomponent nonlinear chromatography

in Table 2. The first gradient in Fig. 5 is the same as the linear gradient in Fig. 3. At \( \tau = 3.1 \) the slope of the gradient increases. This increase does not affect the resolution of the first two peaks since this increase is only in effect trailing the second peak as shown by the comparison of the first two peaks in Figs 3 and 5. This is also revealed by the effluent histories of the three eluents and the modulator in Fig. 5. The separation time in Fig. 5 is slightly shorter than that in Fig. 4. The third eluite peak, which is affected by the second gradient in Fig. 5, has a higher peak height than that of Fig. 4.

The eluite–modulator relationship used in this work accounts for both electrostatic and hydrophobic interactions. Figure 6 shows the eluite–modulator relationship of a system with two eluents and one modulator. At the lower modulator concentration range the affinities of the two eluents are very close. Gradient elution must be operated at the lower modulator concentration range. In this hydrophobic interaction range, the affinities of the eluents decrease with decreasing the modulator concentration. Negative gradients instead of the common positive gradients are needed for the separation. Figure 7 is an isocratic elution of the two eluents in Fig. 6. The second peak in Fig. 7 has a long tail due to the nonlinearity of its isotherm. With a linear gradient to reduce the affinity of the second eluite the separation time can be reduced and its effluent concentration can be increased. This can be carried out with a negative linear gradient as shown in Fig. 8. The gradient takes effect when the peak front of the first eluite has passed
the column. The negative gradient decreases the modulator concentration which thus reduces the affinity of the second eluite (Fig. 6). This helps to reduce the retention time and tailing of the second eluite.

In some systems the relative positions of the eluite–modulator relationship curves are not as desirable as those shown in Figs 1 and 6. Situations with cross-over and mix-up of convergent and divergent curves may occur (Antia and Horvath, 1989; Melander et al., 1989). In such cases, strategies for choosing proper gradient profiles may be more complicated. Further studies are needed.

CONCLUSIONS

In this work, a general rate model has been developed for the study of gradient elutions in nonlinear chromatography. The model is suitable for preparative- and large-scale chromatography since various dispersive effects, such as axial dispersion, film mass transfer, intraparticle diffusion, are considered in the model. A robust and efficient numerical method has been used for the solution of the model. The eluite–modulator relationship in the model accounts for both electrostatic and hydrophobic interactions. Simulations for the comparison of isocratic elution and gradient elution clearly demonstrate the advantage of gradient elution in terms of shortened separation time and increased peak heights with no loss of resolutions. This work also shows that nonlinear gradient is superior to linear gradient when there are more than two eluites present. Stepwise linear gradients can be used to replace nonlinear gradients. The general rate model presented in this work provides a
Modeling of gradient elution in multicomponent nonlinear chromatography

Fig. 7. Isocratic elution of a sample with two eluites.

Fig. 8. Gradient elution with a decreasing modulator concentration.

very useful tool for studying various important aspects of gradient elution in nonlinear chromatography.

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NOTATION

$\alpha_i$ constant in Langmuir isotherm for component $i$ ($= b_i C_i^*$)

$b_i$ adsorption equilibrium constant for component $i$ ($= k_{ai}/k_{di}$)

$B_{li}$ Biot number of mass transfer for component $i$ ($= k_{ai}/(k_{li} D_{li})$)

$C_{ai}$ bulk phase concentration of component $i$, M

$C_{fi}$ feed concentration profile of component $i$, a time-dependent variable, M

$C_0$ concentration used for nondimensionalization [$= \max \{C_{fi}(t), i \neq Ns\}$, M

$C_{0,Ns}$ reference concentration for the modulator (component $Ns$), M

$C_{pi}$ concentration of component $i$ in the stagnant fluid phase inside particle macro pores, M

$C_{m}$ modulator concentration, M

$C_{m0}$ initial modulator concentration, M

$C_{s,i}$ concentration of component $i$ in the solid phase of particle (mole adsorbate/liter of particle skeleton)

$C_{s,0}$ adsorption saturation capacity for eluites (mole adsorbate/liter of particle skeleton)

$C_{0,i}$ concentration of component $i$ in the solid phase of particle (mole adsorbate/liter of particle skeleton)
\[ c_i^{\text{a}} = c_i^{\text{c}}/C_{p0} \]

- **D_{pl}**: axial or radial dispersion coefficient of component \( i \)
- **\( D_{pl} \)**: effective diffusivity of component \( i \), porosity not included
- **\( D_{\text{ad}} \)**: Damköhler number for adsorption \( = L(k_{\text{ad}} C_{i0})/v \)
- **\( D_{\text{des}} \)**: Damköhler number for desorption \( = L(k_{\text{des}} C_{i0})/v \)
- **\( k_i \)**: retention factor
- **\( k_{\text{ad}} \)**: adsorption rate constant for component \( i \)
- **\( k_{\text{des}} \)**: desorption rate constant for component \( i \)
- **\( L \)**: column length
- **\( N \)**: number of interior collocation points
- **\( N_e \)**: number of quadratic elements
- **\( N_s \)**: number of components
- **\( P_{\text{el}} \)**: Péclet number of axial dispersion for component \( i \) \( = v L/D_{pl} \)
- **\( R \)**: radial coordinate for particle
- **\( R_p \)**: particle radius \( = R/R_p \)
- **\( t \)**: time
- **\( v \)**: interstitial velocity
- **\( Z \)**: axial coordinate
- **\( z \)**: (effective) dimensionless time duration for a rectangular pulse of the sample
- **\( \phi \)**: phase ratio

**Greek letters**

- **\( \alpha_i \)**: correlation parameters for component \( i \)
- **\( \beta_i \)**: bed void volume fraction
- **\( \eta_i \)**: particle porosity
- **\( \eta \)**: dimensionless constant \( = \eta \rho D_{pl} L/(R^2 \nu) \)
- **\( \xi_i \)**: dimensionless constant for component \( i \) \( = 3B \eta_i (1 - c_i) / c_i \)
- **\( \tau \)**: dimensionless time \( = vt/L \)
- **\( \tau_{\text{imp}} \)**: dimensionless time duration for a rectangular pulse of the sample

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