Chemical Engineering Science, Vol. 47, No. 1, pp. 253–262, 1992.
Printed in Great Britain.

0009–2509/92 $5.00 + 0.00

MODELING OF GRADIENT ELUTION IN MULTICOMPONENT NONLINEAR CHROMATOGRAPHY

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(*Received for publication* 16 *May* 1991)

Abstract—Gradient elution in nonlinear chromatography is a very powerful tool for the separation of components having a wide range of retentivity. It is not only used for chemical analysis, but also used in preparative- and large-scale chromatography for the separation of many macromolecules, such as proteins. In this work, a general rate model has been presented for the study of gradient elution in nonlinear chromatography. The model considers axial dispersion, external film mass transfer, intraparticle diffusion and kinetic effects. The modulator–eluite relationship used in the model accounts for both electrostatic and hydrophobic interactions. Examples of simulations have been given to demonstrate the efficiency and robustness of a computer code based on a numerical procedure for the general rate model, which uses the finite-element, the orthogonal-collocation and Gear's stiff methods. The examples also show the advantage of gradient elution over isocratic elution in multicomponent elutions, and the comparisons among linear, nonlinear and stepwise linear gradients.

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 eluites 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 eluites in different ways. Two types of interaction between the modulator and the eluites 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 eluites 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 Mazsaroff, 1987). Then, the modulator can be treated as a competing component in the

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Table 1. Comparison of mathematical models for gradient elution chromatography

Reference	Correlation† of k' vs C_m	Isotherm	Interaction‡ type	Gradient method	Axial dispersion	Film mass transfer	Intraparticle diffusion	Kinetic effect
Pitt (1976)	(1)	Linear	(a)	Linear	Yes	No	No	Linear
Jandera and Churacek (1980, 1981)	(1), (2)	Linear	(b)	Linear	No	No	No	No
Hearn and Grego (1983, 1985)	(1), (2)	Linear	(b)	Linear	No	No	No	No
Armstrong and Boehm (1984)	(2)	Linear	(b)	Linear	No	No	No	No
Kennedy *et al.* (1986)	(1)	Linear	(a)	Linear	No	No	No	No
Furusaki *et al.* (1987)	(3)	Linear	(b)	Linear	Yes	No	Yes	No
Yamamoto *et al.* (1987)	(3)	Linear	(b)	Linear	No	No	No	No
D'Agostino *et al.* (1988)	(5)	Linear	(b)	Linear	No	No	No	No
Ghrist and Snyder (1988)	(2)	Linear	(b)	Linear and stepwise linear	No	No	No	No
Markowski and Golkiewicz (1988)	(1), (2)	Linear	(b)	Stepwise	No	No	No	No
Martin (1988)	(2)	Linear	(b)	Linear	No	No	No	No
Merengo *et al.* (1988)	(6)	Linear	(b)	Linear	No	No	No	No
Antia and Horvath (1989)	(1), (2)	Langmuir (multicomponent)	(b)	Linear	Yes	No	No	No
Kang and McCoy (1989)	(3)	Linear	(b)	Linear	Yes	No	No	No
This work	(4)	Langmuir (multicomponent)	(b)	Linear Stepwise linear and nonlinear	Yes	Yes	Yes	2nd order

†(1) $\log k' = \alpha - \beta \log C_m$; (2) $\log k' = \alpha - \beta C_m$; (3) $\log (k' - \alpha) = -\beta C_m$; (4) $\log k' = \alpha - \beta \log C_m + \gamma C_m$; (5) $\log k' = \alpha_0 + \alpha_1 C_m + \alpha_2 C_m^2 + \cdots$; (6) $k' = \alpha_0 + \alpha_1 C_m + \alpha_2 C_m^2 + \cdots$.

‡(a) Modulator competes with eluites for binding sites, but does not affect the k' values of the eluites. (b) Modulator affects the k' values of the eluites, but has negligible adsorption on the stationary phase.

multicomponent isotherm together with the eluites. In other cases, the modulator affects the retentivity of an eluite primarily by changing its adsorption equilibrium constant.

For smaller molecules, the linear dependency between $\log k'$ vs $\log C_m$, $\log k'$ vs C_m, or $\log (k' - \alpha)$ vs C_m, may be anticipated (Table 1). However, for macromolecules such as polypeptides, the linear dependences are true only over relatively narrow modulator concentration ranges (Aguilar *et al.*, 1985). Furthermore, the β values (Table 1) can be negative, i.e. the retention time of the solute increases when the modulator concentration increases. The α 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_m or $\log C_m$ 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 concentrations 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 interaction 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 transfer, intraparticle diffusion, second-order kinetics, and uses the eluite–modulator relationship proposed by Melander *et al.* (1989). The model is capable of simulating various gradient operations including linear, nonlinear and stepwise linear operations. An efficient and robust numerical procedure is used for the solution 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 direction of the bed are negligible.
(3) The adsorption and desorption (dissociation) follow the second-order kinetics.
(4) The diffusional and mass transfer coefficients are constant and independent of the mixing effects of the components.

Model formulation

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

$$-D_{bi}\frac{\partial^2 C_{bi}}{\partial Z^2}+v\frac{\partial C_{bi}}{\partial Z}+\frac{\partial C_{bi}}{\partial t}+\frac{3k_i(1-\varepsilon_b)}{\varepsilon_b R_p}\times(C_{bi}-C_{pi,R=R_p})=0 \quad (1)$$

$$(1-\varepsilon_p)\frac{\partial C^s_{pi}}{\partial t}+\varepsilon_p\frac{\partial C_{pi}}{\partial t}-\varepsilon_p D_{pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\times\left(R^2\frac{\partial C_{pi}}{\partial R}\right)\right]=0. \quad (2)$$

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

$$\frac{\partial C^s_{pi}}{\partial t}=k_{ai}C_{pi}\left(C^\infty-\sum_{j=1}^{Ns}C^s_{pj}\right)-k_{di}C^s_{pi}. \quad (3)$$

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

$$C_{bi}=C_{bi}(0,Z) \quad (4)$$

$$t=0, \qquad C_{pi}=C_{pi}(0,R,Z) \quad (5)$$

$$C^s_{pi}=C^s_{pi}(0,R,Z) \quad (6)$$

$$Z=0, \qquad \frac{\partial C_{bi}}{\partial Z}=\frac{v}{D_{bi}}[C_{bi}-C_{fi}(t)] \quad (7)$$

$$Z=L, \qquad \frac{\partial C_{bi}}{\partial Z}=0 \quad (8)$$

$$R=0, \qquad \frac{\partial C_{pi}}{\partial R}=0 \quad (9)$$

$$R=R_p, \qquad \frac{\partial C_{pi}}{\partial R}=\frac{k_i}{\varepsilon_p D_{pi}}(C_{bi}-C_{pi,R=R_p}). \quad (10)$$

Equations (1) and (2) are coupled via $C_{pi,R=R_p}$ which is the concentration of component i at the surface of a particle. In eqs (2) and (3), C^s_{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

$$c_{bi}=C_{bi}/C_{0i}, \quad c_{pi}=C_{pi}/C_{0i}, \quad c^s_{pi}=C^s_{pi}/C_{0i}$$

$$r=R/R_p, \quad z=Z/L, \quad \tau=vt/L$$

$$Pe_{Li}=vL/D_{bi}, \quad Bi_i=k_iR_p/\varepsilon_p D_{pi}$$

$$\eta_i=\varepsilon_p D_{pi}L/R_p^2 v, \quad \xi_i=3Bi_i\eta_i(1-\varepsilon_b)/\varepsilon_b$$

$$Da^a_i=L(k_{ai}C_{0i})/v, \quad Da^d_i=Lk_{di}/v$$

the PDE system can be transformed into the following

dimensionless forms.

$$-\frac{1}{Pe_{Li}}\frac{\partial^2 c_{bi}}{\partial z^2}+\frac{\partial c_{bi}}{\partial z}+\frac{\partial c_{bi}}{\partial \tau}+\xi_i(c_{bi}-c_{pi,r=1})=0 \tag{11}$$

$$(1-\varepsilon_p)\frac{\partial c_{pi}^s}{\partial \tau}+\varepsilon_p\frac{\partial c_{pi}}{\partial \tau}-\eta_i\left[\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_{pi}}{\partial r}\right)\right]=0 \tag{12}$$

$$\frac{\partial c_{pi}^s}{\partial \tau}=Da_i^a c_{pi}\left(c^\infty-\sum_{j=1}^{Ns}\frac{C_{0j}}{C_{0i}}c_{pj}^s\right)-Da_i^d c_{pi}^s. \tag{13}$$

I.C.

$$c_{bi}=c_{bi}(0,z) \tag{14}$$

$$\tau=0, \qquad c_{pi}=c_{pi}(0,r,z) \tag{15}$$

$$c_{pi}^s=c_{pi}^s(0,r,z) \tag{16}$$

B.C.

$$z=0, \qquad \frac{\partial c_{bi}}{\partial z}=Pe_{Li}(c_{bi}-C_{fi}(\tau)/C_{0i}) \tag{17}$$

$$z=1, \qquad \frac{\partial c_{bi}}{\partial z}=0 \tag{18}$$

$$r=0, \qquad \frac{\partial c_{pi}}{\partial r}=0 \tag{19}$$

$$r=1, \qquad \frac{\partial c_{pi}}{\partial r}=Bi_i(c_{bi}-c_{pi,r=1}). \tag{20}$$

If the adsorption and desorption rates, or the adsorption and desorption Damköhler numbers, Da_i^a and Da_i^d, 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.

$$C_{pi}^s=\frac{a_i C_{pi}}{1+\sum_{j=1}^{Ns} b_j C_{pj}}, \text{ i.e. } c_{pi}^s=\frac{a_i c_{pi}}{1+\sum_{j=1}^{Ns}(b_j c_{0j})c_{pj}} \tag{21}$$

where $b_i=k_{ai}/k_{di}$ and $a_i=b_iC^\infty$. It is obvious that $b_iC_{0i}=Da_i^a/Da_i^d$.

Initial and boundary conditions for gradient elution

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

$$\log b_i=\alpha_i-\beta_i\log C_{p,Ns}+\gamma_i C_{p,Ns} \tag{22}$$

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 $k'=\phi C^\infty b$. The adsorption equilibrium value b 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 ϕC^∞ can be separated from ϕb and lumped into the α term in correlation (4) to obtain eq. (22). It has been assumed that eluites do not interfere with each other's correlation parameters, α_i, β_i and γ_i. The saturation capacities for all the eluites are the same (C^∞) and they are not affected by the modulator concentration. This correlation implies that when the modulator concentration is zero, the b_i values for eluites are infinity which cause irreversible bindings. It is desirable that the column should be presaturated with a small nonzero modulator concentration (denoted C_{m0}).

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 Damköhler numbers for adsorption and desorption to high values (say, no less than 1000) and keep the ratio $Da_i^a/Da_i^d=b_iC_{0i}$ for eluites ($i=1,2,\ldots,Ns-1$).

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

for the eluites ($i=1,2,\ldots,Ns-1$)

$$c_{bi}=c_{pi}=c_{pi}^s=0 \tag{23}$$

and for the modulator ($i=Ns$)

$$c_{bi}=c_{pi}=C_{m0}/C_{0i}=c_{m0}, \tag{24}$$

$$c_{pi}^s=0. \tag{25}$$

The dimensionless feed concentration profiles for the boundary conditions at the column inlet [eq. (17)] are

for the eluites ($i=1,2,\ldots,Ns-1$)

$$C_{fi}(\tau)/C_{0i}=\begin{cases}1 & 0\leqslant\tau\leqslant\tau_{\text{imp}}\\ 0 & \tau>\tau_{\text{imp}}\end{cases} \tag{26}$$

and

for the modulator ($i=Ns$)

$$C_{fi}(\tau)/C_{0i}\begin{cases}=C_{m0}/C_{0i} & -\infty<\tau\leqslant\tau_{\text{imp}}\\ \geqslant(\text{or}\leqslant)C_{m0}/C_{0i} & \tau>\tau_{\text{imp}}.\end{cases} \tag{27}$$

The upper boundary values of the rectangular sample pulse of the eluites are taken as their reference concentration values, C_{0i}. For the modulator, its reference concentration value can take any convenient value, such as the C_{m0} 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 $\tau>\tau_{\text{imp}}$. 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. $C_{f,Ns}(\tau)/C_{0,Ns}$ vs τ is nonlinear for $\tau>\tau_{\text{imp}}$, 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 $Ns(2Ne + 1)(2N + 1)$ 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_{pi}^s in eq. (12). This makes the left-hand side of eq. (12) too complicated for the evaluation of time derivatives since a_i and b_i are also time-dependent variables. The chain rule method used for decoupling the time derivatives of c_{pi}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 $\tau_{imp} = 0.3$, 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 affinites 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 $\tau_{imp} = 0.3$. 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 2. Parameter values used for simulation†

Figure	Species	Physical parameters					Numerical parameters	
		Pe_i	η_i	Bi_i	C_{0i}	C^∞	Ne	N
2–5	1	350	4	30	5×10^{-5}	1×10^{-4}	9–11	2
	2	350	4	30	1×10^{-5}	1×10^{-4}		
	3	400	6	20	1×10^{-5}	1×10^{-4}		
	4	400	100	5	0.1	0		
7, 8	1	400	3	25	2×10^{-4}	2×10^{-4}	14–15	2
	2	400	6	20	2×10^{-4}	2×10^{-4}		
	3	400	100	5	10	0		

† Dimensionless initial modulator concentrations are: Fig. 2, 0.027; Figs 3–5, 0.015; Fig. 7, 0.217; Fig. 8, 0.25. Gradient profiles $C_{f,Ns}(\tau)/C_{0,Ns}$ are: Fig. 3, $0.015 + 0.015(\tau - \tau_{imp})$ for $\tau \geqslant \tau_{imp}$; Fig. 4, $0.015 + 0.007(\tau - \tau_{imp})^2$ for $\tau \geqslant \tau_{imp}$; Fig. 5, $0.015 + 0.015(\tau - \tau_{imp})$ for $\tau_{imp} \leqslant \tau < 3.1$, and $-0.223 + 0.1(\tau - \tau_{imp})$ for $\tau \geqslant 3.1$; Fig. 8, $0.25 - 0.05(\tau - \tau_{imp})$ for $\tau \geqslant \tau_{imp}$. 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.

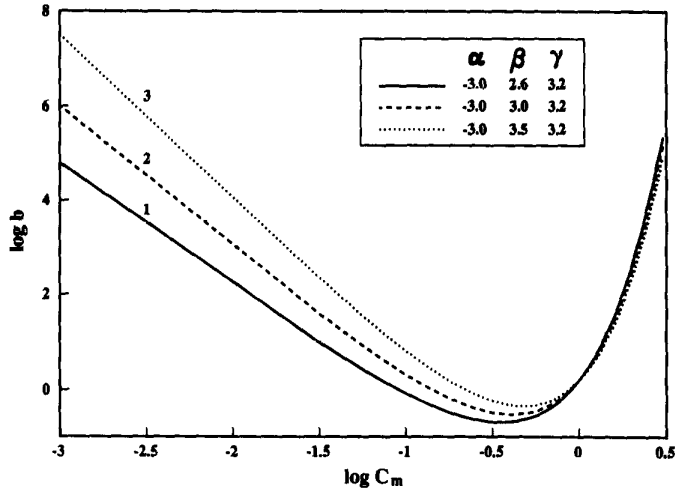

Fig. 1. Modulator vs eluite relationship for a system with three eluites and one modulator.

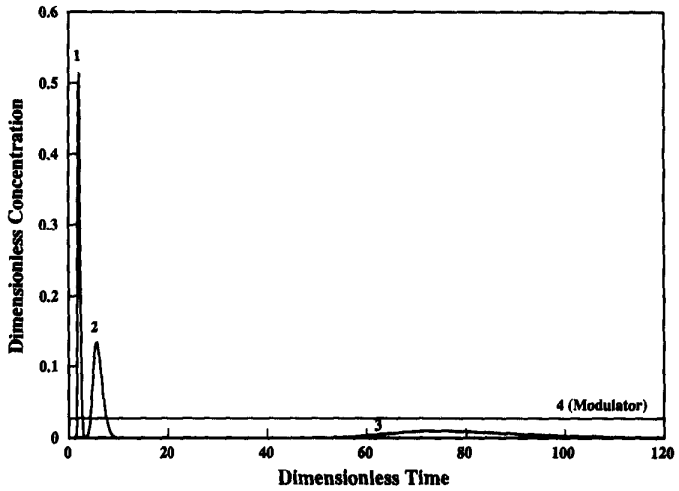

Fig. 2. Isocratic elution of a sample with three eluites.

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

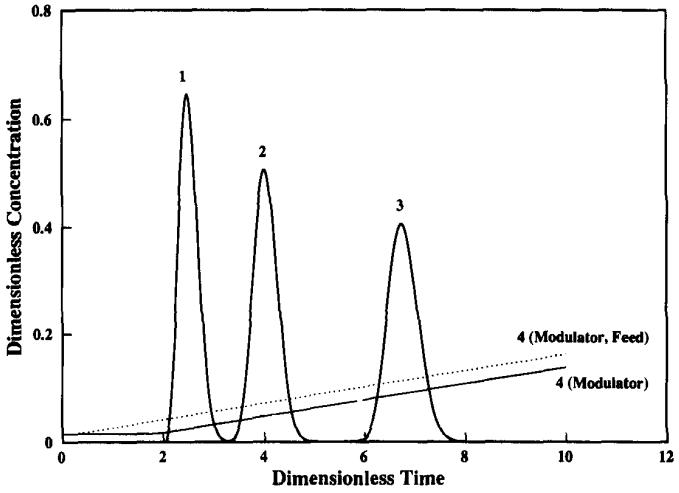

Fig. 3. Linear gradient elution of a sample with three eluites.

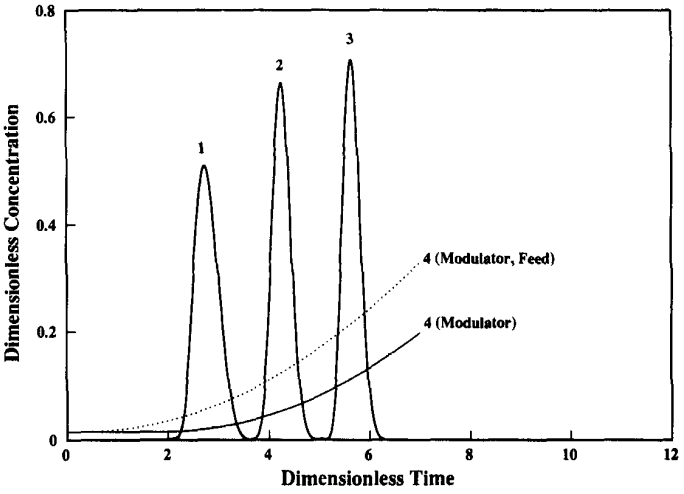

Fig. 4. Nonlinear gradient elution of a sample with three eluites.

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 eluites 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 eluites and one modulator. At the lower modulator concentration range the affinities of the two eluites are very close. Gradient elution must be operated at the lower modulator concentration range. In this hydrophobic interaction range, the affinities of the eluites 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 eluites 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

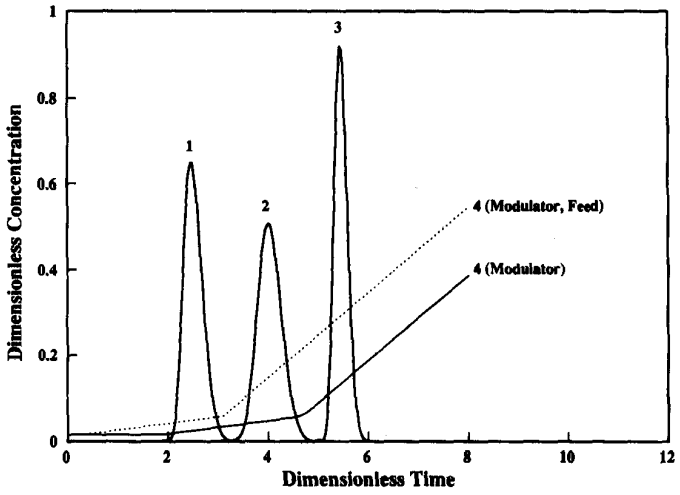

Fig. 5. Gradient elution with two consecutive linear gradients of a sample with three eluites.

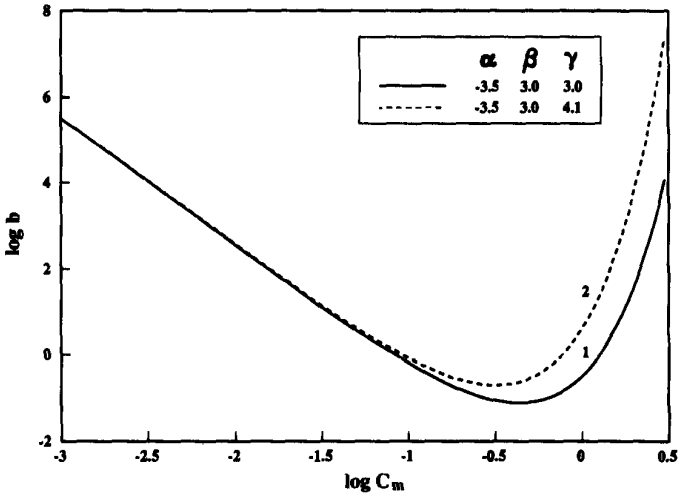

Fig. 6. Modulator vs eluite relationship for a system with two eluites and one modulator.

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

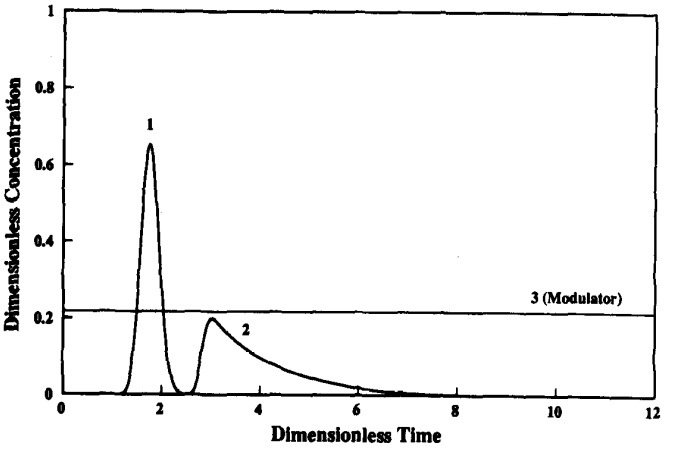

Fig. 7. Isocratic elution of a sample with two eluites.

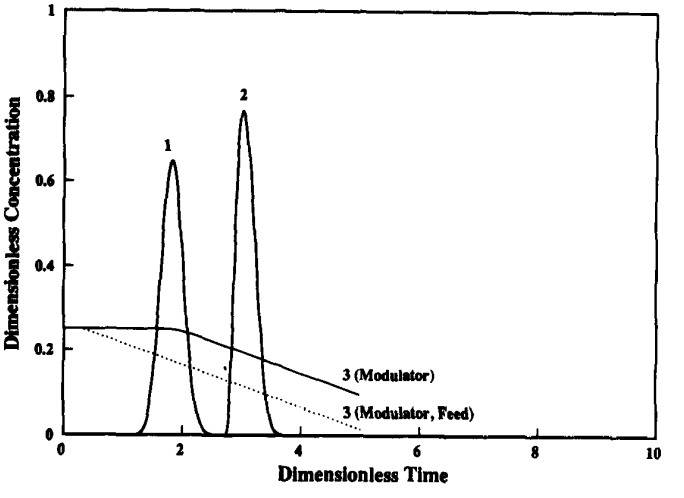

Fig. 8. Gradient elution with a decreasing modulator concentration.

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

Acknowledgement—We acknowledge financial support from National Science Foundation Grant EET-8613167A2.

NOTATION

a_i constant in Langmuir isotherm for component i ($= b_i C_i^{\infty}$)

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

Bi_i Biot number of mass transfer for component i [$= k_i R_p/(\varepsilon_p D_{pi})$]

C_{bi} bulk phase concentration of component i, M

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

C_{0i} 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 macropores, M

C_m modulator concentration, M

C_{m0} initial modulator concentration, M

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

C^{∞} adsorption saturation capacity for eluites (mole adsorbate/liter of particle skeleton)

c_{bi} ($= C_{bi}/C_{0i}$)

c_m ($= C_m/C_{0,Ns}$)

c_{m0} ($= C_{m0}/C_{0,Ns}$)

c_{pi} ($= C_{pi}/C_{0i}$)

c_{pi}^s ($= C_{pi}^s/C_{0i}$)

c^{∞}	$(= C^{\infty}/C_{0i})$
D_{bi}	axial or radial dispersion coefficient of component i
D_{pi}	effective diffusivity of component i, porosity not included
Da_i^a	Damköhler number for adsorption $[= L(k_{ai}C_{0i})/v]$
Da_i^d	Damköhler number for desorption $(= Lk_{di}/v)$
k'	retention factor
k_i	film mass transfer coefficient of component i
k_{ai}	adsorption rate constant for component i
k_{di}	desorption rate constant for component i
L	column length
N	number of interior collocation points
Ne	number of quadratic elements
Ns	number of components
Pe_{Li}	Peclet number of axial dispersion for component i $(= vL/D_{bi})$
R	radial coordinate for particle
R_p	particle radius
r	$(= R/R_p)$
t	time
v	interstitial velocity
Z	axial coordinate
z	$(= Z/L)$

Greek letters

$\alpha_i, \beta_i, \gamma_i$	correlation parameters for component i
ε_b	bed void volume fraction
ε_p	particle porosity
η_i	dimensionless constant $[= \varepsilon_p D_{pi}L/(R_p^2 v)]$
ξ_i	dimensionless constant for component i $[= 3Bi_i\eta_i(1-\varepsilon_b)/\varepsilon_b]$
τ	dimensionless time $(= vt/L)$
τ_{imp}	dimensionless time duration for a rectangular pulse of the sample
ϕ	phase ratio

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