Journal of Chromatography, 484 (1989) 1–27
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 047

GRADIENT ELUTION IN NON-LINEAR PREPARATIVE LIQUID CHROMATOGRAPHY

FIROZ D. ANTIA and CSABA HORVÁTH*
Department of Chemical Engineering, Yale University, P.O. Box 2159 Yale Station, New Haven, CT 06520 (U.S.A.)

SUMMARY

The effect of column overload in gradient elution is studied theoretically using a model based on a differential mass balance equation that is solved numerically by an orthogonal collocation method. First elution profiles of a single component and then the separation of binary mixtures having both constant and non-constant separation factors with changing modulator concentration are examined and contrasted with results obtained by isocratic elution under comparable conditions. In overloaded chromatography of binary mixtures with constant separation factor gradient elution is shown to be superior to isocratic elution for the cases studied on the basis of production rate, yield and enrichment factor. For the examination of the results obtained by gradient elution with binary mixtures having non-constant separation factors, a logarithmic mean separation factor, which offers a simple means for comparison and indicates whether or not separation will occur in overloaded conditions, is introduced. The effect of selectivity inversion due to changes in eluent strength and the role played by the logarithmic mean separation factor are illustrated.

INTRODUCTION

In elution chromatography, also called wave or zonal chromatography, a pulse of a mixture is fed into the column and its components are eluted by a continuous stream of an eluent that binds less strongly to the stationary phase than any of the eluites. Elution is isocratic when the eluent strength is kept constant throughout the separation. In gradient elution, introduced almost four decades ago[1], the eluent strength is increased continuously during the chromatographic run by suitable manipulation of the mobile phase composition upon increasing or decreasing the concentration of an appropriate additive termed the mobile phase modulator. This facilitates the separation, in a single run, of all the components of the mixture, whether they are initially weakly or strongly bound to the stationary phase. For this reason, gradient elution is widely used for the separation of mixtures whose components display a broad range of retentivity. Furthermore, gradient elution is employed frequently in the separation of biopolymers in order to take advantage of the concomitant increase in the peak capacity[2].

0021-9673/89/$03.50

In most analytical applications, linear elution chromatography (where analytes are present at low concentrations and their adsorption is governed by Henry's law) is employed so that the migration of a component is not perturbed by the presence of other eluites. In preparative chromatography, however, feed concentrations can be high enough so that the migrating molecules interfere with each other's adsorption. As a result the adsorption isotherms are non-linear and, under these conditions, the process is termed non-linear, or overloaded, chromatography.

Gradient elution chromatography with linear adsorption isotherms has been studied theoretically since the introduction of the technique[3–10]. The most comprehensive treatment has been provided by Snyder[6]. Since the results of these theories do not apply directly in non-linear conditions, attempts have been made recently to model overloaded gradient elution with the help of Craig simulations[11–14]. The goal of our work is to compare gradient and isocratic elution chromatography under overloaded conditions using a mathematical description based on a differential mass balance[15]. We first outline our model and test it under linear conditions to verify that it matches the extant theory for this case. The model is then used to compute elution profiles of single components and of binary mixtures in non-linear gradient elution chromatography. Next, a comparison of performance in gradient and isocratic elution for a binary mixture having a constant separation factor is presented. Finally, the phenomenon of selectivity reversal with changing eluent strength and its effects on the separation of binary mixtures by gradient elution under overloaded conditions are discussed.

MODEL

The adsorption isotherm

In chromatography, the migration rate of the components of a mixture, and thus their separation, depends on how they distribute between the stationary and mobile phases. In overloaded chromatography, the concentration of an eluite i in the stationary phase, q_i, depends on its own local concentration, c_i, as well as on the local concentrations of all the other eluites and of the mobile phase modulator, φ. Thus the adsorption isotherm, which formally describes the equilibrium concentrations of the components in the two phases, must account for the interdependence of the sorption behavior of the eluites and the modulator. The simplest multicomponent adsorption isotherm is the Langmuir isotherm, given by[16,17]

$$q_i = \frac{a_i c_i}{1 + \sum_{j=1}^{n} b_j c_j} \qquad i = 1, 2, \ldots, n \tag{1}$$

where a_i and b_i are the parameters of the respective single component isotherms and n is the total number of eluites. The parameter a_i is dimensionless and represents the initial slope of the isotherm, b_i has the dimension of reciprocal concentration and the ratio a_i/b_i is equal to λ_i, the saturation capacity per unit volume of the sorbent for species i. In the limit of infinite dilution the isotherm is linear and the retention factor, k'_i, is equal to Φa_i, where Φ is the ratio of stationary to mobile phase volumes (the phase ratio) which, for simplicity, is set to unity in this study. The isotherm in eqn. 1 predicts that the

separation factor for two components, i and j, defined as $q_j c_i / q_i c_j$, is constant. For this reason, when the saturation capacities of the various components are unequal, eqn. 1 does not satisfy the surface analogue of the Gibbs–Duhem equation (the so-called Gibbs' isotherm) and is therefore thermodynamically inconsistent[18,19]. Nevertheless, use of the Langmuir isotherm has been found to give results that are in reasonable concord with experimental data obtained under various conditions when there are no drastic changes in separation factor with eluite concentration[20–22].

In view of eqn. 1, the modulator could be treated as one of the n species so that at low eluite concentrations, the dependence of the retention factor on the modulator concentration, φ, would be given by

$$k'_i = \frac{\Phi a_i}{1 + b_\varphi \varphi} \tag{2}$$

where b_φ is the pertinent isotherm parameter for the modulator. However, the data in the literature of analytical chromatography for the retention factor *versus* modulator concentration do not support eqn. 2. For example in reversed-phase chromatography the data appear to conform to a relationship of the type[6]

$$\kappa_i = \kappa_{0,i} - S_i \varphi / 2.3 \tag{3}$$

Here κ is $\log_{10} k'_i$, κ_0 is $\log_{10}(\Phi a_{0,i})$ where $a_{0,i}$ is the value of a_i in the absence of modulator and $-S_i/2.3$ is the slope of the $\kappa - \varphi$ plot. Assuming that the saturation capacity for a component is constant and independent of the modulator concentration, substituting eqn. 3 into eqn. 1 yields the isotherm

$$q_i = \frac{a_{0,i} \exp(-S_i \varphi) c_i}{1 + \sum_{j=1}^{n} \frac{a_{0,j}}{\lambda_j} \exp(-S_j \varphi) c_j} \qquad i = 1, 2, \ldots, n \tag{4}$$

Similarly, in ion-exchange (electrostatic interaction) chromatography with a salt as the modulator, retention is related to the salt molality in the mobile phase, φ_s, via[23]

$$\kappa_i = \log_{10}(\Phi a_{1,i}) - Z \log \varphi_s \qquad i = 1, 2, \ldots, n \tag{5}$$

where $a_{1,i}$ is the projected value of a_i at $\varphi_s = 1$ and Z is a constant representing the ratio of the apparent valence of the eluite to the valence of the salt counterion. Substitution of eqn. 5 in eqn. 1 yields the isotherm

$$q_i = \frac{a_{1,i} \varphi_s^{-z} c_i}{1 + \sum_{j=1}^{n} \frac{a_{1,j}}{\lambda_j} \varphi_s^{-z} c_j} \qquad i = 1, 2, \ldots, n \tag{6}$$

A shortcoming of eqn. 6 is that it becomes indeterminate at $\varphi_s = 0$. Alternatively, the rigorous isotherm for ion-exchange could be employed[24,25], but it has the disadvantage of being implicit in q_i.

In this study we use only the isotherm in eqn. 4 which is expected to hold under conditions where eqn. 3 is valid, *i.e.*, in reversed-phase chromatography with organic solvent as the modulator, and, with appropriate changes of sign, in reversed-phase or hydrophobic interaction chromatography with salt as the modulator. The groups $a_{0,i}\exp(-S_i\varphi)$ and $a_{0,i}\exp(-S_i\varphi)/\lambda_i$ can be viewed as modified Langmuir parameters a_i and b_i, respectively. Although the assumption of constant λ is not expected to hold over a wide range of modulator concentration, there is some experimental support for eqn. 4 from data on the variation of single component Langmuir isotherm parameters with modulator concentration[20,22]. More information is needed to ascertain the true nature of multicomponent adsorption isotherms of various species, particularly of biopolymers at large and the effect of modulators in particular.

Dispersive effects

In chromatography, band spreading occurs due to various factors, including diffusion in the mobile phase, flow maldistribution arising from non-uniformities in the column packing structure, mass transfer to, from and within the sorbent beads, slow sorption kinetics, and non-linearities in the adsorption isotherms. It is assumed that the effect of all dispersive processes, with the exception of those due to slow sorption kinetics and isotherm non-linearities, can be lumped into an effective dispersion coefficient, $\mathscr{D}$, whose value is estimated from chromatograms of the eluites in linear chromatography[26]. The value for $\mathscr{D}$ can be determined theoretically from the sorbent particle size, the column packing structure, the flow velocity of the mobile phase and the diffusivity and retention factor of the migrating components[27]. Although in non-linear systems $\mathscr{D}$ is likely to depend on the eluite concentrations, in the present study it is assumed to be concentration independent. Moreover, a single value of $\mathscr{D}$ is used to describe the dispersion of all the eluites. This appears to be acceptable for closely related components under some circumstances[26], but needs more support under conditions when diffusivities are strongly concentration dependent. Bandspreading due to slow sorption is neglected in the model since kinetic limitations are assumed to be absent.

Governing equations

With these assumptions, the chromatographic process can be described by an isothermal one-dimensional differential mass balance as[15,26]

$$\frac{\partial c_i}{\partial t} + \Phi\frac{\partial q_i(c_1, c_2, \ldots, c_n, \varphi)}{\partial t} + u_0\frac{\partial c_i}{\partial z} = \mathscr{D}\frac{\partial^2 c_i}{\partial z^2} \qquad i = 1, 2, \ldots, n \quad (7)$$

with the initial and boundary conditions for elution of the feed components being[28]

$$c_i(0,z) = 0 \qquad 0 \le z \le L$$

$$c_i(t,0) = c_{0,i} \qquad 0 < t \le t_{\text{inj}} \qquad i = 1, 2, \ldots, n \quad (8)$$

$$\left(\frac{\partial c_i}{\partial z}\right)_{z=L} = 0 \qquad t \ge 0$$

Here $q_i(c_1, c_2, \ldots, c_n, \varphi)$ indicates that the stationary phase concentration is a function of the eluite and modulator concentrations as given by an adsorption isotherm such as those in eqns. 4 or 6, u_0 is the mean velocity of the bulk mobile phase which is considered not to be bound to the stationary phase[26], t is time, z is the distance in the direction of bulk flow, L is the column length, t_{inj} is the time of feed introduction, and $c_{0,i}$ is the concentration of component i in the feed.

The progress of the eluent modulator proper through the system ought to be described by an equation similar to eqn. 7 with appropriate boundary conditions. However, for simplicity, adsorption of the modulator onto the stationary phase is assumed to be negligible compared to that of the eluites. Furthermore, the modulator molecule is often smaller than the eluites and therefore is expected to disperse to a much lesser extent. With these approximations the mass balance for the modulator is

$$\frac{\partial \varphi}{\partial t} + u_0 \frac{\partial \varphi}{\partial z} = 0 \tag{9}$$

and the initial and boundary conditions are

$$\begin{aligned} &\varphi(t_{inj},z) = 0 \quad 0 \leq z \leq L \\ &\varphi(t,0) = g(t - t_{inj}) \quad t > t_{inj} \end{aligned} \tag{10}$$

where g is an arbitrary function that represents the gradient profile, given by the modulator concentration as a function of time at the column inlet. Here the gradient is assumed to begin immediately after the feed is introduced, *i.e.*, at time t_{inj}. Any appropriate delay time to account for system dwell volume between the gradient former and column can be readily introduced by adding this to t_{inj}, but this is neglected here . The solution to eqns. 9 and 10 at any axial location z is simply $\varphi = g(t - z/u_0 - t_{inj})$. We will consider only linear forms for the gradient profile, *i.e.*, the rate of change of modulator concentration is a constant, β, so that

$$\varphi(t,z) = \varphi_0 + \beta\left(t - \frac{z}{u_0} - t_{inj}\right) \tag{11}$$

where φ_0 is the modulator concentration in the eluent at the start of the gradient.

Our model for overloaded gradient elution chromatography is completely specified by eqns. 4, 7, 8 and 11 that must be solved numerically. To facilitate the calculations we introduce the dimensionless independent variables $\theta (= tu_0/L)$ for time and $X (= z/L)$ for distance. In terms of the dimensionless variables the model for gradient elution chromatography is written as

$$\begin{aligned} &\frac{\partial c_i}{\partial \theta} + \Phi \frac{\partial q_i}{\partial \theta} + \frac{\partial c_i}{\partial X} = \frac{1}{2N} \frac{\partial^2 c_i}{\partial X^2} \\ &c_i(0,X) = 0 \qquad 0 \leq X \leq 1 \end{aligned} \tag{12}$$

$$c_i(\theta,0) = c_{0,i} \quad 0 < \theta \leq \tau \quad i = 1, 2, \ldots, n \tag{12}$$

$$\left(\frac{\partial c_i}{\partial X}\right)_{X=1} = 0 \qquad \theta \geq 0$$

$$q_i = q_i(c_1, c_2, \ldots, c_n, \varphi)$$

$$\varphi = \varphi_0 + \beta t_0(\theta - X - \tau)$$

Here t_0 is L/u_0, τ is t_{inj}/t_0 and the expression for q_i is given by eqn. 4.

The parameter N is a Peclet number given by $u_0L/2\mathscr{D}$ that equals the plate number under conditions of linear isocratic chromatography. The effective dispersion coefficient, $\mathscr{D}$, in eqn. 7 is defined so as if all dispersive process would occur in the time that the eluite molecules spend in the mobile phase. Thus when relating N and $\mathscr{D}$, only the time spent by the eluite in the mobile phase, and not the total retention time, must be considered.

Presentation of results

It is convenient to introduce a dimensionless gradient steepness parameter, G[6] given by

$$G \equiv \frac{S_{ref}\beta t_0}{2.3} \tag{13}$$

where S_{ref} is the S value (see eqn. 3) for one of the eluting components chosen as a reference. Similarly a dimensionless load parameter, $\mathscr{L}$ is defined by the mass of the reference feed component normalized to the saturation capacity of the column for that component under conditions prevalent at the start of the run.

$$\mathscr{L} \equiv \frac{\tau c_{0,ref}}{\Phi\lambda_{ref}} \tag{14}$$

In general any feed components may be chosen as reference for G and $\mathscr{L}$; when the S and λ values and feed concentrations are the same for each eluite G and $\mathscr{L}$ are independent of the choice of reference. Selection of the reference component in other cases is discussed later.

For a given feed mixture, results calculated from eqn. 12 are illustrated as "chromatograms" showing the outlet concentration of the components *versus* column volumes of mobile phase passed through the system (numerically the same as the dimensionless time, θ) for specified values of the parameters φ_0, N, G and $\mathscr{L}$. Other attributes of the chromatogram may be calculated from these results, as discussed later. When $a_{0,i}\exp(-S_i\varphi_0)$, *i.e.*, the "$a$" parameters at the start of the run, G and the ratio of S values of each component to the reference component are specified, the results are independent of the particular S values.

Calculations

The model for gradient elution is given by a set of partial differential equations, one for each component, which must be integrated numerically. When substituting eqn. 4 into eqn. 12, use of the chain rule yields a set of equations of the form

$$\boldsymbol{A}y = \boldsymbol{B} \tag{15}$$

where y is a vector of temporal derivatives with elements $\partial c_i/\partial\theta$, $\boldsymbol{B}$ is a vector whose elements contain spatial derivatives and the terms $(\partial q_i/\partial\varphi)(\partial\varphi/\partial\theta)$, and $\boldsymbol{A}$ is a matrix of coefficients that depend on $\partial q_i/\partial c_j$. Orthogonal collocation on finite elements is used to estimate the spatial derivatives (subroutine DSS046 in the software package DSS/2 supplied by Prof. W. E. Schiesser at Lehigh University, Bethlehem, PA, U.S.A.), and eqn. 15 is then solved for the temporal derivatives using a standard linear algebraic equation solver (routine SGEFCS, written at Argonne National Labs., and available in the Numerical Analysis Program Library at the Yale Computer Center). The resulting set of ordinary differential equations, n for each collocation point, are integrated using a standard ODE solver (routine LSODE, available in the DSS/2 library and elsewhere). Sharp transitions at the beginning of the gradient profile are smoothened by the arc of a circle so as to ensure that the time derivative of the modulator concentration is defined at every point. Similar orthogonal collocation schemes have been used to simulate fixed-bed absorption column performance[29].

All calculations were performed on either a MicroVAX computer in the Yale Chemical Engineering Department or on a VAX 8600 computer in the Yale Computer Center. Results were plotted using software from Passage Software (Ft. Collins, CO, U.S.A.) on a Macintosh computer. A fourth order collocation scheme with the column divided into about 80 identical elements was used. The time required for the simulation of a single chromatographic run on the VAX 8600 computer varied from 2 to 10 min for a single component and from 7 to 45 min for a binary mixture, depending on the values of the parameters.

RESULTS AND DISCUSSION

Validation of numerical procedure for linear chromatography

The accuracy of the numerical procedure *per se* was confirmed in both isocratic and gradient elution with linear isotherms ($\lambda_j \to \infty$ in eqn. 4) for $100 < N < 2000$ and k' values up to 10. In isocratic elution at very low loads, plate numbers measured by standard methods[30] from simulated chromatograms matched within 5% the plate number used in our calculations. In gradient linear elution with low loads, bandwidths predicted by our model under linear conditions tallied closely with those calculated using Snyder's linear solvent strength (LSS) theory of gradient elution[6,31]. Details of these calculations are provided in the Appendix. A comparison of the results obtained by the two approaches is shown in Fig. 1, where the ratio of the peak variance in isocratic elution to that in gradient elution, $\sigma^2_{is}/\sigma^2_{gr}$, is plotted against gradient steepness for different values of the apparent retention factor, k'_{app}. Linear adsorption isotherms were used and, for the comparison, retention times were taken to be the same in both isocratic and gradient elution. It was found that in agreement with the LSS theory the predictions of the method did not depend on the plate number.

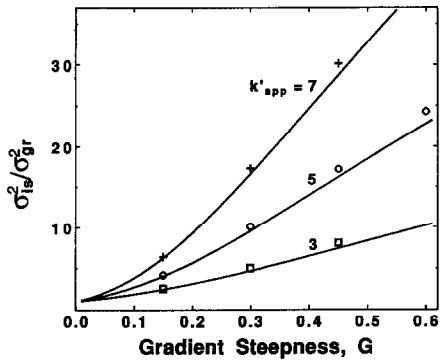

Fig. 1. The ratio of peak variances in isocratic and gradient elution for components having linear isotherms ($\lambda_j \rightarrow \infty$ in eqn. 4) as a function of the gradient steepness with the apparent retention factor, k_{app}, as the parameter. Solid lines were calculated according to eqn. A6 whereas symbols represent values calculated using eqn. 12 at very low loads.

In calculating the results in Fig. 1 by the LSS theory the correction factor for "anomalous" band broadening[6] was not used. The close match between the band variances calculated from our model and those from the linear theory in the absence of excessive broadening suggests that the origins of the anomaly lie in processes such as slow sorption kinetics that are not accounted for explicitly in either model. Since the anomalous broadening is not universally observed[32], and axial dispersion is overshadowed by band spreading due to isotherm non-linearity[33], correction for anomalous band broadening may not be necessary in overloaded elution.

Concentration profiles of single components

Although the behavior of a single component yields little information on the separation proper in non-linear chromatography, it is instructive to examine the concentration profiles of single components under overloaded conditions. In Fig. 2 single peaks obtained in isocratic elution are compared to those in gradient elution for three values of the gradient steepness parameter, G, and five values of the load parameter, $\mathscr{L}$. The value of a_0 is adjusted such that all peaks have a common end point, keeping τ constant so that load is varied only by changing the concentration (overload at constant volume). Therefore the peaks at different loads have coincident tails.

In Fig. 2, there is a regular progression from the concave upward rear envelope of the isocratic bands to the accentuated concave downward rear profile at high values of G. In gradient elution the strength of the eluent is greater at the rear of the peak than at its front: when the isotherm is linear, this causes molecules at the rear to move faster than those at the front, giving rise to a peak compression that counteracts dispersion. Although under overloaded conditions the velocity of the molecules depends not only on the local concentration of the modulator, but also on that of the eluite, the compressive effect tends to oppose band broadening arising from isotherm non-linearity and gives the rear of the band its characteristic concave downward shape.

For a given value of $\mathscr{L}$, the maximum concentration of the bands in Fig. 2 first decreases and later increases with increasing G, as indicated in Table I. This occurs

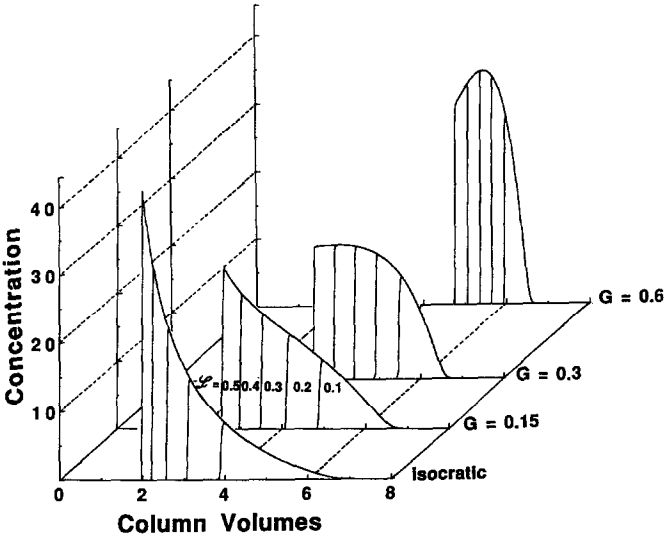

Fig. 2. Effect of elution conditions and column overloading on the peak profile of a single component. Peaks obtained for different loads under otherwise identical conditions are superposed in each case. Conditions: $N = 500$, $\Phi = 1$, $\tau = 0.5$, $\lambda = 100$; in isocratic elution, $a = 5$; in gradient elution $\varphi_0 = 0$, a_0 adjusted such that $k'_{app} = 5$. Concentration units are arbitrary.

because, in order to ensure a common elution time for all the peaks, conditions have been adjusted so that the binding strength at the start of the process is larger for runs with higher gradient steepness. Thus the progress of the band immediately following injection is arrested more strongly with increasing G, with a concommitant decrease in the concentration of the peak front. The concentration at the peak front is the maximum band concentration until at higher G band compression becomes significant and the maximum concentration increases. Unlike the maximum concentration the mean peak concentration always increases with increasing G, as summarized in Table I.

TABLE I

COMPARISON OF SINGLE COMPONENT PEAKS IN ISOCRATIC AND GRADIENT ELUTION[a] AS MEASURED BY THE MAXIMUM CONCENTRATION, c_{max}, THE MEAN CONCENTRATION, $\bar{c}$, THE FIRST MOMENT AT $\mathscr{L} = 0.5$ RELATIVE TO THAT AT $\mathscr{L} = 0.1$, $\mu_1(0.5)/\mu_1(0.1)$, THE SECOND CENTRAL MOMENT, μ_2^*, THE APPARENT PLATE NUMBER, μ_1^2/μ_2^*, AND THE SKEWNESS[b], μ_3^*/μ_2^{*3}

G	c_{max}	$\bar{c}$	$\frac{\mu_1(0.5)}{\mu_1(0.1)}$	μ_2^*	μ_1^2/μ_2^*	μ_3^*/μ_2^{*3}
0.0[c]	42.4	10.3	0.652	0.988	9.86	1.081
0.15	23.8	12.1	0.721	0.978	16.02	0.509
0.3	19.9	15.4	0.805	0.612	36.48	0.231
0.6	34.5	26.7	0.897	0.198	155.1	0.144

[a] $\mathscr{L} = 0.5$. Conditions as in Fig. 2.
[b] *cf.* ref. 30.
[c] Isocratic elution.

Quasi-linearization due to the gradient. When gradient elution produces bands of reduced asymmetry as compared with corresponding isocratic band profiles, the effect may be termed as a quasi-linearization due to the gradient. Several characteristics of the overloaded isocratic and gradient elution peaks for $\mathscr{L} = 0.5$ are summarized in Table I. The trends for peaks at other loads are similar. In overloaded chromatography, unlike under linear conditions, the first moments of the peaks do vary with load, as indicated by values of the first moments of peaks at $\mathscr{L} = 0.5$ normalized to those at $\mathscr{L} = 0.1$, but the variation decreases with increasing gradient steepness. The apparent efficiency of the column may be expressed by the ratio of the square of the first moment to the second central moment (μ_1^2/μ_2^*), which follows from the definition of the plate number under analytical isocratic conditions. The results in Table I show that this ratio increases fifteen-fold as G is increased from 0 to 0.6 and the skewness of the peaks, larger positive values of which indicate a greater extent of tailing[30], decreases significantly with increasing G. Thus, at higher gradient steepness, peak shapes approach more closely the symmetrical peaks encountered in analytical chromatography and this is an indication of the quasi-linearization due to the gradient. In turn, slight column overloading in analytical chromatography is less likely to lead to asymmetrical peaks with gradient elution than with isocratic elution. However, quasi-linearization is meaningful only at low levels of overload, since in multicomponent separations at higher loads interference between eluites is important and distorts peak shapes considerably, as discussed later.

Concentration and volume overload in gradient elution. In preparative chromatography with isocratic elution two limiting cases are distinguished: concentration overload, when the feed is introduced as an impulse of infinitesimal volume, and volume overload, when the feed is introduced in a finite volume at sufficiently low concentrations such that the adsorption isotherms are linear. In practice, conditions lie somewhere in between these two extremes. In non-linear isocratic elution, the effect of large sample volume becomes noticeable when τ exceeds more than half the bandwidth of a concentration overloaded peak having the same mass load[33,34]. However, in gradient elution, when the initial loading step is carried out under strong binding conditions ($a_0 > 100$) the adsorption isotherm is almost rectangular[25]. Therefore, immediately following feed introduction, the sample, regardless of its input concentration, is confined to a very narrow band in the column. Consequently, the band shape observed when the peak is eluted under gradually weaker binding conditions is practically independent of the load volume. Thus, in gradient elution with initially strong binding conditions, only the magnitude of the mass load counts and the concept of volume overloading is not pertinent.

Concentration profiles for binary mixtures with constant separation factor

Because of interference between migrating species, peak profiles in multicomponent chromatography have little resemblance to the corresponding single component peak shapes until they are completely separated and interference ceases, as evidenced by theoretical and experimental examples of non-linear isocratic elution[22,35,36]. Representative peak profiles of a 1:1 binary mixture calculated for non-linear gradient elution at increasing values of $\mathscr{L}$ for fixed values of the gradient steepness and initial modulator concentration, φ_0, are shown in Fig. 3. For both components the value of S is the same and thus the separation factor, $q_B c_A/q_A c_B$, *i.e.*, the selectivity, is

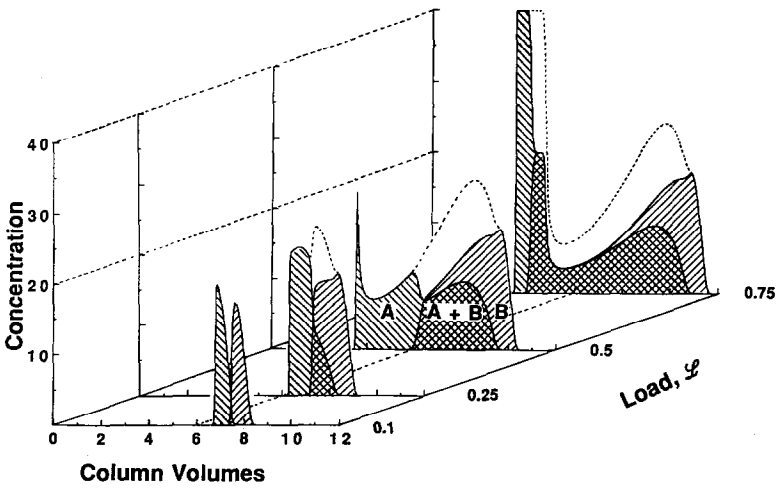

Fig. 3. Elution profiles of a 1:1 binary mixture in non-linear gradient elution at different loads. An envelope representing the response of a non-specific detector, *i.e.*, the total concentration, is shown in addition to the concentration profiles of the two components. Conditions: $\varphi_0 = 0, G = 0.45, N = 250, \Phi = 1; \lambda_A = \lambda_B = 100, a_{0,A} = 600, a_{0,B} = 1000, S_A = S_B$. Component A elutes first.

independent of modulator concentration. Separations with non-constant selectivity are discussed later. In the calculated chromatograms shown in Fig. 3, at a loading of approximately 50% of the total stationary phase capacity for each component, peak splitting occurs. Note that no split portion of the peak appears at $\theta = 1$, as it would in split-peaks arising from slow adsorption kinetics[37] where the early eluting split fraction moves with the mobile phase velocity. In our case it is a result of interference during the feed step, which is essentially foreshortened frontal chromatography: a fraction of the first component is pushed ahead by the second one, and, due to the concave downward shapes of the isotherms, is concentrated to a level greater than its concentration in the feed[38,39]. Furthermore, at high load the feed time and consequently the value of the gradient delay is large, and the first component front travels a significant distance (more than halfway through the column in this case) before the gradient is introduced at the column inlet. As a result, the front of the first component traverses the column by isocratic elution leaving behind a long tail. The compressive effect of the gradient later acts to concentrate the trailing portion of the first component peak. The trailing portion of component A forms two distinct humps as a result of the abrupt change in its isotherm parameters across the second component front. Such complex peak shapes, which are the result of interfering adsorption behavior of the components, are of course not observed with single components. At even higher loads, the fronts of both the first and second component may pass through the column under isocratic conditions during the long feed time and the process increasingly exhibits the features of frontal chromatography followed by gradient elution.

Design parameters in preparative chromatography

The goal of a preparative separation is to recover from a mixture the largest

quantities of a set of desired substances at a specified purity and concentration in the shortest possible time. These aims can be quantified in terms of three design parameters[28,40]:

(i) The production rate, P_i, defined as the mass of the desired substance i produced per run at a specified purity divided by the cycle time, θ_c, that includes any wash and regeneration steps. In dimensionless terms

$$P_i = \frac{\int_{\theta_{1,i}}^{\theta_{2,i}} c_i(\theta,1)\mathrm{d}\theta}{\Phi\lambda_{\mathrm{ref}}\theta_c} \tag{16}$$

where $c_i(\theta,1)$ is the outlet concentration of the product i, and $\theta_{1,i}$ and $\theta_{2,i}$ are "cut" points between which it is collected. The cut points are chosen such that the product is of the specified purity. For P_i to be a meaningful parameter for scaling purposes, normalization is done in the same manner as for the load parameter in eqn. 14. For simplicity, results reported here for both isocratic and gradient elution are based on a cycle time that extends from the start of the run to the point where the last traces of the last component elute. Actual dimensional production rates would depend upon the value of the flow-rate: detailed analysis of flow-rate effects can be found in the literature[41].

(ii) The yield, Y_i, which is the fraction of the desired substance i fed into the column that is recovered as product.

(iii) The enrichment factor, E_i, given by concentration of product i normalized to its concentration in the feed.

Separation of a binary mixture with constant separation factor

Optimization of isocratic elution. Our aim here is to compare preparative separations of a given binary mixture in isocratic and gradient elution on the basis of the three design parameters. For this purpose the separation must first be optimized under isocratic conditions. We will examine the separation of a 1:1 binary mixture with a constant separation factor of 1.67 on a column having 250 plates. In order that the separation factor remains constant, the κ–φ plots for the two components must be parallel, *i.e.*, they must have the same slope: this is often true of closely related compounds[6]. In non-linear elution, when $\mathscr{L}$ is greater than 0.1, the bandwidth is essentially independent of plate number for N greater than 200[33]; hence the low value used for the plate number to facilitate the numerical calculations is justified. On such a column under linear isocratic conditions at analytical loads the resolution between the components, R_s[30], would lie between 1.1 and 1.4, depending on the value of a_B.

There are two independent variables in the optimization: the load, $\mathscr{L}$, and the modulator concentration, or, equivalently, the Langmuir parameter, a_B, which may be manipulated by changing the modulator concentration. In Fig. 4, the production rates, yields and enrichment factors in isocratic elution for 98% pure A and B are plotted against load for different values of a_B. For each a_B value the production rates for both components as functions of load go through maximas, yields fall monotonically with increasing load, and enrichment factors reach a plateau at high load, as has been

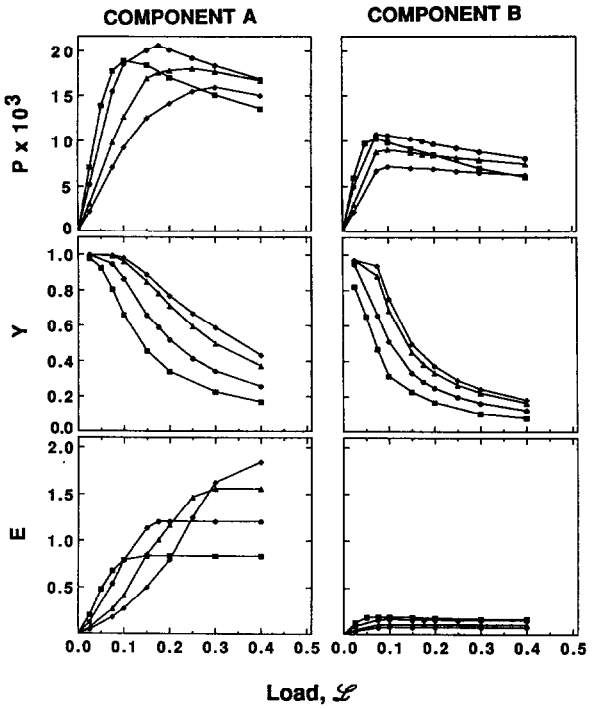

Fig. 4. Production rate, P (see eqn. 17), yield, Y, and enrichment factor, E, for the components of a 1:1 binary mixture in overloaded isocratic elution as functions of load, $\mathscr{L}$. Conditions not indicated here are as in Fig. 3; $a_{0,B}/a_{0,A} = 1.67$. ◆ = $a_B = 7.5$; ▲ = $a_B = 5.0$; ● = $a_B = 2.5$; ■ = $a_B = 1.5$.

reported elsewhere[15,40,42]. Values of a_B and $\mathscr{L}$ can be found such that the production rate is maximized. In the example shown, maximal production for component A is achieved with $a_B = 2.5$ and $\mathscr{L} = 0.175$, and for component B at the same value for a_B but at $\mathscr{L} = 0.075$. Production rates are significantly higher for the first component as compared with the second. This is because component A tails into the most concentrated region of peak B. Since the isotherm of A is suppressed in the presence of B due to competition for adsorption sites on the stationary phase (*vide* eqn. 4), A is desorbed and consequently displaced by B, resulting in enrichment factors which are higher for the first than for the second component. The tailing is reduced and the displacement enhanced when B is present in higher concentrations than A in the feedstock[42] and also if $\lambda_A < \lambda_B$, as discussed later.

In general, choice of optimal conditions depends on the relative importance of the three design parameters for the application at hand, and on the component designated as the product. In many cases production rate and enrichment factor are the most significant parameters. The yield is often of minor importance, since it is always possible, in principle, to recycle impure fractions. However, in such circumstances, the feedstock composition would change from run to run, and a careful analysis of viable recycle schemes is warranted.

Optimization of gradient elution and comparison with isocratic elution. For

a binary mixture with constant separation factor under analytical conditions, the resolution in gradient elution is lower than in a corresponding isocratic separation (see Appendix). Thus in linear chromatography of such binary mixtures, gradient elution can actually be detrimental to the separation. On the other hand, in preparative chromatography, resolution has little significance, and comparison of the two techniques should be based on the three design parameters discussed earlier.

In Fig. 5, production rates normalized with respect to the corresponding maximum production rate in isocratic elution ($P_{gr}/P_{max,is}$), yields and enrichment factors for components A and B in gradient elution are plotted against load for different values of G with fixed initial gradient conditions (*i.e.*, fixed $a_{0,B}$). The general trends followed in gradient elution mirror those in isocratic elution: production rates and enrichment factors are higher for the first component as compared with the second. Enrichment factors are significantly larger for both components in gradient elution as compared with isocratic elution. This is a result of band compression, and is also partly due to the increased displacement of A by B since the latter is maintained at higher concentrations in gradient as compared with isocratic elution. The enrichment factor for component A goes through a minima as a result of the peak splitting illustrated in Fig. 3. At high loads, component A is essentially recovered by frontal chromatography and the plateau value of the enrichment factor reflects the concentration reached in a frontal process.

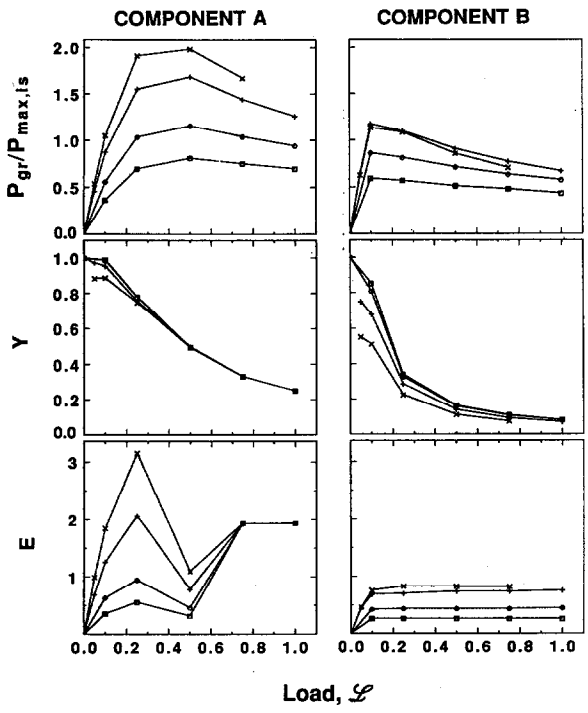

Fig. 5. Production rate normalized to the maximum production rate under isocratic conditions ($P_g/P_{max,is}$), yield, Y, and enrichment factor, E, for the components of a 1:1 binary mixture in overloaded gradient elution as functions of load, $\mathscr{L}$. Conditions not indicated here are as in Fig. 3. × = G = 1.50; + = G = 0.95; ○ = G = 0.45; □ = G = 0.25.

As shown in Fig. 5, production rates in gradient elution can be considerably higher than in isocratic elution for the first component whereas there appears to be no significant advantage for the second component. In gradient elution, the production rate for the second component can be improved by appropriately changing the initial gradient conditions by changing the initial modulator concentration. For a binary mixture with constant separation factor of 1.67, the highest production rate for B is achieved with $G = 0.6$ and $a_{0,B}\exp(-S\varphi_0) = 50$ at $\mathscr{L} = 0.1$ and is approximately 40% higher than the maximum isocratic production rate shown in Fig. 4. This gain may be important in certain large scale industrial applications. However, the advantages may well be vitiated if gradient formation and column regeneration in the gradient elution cycle are significant economic factors. Since the production rate is far less for B than for A in both gradient and isocratic elution, when the purification of component B is the goal, displacement chromatography[28,39,43] may be the method of choice.

After gradient elution, the column must be re-equilibrated with the initial mobile phase before subsequent runs, and this adds to the cycle time. Furthermore in isocratic elution it is possible to introduce the next feed batch before the components of the previous one exit the column and this reduces the cycle time somewhat. However, since it is a good practice to wash the column to remove contaminants after every run, isocratic or gradient, re-equilibration considerations depend largely on the particular application and must be addressed independently in each case.

The results shown in Figs. 4 and 5 are for a case in which the stationary phase has equal saturation capacities, λ, for both components of the binary mixture. If the capacity for the early eluting component A is less than that for component B, simulations with either our or other[14] models show a considerably enhanced displacement effect in both gradient and isocratic elution, with concomitantly higher production rates and yields, for both components, than those reported in this study. Although for such conditions the Langmuir isotherm is thermodynamically inconsistent, its employment has some experimental support[44]. In the opposite case, however, when $\lambda_A > \lambda_B$, models based on the Langmuir isotherm fail to provide adequate descriptions of observed phenomena[21,45].

Separation of binary mixtures with non-constant separation factor

Thus far we have been concerned with the special case of binary mixtures where the separation factor between the components is assumed to be constant, regardless of their concentrations or that of the modulator. In practice, however, the separation factor may vary with the concentration of the components in the feed (concentration dependent selectivity), or the modulator (modulator dependent selectivity), or both (concentration and modulator dependent selectivity). Our model for non-linear gradient elution is based on the isotherm relationship in eqn. 4, which predicts constant separation factors at a given modulator concentration and thus does not account for concentration dependent selectivity. However, it allows us to treat modulator dependent selectivity: here we examine those cases where values of the slope of the κ–φ plots are different for the individual components and thus selectivity varies with modulator concentration.

Perusal of the literature (*cf.* refs. 32, 46 and 47) shows that the κ–φ plots for a pair of eluites with fundamentally different molecular structures may converge or diverge

from their initial value at $\varphi = 0$, as shown in the insets in Fig. 6, and we may call them convergent or divergent eluite pairs, respectively. The κ–φ plots for a convergent pair always cross, leading to selectivity reversal over a defined modulator concentration range, although this range may or may not be significant in practice. For convenience, in the following study of binary mixtures only the parameters of component A are altered, whereas those of component B are left unchanged. With fixed values of the parameters for B ($a_{0,B} = 1000$, $\lambda_B = 100$) the retention behavior of the pair in gradient elution is completely specified by a value for the selectivity in the absence of modulator, α_0 ($=a_{0,B}/a_{0,A}$), the ratio of slopes S_B/S_A, and a value for λ_A, if different from λ_B. With $\varphi_0 = 0$ and given values of G the results as calculated from eqn. 12 are independent of the particular value of S_B. For illustration, however, κ–φ plots are drawn with $S_B = 20$.

Comparison of convergent, divergent and parallel eluite pairs. A comparison of the behavior of convergent, divergent and parallel pairs having the same value of α_0 in overloaded gradient elution is shown in Fig. 6. The chromatograms indicate that at a given load, initial modulator concentration (fixed at zero in this study) and gradient steepness, separation appears to be inferior for a convergent and superior for a divergent pair as compared with that for the parallel pair. This behavior is intuitively reasonable, and has been observed in a broad range of α_0.

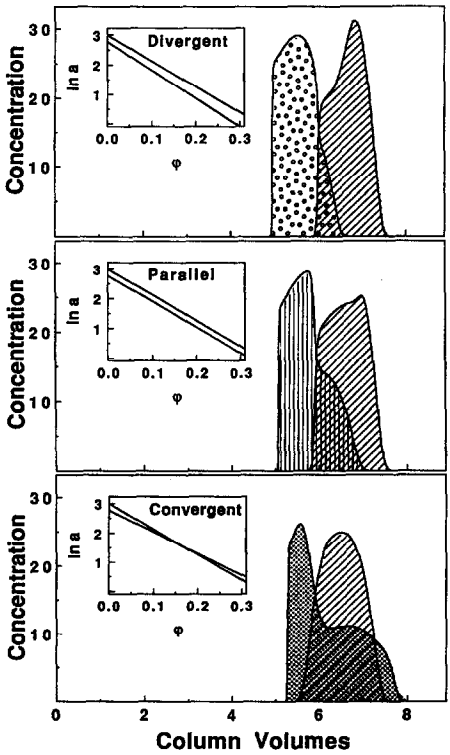

Fig. 6. Elution profiles of a 1:1 binary mixture with convergent, parallel and divergent κ–φ plots with $\alpha_0 = 1.67$ as shown in the insets. Conditions: $\varphi_0 = 0$, $G = 0.45$, $\mathscr{L} = 0.325$; convergent plot, $S_B/S_A = 1.176$; divergent plot, $S_B/S_A = 0.91$. Other conditions as in Fig. 3. Elution profiles are independent of S_B but $S_B = 20$ is shown in insets for illustration.

In order to compare such systems with modifier dependent selectivity, we introduce on the basis of the κ–φ plot a logarithmic mean separation factor, $\bar{\alpha}_{ln}$, defined by

$$\bar{\alpha}_{ln} \equiv \frac{\alpha_{\varphi_0} - \alpha_{\varphi^*}}{\ln\left(\frac{\alpha_{\varphi_0}}{\alpha_{\varphi^*}}\right)} \tag{17}$$

where α_{φ_0} is the separation factor at the initial modulator concentration and α_{φ^*} is evaluated at the modulator concentration, φ^*, at which Φa for the less retained component is unity. The concentration φ^* is chosen because in gradient linear chromatography when the isocratic retention factor, k', *i.e.* the value of Φa, is initially high (> 100), the components elute when Φa is $1/2.3G$, the value of which is approximately unity in practice[6]; thus, the modulator concentration is near φ^* at the end of the separation. For a pair of components with given α_{φ_0} and ratio of slopes S_B/S_A, $\bar{\alpha}_{ln}$ is independent of the value chosen for S_B, as may be verified by substituting into eqn. 17 the pertinent relationships for κ *versus* φ given by eqn. 3.

A comparison of the behavior of convergent, divergent and parallel eluite pairs having the same $\bar{\alpha}_{ln}$ is shown in Fig. 7. For a given load, initial modulator

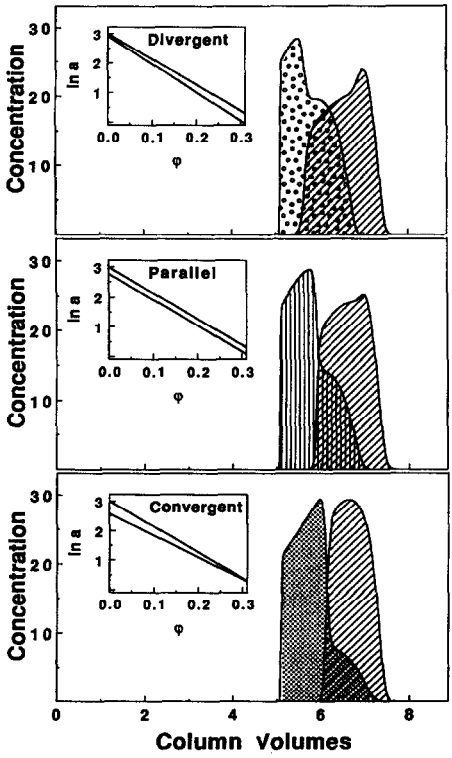

Fig. 7. Elution profiles of a 1:1 binary mixture with convergent, parallel and divergent κ–φ plots with $\bar{\alpha}_{ln} = 1.67$ as shown in the insets. Conditions: convergent plot, $\alpha_0 = 2.682$; divergent plot, $\alpha_0 = 1.212$. Other conditions as in Fig. 6.

concentration and gradient steepness, the extent of separation is approximately the same although the three chromatograms show somewhat different elution profiles. Indeed, given the necessarily *ad hoc* nature of eqn. 17, separations of different pairs of components having different S_B/S_A ratios but the same $\bar{\alpha}_{ln}$, exhibit remarkably similar separation characteristics. The similarities tend to break down at low loads and for very large or very small S_B/S_A ratios. By and large, however, under overloaded gradient conditions, different eluite pairs with the same logarithmic mean separation factor and reference component chromatographed under otherwise identical conditions show similar separation behavior. We conclude that the logarithmic mean separation factor is a useful parameter in such studies. Separations of components is expected to improve with increasing values of $\bar{\alpha}_{ln}$ as discussed below.

Modulator dependent selectivity reversal. Convergent pairs are most interesting since they may be subject to modulator dependent selectivity reversal. In the following we consider eluite pair I (A_I–B) and eluite pair II (A_{II}–B), with $\bar{\alpha}_{ln}$ of 1 and 2, respectively. Their convergent κ–φ plots that have the same ratio of slopes (1.5) are shown in Fig. 8: the two cross each other at different points and the k' (or "a" parameter, since we consider $\Phi = 1$) values at which the separation factor is unity are approximately 50 and 8 for pairs I and II, respectively.

Although the selectivity of both eluite pairs is reversed over a defined modulator range, in linear chromatography under practical operating conditions the reversal is evident only for pair II. In Fig. 9 resolution of eluite pair II by a 250-plate column is plotted against the apparent retention of component B, $k_{app,B}$, under linear conditions for different values of the gradient steepness, G. The resolution for pair I at $G = 0.3$ is also shown for comparison. Details of the necessary calculations are outlined in the Appendix. The low plate number is used only for consistency with the simulations under overloaded conditions: values in the plot may be scaled for any N by multiplying the ordinate by $\sqrt{N/250}$. Since chromatography with $k_{app,B}$ larger than 20 is not likely to be feasible due to excessively long run times, the $k_{app,B}$ span in the plot represents the practical working range for the separations. In this range eluite pair II shows selectivity

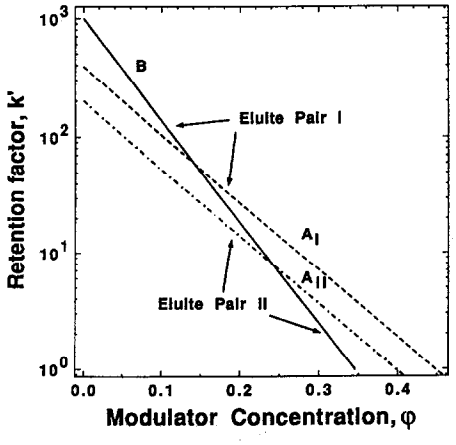

Fig. 8. Two pairs of convergent κ–φ plots both with $a_{0,B} = 1000$, $S_B = 20$ and $S_B/S_{AI} = S_B/S_{AII} = 1.5$; pair I, $a_{0,AI} = 400$; pair II, $a_{0,AII} = 200$.

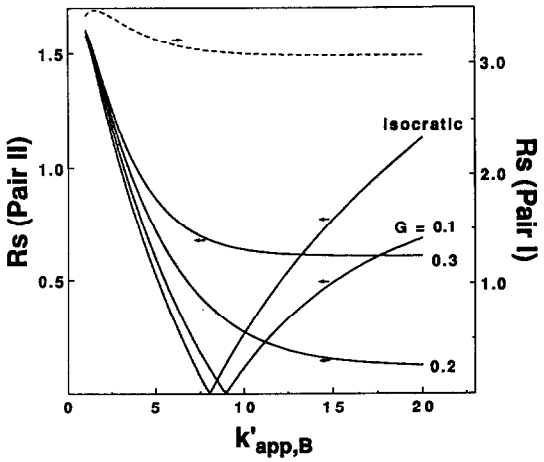

Fig. 9. Plots of the resolution, R_s, of the two eluite pairs whose κ–φ plots are shown in Fig. 8 *versus* $k'_{app,B}$, the apparent retention factor of component B, under analytical conditions. For eluite pair II, values of gradient steepness, G, based on component B (see eqn. 13) are shown on the plot. For eluite pair I, one curve with $G = 0.3$ is shown. Details of the calculations are given in the Appendix.

reversal for G values up to and slightly above 0.1, with increasing $k_{app,B}$ the resolution first decreases to zero and then increases). At $k_{app,B}$ values below the point of zero resolution, B elutes prior to A_{II} and *vice versa*. For all other values of G, and for all separations, whether isocratic or gradient, of eluite pair I in the range of interest, B is the early eluting component. Unlike the case of binaries with invariant selectivity, there is an operating range in which gradient is superior to isocratic elution under linear conditions.

Under overloaded conditions separations of both eluite pairs in gradient elution are greatly influenced by selectivity inversion. Chromatograms for eluite pairs I and II at increasing loads under fixed gradient conditions are shown in Figs. 10 and 11

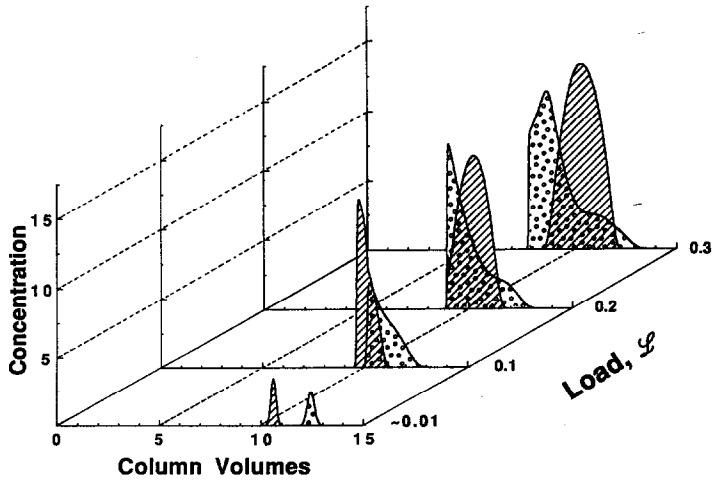

Fig. 10. Elution profiles of a 1:1 binary mixture of eluite pair I in Fig. 8 obtained with gradient elution at different loads. Conditions: $\varphi_0 = 0$, $G = 0.3$, $N = 250$, $\lambda_{AI} = 80$, $\lambda_B = 100$.

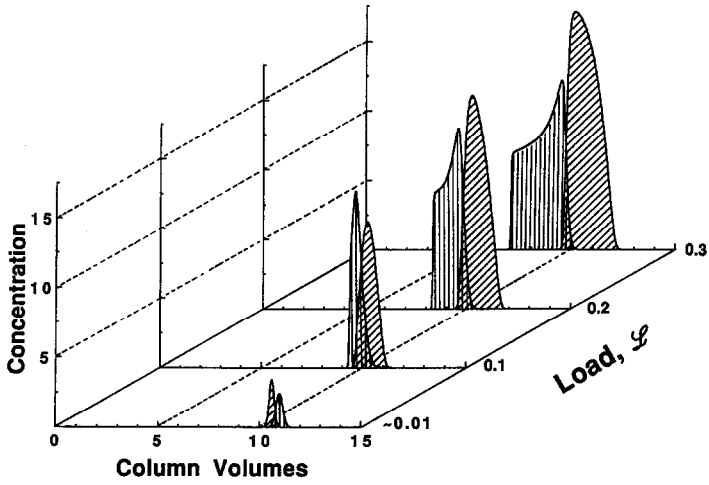

Fig. 11. Elution profiles of a 1:1 binary mixture of eluite pair II in Fig. 8 obtained with gradient elution at different loads. $\lambda_{AII} = 80$, other conditions as in Fig. 10.

respectively. At the low loads representative of linear chromatography pair I is well resolved but pair II is poorly resolved, as anticipated by the preceding analysis. The separation for pair I deteriorates drastically with increasing load, and eventually the "peak" of B is engulfed within that of A_I. On the other hand, the separation for pair II improves at higher loads, with A_{II} eluting prior to B and a sharp boundary between the bands. These observations are readily explained by phenomena that occur during the initial stages of the process. In both cases the feed step is essentially frontal chromatography under isocratic conditions with a separation factor greater than unity; under conditions of column overload, a pure fraction of component A_I or A_{II} is pushed ahead by component B to a greater extent than at low loads. Upon increasing the modulator concentration during the gradient, selectivity is reversed for both pairs, but the reversal occurs at lower modulator concentration, *i.e.*, earlier in the separation, for pair I than for pair II, as depicted in Fig. 8. In the case of pair I, the early separation between A_I and B is destroyed. In the case of pair II, however, the components have migrated a fair distance down the column before the inversion of selectivity occurs, and component B continues to displace component A_{II} throughout the separation process. As a result sharp boundaries form between the bands; this has also been observed by Snyder and co-workers[14,44].

We have found in other simulations that for eluite pair II, the sharpness of zone boundaries between A and B decreases with increasing initial modulator concentration, φ_0, *i.e.*, increases with the strength with which the components are bound to the stationary phase prior to the start of the gradient. This is in agreement with other observations from our laboratory that strong initial binding is often favorable in preparative chromatography at large. In the light of this, and the results shown in Figs. 10 and 11, we can draw the following conclusion about separations of binary mixtures with convergent κ–φ plots: for a given ratio of slopes and initial modulator concentration there is a critical value of the initial selectivity, $\alpha^*_{\varphi_0}$, above which separation by gradient elution improves with increasing load and below which the

opposite is true. The value of $\alpha^*_{\varphi_0}$ depends on those factors that determine the magnitude of the displacement effect. For instance, an increase in the proportion of component B or a decrease in the ratio of column adsorption capacities for the two components, λ_A/λ_B, would enhance the displacement of A by B and thus lower the value of $\alpha^*_{\varphi_0}$. Whether or not separation will improve with increasing load in such cases can roughly be estimated from the magnitude of the logarithmic mean separation factor, which is evaluated from the pertinent κ–φ plots. In the cases discussed above, eluite pair I, having a $\bar{\alpha}_{ln}$ of unity is not separated at high load, but pair II, with a $\bar{\alpha}_{ln}$ value of 2, is.

The κ–φ plots, *i.e.*, an examination of the separation factors at different φ values, also offer a means to predict whether isocratic or gradient elution is more suitable to bring about the separation of a given binary mixture under conditions of column overload. In practice the upper limit for k'_B in isocratic elution is about 15. Therefore in the cases shown by the plots in Fig. 8, isocratic elution is feasible for pair I but not for pair II because of the very small separation factor in the latter. Thus for the separation of pair I at high load, isocratic elution is likely to outperform gradient elution, whereas for the separation of pair II, gradient elution would be strongly favored over isocratic elution when the column is overloaded.

CONCLUSIONS

We have compared isocratic and gradient elution under overloaded conditions for single components and binary mixtures. The approach used here is rigorous and allows the investigation of the effect of gradient elution over a wide range of operating conditions and thermodynamic properties, which may not readily accessible in an experimental study. The insight gained on the separation of binary mixtures from this investigation can serve as a starting point for developing rational methods for the selection of conditions for the preparative separation of ternary and higher mixtures.

In the chromatography of a single component at high column load, simulated concentration profiles illustrate that gradient elution as compared with isocratic elution exhibits distinctly improved characteristics, in terms of apparent column efficiency. Whether or not this improvement is manifest in the separation of mixtures depends on a number of factors, the foremost being the separation factor and its dependence on the modulator concentration. Our results for binary mixtures with constant separation factor over the modulator concentration range of interest indicate that under overloaded conditions gradient elution gives better results in terms of production rates and enrichment factors for both components of the mixture with yields comparable with those in isocratic elution. These findings are in contradistinction to those in analytical chromatography where isocratic elution always gives superior results. For binary mixtures with separation factors that depend on the modulator concentration, the logarithmic mean separation factor, $\bar{\alpha}_{ln}$, provides a reasonable estimate of the extent of separation under overloaded conditions, regardless of whether the pertinent κ–φ plots of the components converge or diverge. In systems with convergent κ–φ plots where selectivity reversal occurs in the course of gradient elution over the modulator concentration range of interest, separation behavior changes dramatically with column load. The logarithmic mean separation factor can be used to predict whether or not separation will improve with increasing

load. The results suggest that isocratic elution is most useful when $\bar{\alpha}_{ln}$ is near unity, and that gradient elution is advantageous for higher values of $\bar{\alpha}_{ln}$.

ACKNOWLEDGEMENTS

This work was supported by Grants Nos. CA21948 and GM20993 from the National Institutes of Health, U.S. Department of Health and Human Resources, and by the National Foundation for Cancer Research.

APPENDIX

Here we briefly summarize the important relationships of the linear solvent strength (LSS) theory of gradient elution, which is applicable only to linear elution chromatography[6]. These expressions are then rearranged so that calculations for Figs. 1 and 9 can be made. With the help of the relationships it is shown for binary mixtures with constant selectivity over the modulator range of interest that resolution in isocratic elution is always greater than that in gradient elution.

Summary of results of the LSS theory

The LSS theory is developed for solutes that obey eqn. 3 (see text) and have linear isotherms, under gradient conditions such that eqn. 11 holds[6]. If there is no gradient delay, the retention time, t_R, of a component is given by

$$t_R = t_0 + \frac{t_0 \ln(2.3Gk'_0 + 1)}{2.3G} \tag{A1}$$

where k'_0 is the retention factor at the start of the gradient run, G is the gradient steepness (see eqn. 13) and t_0 is the column holdup time. The standard deviation in time units of a peak in gradient elution, σ_{gr} is given by

$$\sigma_{gr} = \frac{Ct_0}{\sqrt{N}}\left(1 + \frac{k'_0}{2.3k'_0G + 1}\right) \tag{A2}$$

where N is the plate number measured under isocratic conditions for a peak that elutes with a retention time of t_R, and C is a band compression factor given by

$$C = \frac{\left(1 + p + \frac{p^2}{3}\right)^{\frac{1}{2}}}{1 + p} \tag{A3}$$

where

$$p = \frac{2.3k'_0G}{1 + k'_0} \tag{A4}$$

In the above equations, the anomalous band broadening factor, J (ref. 6), is neglected as discussed in the text.

Comparison of the results in isocratic and gradient elution

An apparent retention, k'_{app}, in gradient elution is defined as

$$k'_{app} \equiv \frac{t_R - t_0}{t_0} = \frac{\ln(2.3Gk'_0 + 1)}{2.3G} \tag{A5}$$

The peak variance in isocratic elution, σ^2_{is}, is given by $t_0(1 + k')/\sqrt{N}$, where k' is the retention factor in isocratic linear elution. Combining this with eqn. A2, the ratio of peak variance in isocratic elution to that in gradient elution, $\sigma^2_{is}/\sigma^2_{gr}$, when k' and k'_{app} are the same, is given by

$$\frac{\sigma^2_{is}}{\sigma^2_{gr}} = \frac{(1 + k'_{app})^2}{C^2\left(1 + \dfrac{k'_0}{2.3Gk'_0 + 1}\right)^2} \tag{A6}$$

Here k'_0 is related to k'_{app} by the expression

$$k'_0 = \frac{\exp(2.3Gk'_{app}) - 1}{2.3G} \tag{A7}$$

and C is a function of k'_0 as indicated in eqns. A3 and A4. Equations A5–A7 are used in the calculations for Fig. 1.

The resolution, R_s, between two peaks A and B in linear chromatography is given by[30]

$$R_s = \frac{|t_{R,B} - t_{R,A}|}{2(\sigma_B + \sigma_A)} \tag{A8}$$

where subscripts A and B represent the corresponding peaks. For given values of G, based on component B (see eqn. 13), and $k'_{app,B}$, $k'_{0,B}$ can be calculated from eqn. A7, and $k'_{0,A}$ determined from the expression

$$k'_{0,A} = \Phi a_{0,A}\left(\frac{k'_{0,B}}{\Phi a_{0,B}}\right)^{S_A/S_B} \tag{A9}$$

where the Φa_0 are the retention factors of the respective components in the absence of the modifier and S_B/S_A is the ratio of slopes of their respective κ–φ plots. Eqn. A9 is derived by noting that $k'_{0,i} = \Phi a_{0,i}\exp(-S_i\varphi_0)$ (see eqns. 3, 4 and 11) and eliminating φ_0 between the respective expressions for $k'_{0,A}$ and $k'_{0,B}$. Using eqns. A1–A4, and A7 and A9, t_R and σ for the two components can be determined (for component A, S_AG/S_B must be used in place of G). Eqn. A8 can then be used to calculate the resolution as is done in Fig. 10.

For binary mixtures with constant selectivity, *i.e.*, with $S_B/S_A = 1$, resolution in isocratic elution, R_{is}, can be approximated as[30]

$$R_{is} = \frac{\sqrt{N}}{2}\left(\frac{\alpha - 1}{\alpha + 1}\right)\frac{\overline{k'}}{1 + \overline{k'}} \approx \frac{t_0\overline{k'}}{2\sigma_{is}}\left(\frac{\alpha - 1}{\alpha + 1}\right) \tag{A10}$$

where α is the selectivity ($=k'_B/k'_A$) and $\overline{k'}$ is the mean retention factor of the two components. An analogous approximation for resolution in gradient elution can be written as

$$R_{gr} = \frac{t_0\overline{k'_{app}}}{2\sigma_{gr}}\left(\frac{\alpha_{gr} - 1}{\alpha_{gr} + 1}\right) \tag{A11}$$

where α_{gr} is an observed relative retention ($= k'_{app,B}/k'_{app,A}$) which can be derived from eqn. A5 and is given by

$$\alpha_{gr} = \frac{\ln(2.3Gk_{0,B} + 1)}{\ln(2.3Gk_{0,B}/\alpha + 1)} \approx \frac{\ln(2.3G\overline{k'_{app}} + 1)}{\ln(2.3G\overline{k'_{app}}/\alpha + 1)} \tag{A12}$$

Therefore, when $\overline{k'} = \overline{k'_{app}}$ the ratio of the resolution in gradient to that in isocratic elution, R_{gr}/R_{is}, is expressed as

$$\frac{R_{gr}}{R_{is}} = \frac{\sigma_{is}}{\sigma_{gr}}\left(\frac{\alpha_{gr} - 1}{\alpha_{gr} + 1}\right)\left(\frac{\alpha + 1}{\alpha - 1}\right) \tag{A13}$$

For given values of $\overline{k'_{app}}$ and α, R_{gr}/R_{is} can be calculated as a function of G using eqns. A12 and A13. Although it cannot be readily shown algebraically, substitution of $1 < \overline{k'_{app}} < 10$ and $1.01 < \alpha < 1.7$ into the above expressions did confirm that for all finite values of G, $R_{gr}/R_{is} < 1$. This implies that for a given component pair with constant separation factor, assuming that N does not change with $\overline{k'_{app}}$, there exists at least one condition for an isocratic separation at which the resolution is larger than for any gradient separation with finite G.

SYMBOLS

a_i	initial slope of the Langmuir type multicomponent adsorption isotherm for component i when all $c_{j\neq i} = 0$.
$a_{0,i}$	value of a_i without modulator in the mobile phase
$a_{1,i}$	extrapolated value of a_i at $\varphi_s = 1.0\ M$ (eqns. 5 and 6)
b_i	parameter of the Langmuir type multicomponent isotherm: the reciprocal of mobile phase concentration, c_i, at half saturation of the stationary phase for component i when all $c_{j\neq i} = 0$
b_φ	b parameter for mobile phase modulator (eqn. 2)
c_i	concentration of eluite i in the mobile phase

$c_{0,i}$ inlet concentration of component i
$c_{0,\mathrm{ref}}$ inlet concentration of reference component (eqns. 13 and 14)
C band compression factor according to the LSS theory (eqn. A3); same as parameter G in ref. 6
$\mathscr{D}$ lumped effective dispersion coefficient, see discussion preceding eqn. 7
E_i enrichment factor, *i.e.* ratio of product to feed concentration, of component i
g function describing gradient profile (eqn. 10)
G gradient steepness parameter defined in eqn. 13; same as parameter b in ref. 6
k'_{app} apparent retention factor in gradient linear elution (eqn. A5)
$\overline{k'_{\mathrm{app}}}$ mean apparent retention factor of two components (eqn. A11)
k' retention factor in isocratic linear elution
$\overline{k'}$ mean retention factor of two components (eqn. A10)
k'_0 retention factor at start of gradient run (eqns. A4, A5 and A7)
L column length
$\mathscr{L}$ load parameter (eqn. 14)
n total number of eluites
N plate number under conditions of isocratic linear chromatography, $u_0 L/2\mathscr{D}$
p dimensionless group in the expression for C (eqn. A4)
P_i dimensionless production rate (eqn. 16)
q_i concentration of eluite i in the stationary phase
R_{gr} resolution in gradient linear elution (eqn. A11)
R_{is} resolution in isocratic linear elution (eqn. A10)
R_s resolution between two peaks (eqn. A8)
S_i 2.3 times the slope of the κ–φ plot for component i (eqn. 3); it is 2.3 times the value for the S used in ref. 6
t time
t_0 mean hold-up time of the mobile phase in the column, *i.e.*, the residence time of an unretained homomorph of the eluite
t_R retention time in linear elution
t_{inj} time of feed introduction
u_0 chromatographic velocity, L/t_0
X dimensionless axial distance, z/L
Y_i yield of component i, *i.e.*, the ratio of mass recovered in product to that in feed
z axial distance in the column
Z ratio of eluite to salt valence in electrostatic interaction chromatography (eqns. 5 and 6)

Greek

α selectivity or relative retention or separation factor for two components in isocratic linear elution (eqn. A10)
α_{gr} ostensible selectivity for two components, $k'_{\mathrm{app,B}}/k'_{\mathrm{app,A}}$, in gradient linear elution (eqn. A11)
$\bar{\alpha}_{\mathrm{ln}}$ logarithmic mean separation factor (eqn. 17)
α_{φ_0} separation factor at modulator concentration φ_0 (eqn. 17)
α_{φ^*} separation factor at modulator concentration φ^* (eqn. 17)

β linear rate of change of modulator concentration at the inlet, *i.e.*, the difference between final and initial modulator concentration divided by the gradient time

Φ phase ratio, *i.e.*, the ratio of stationary phase to mobile phase volumes within the column; set to unity in this study

φ modulator concentration in the mobile phase

φ_0 modulator concentration at the start of the gradient run

φ^* modulator concentration at which Φa_0 is unity for the least retained component of an eluite pair (eqn. 17)

κ_i $\log_{10} k_i'$ (eqn. 3)

$\kappa_{0,i}$ $\log_{10}(\Phi a_{0,i})$ (eqn. 3)

λ_i parameter of the Langmuir type multicomponent adsorption isotherm for component i, *i.e.*, the saturation value of stationary phase concentration for that component, a_i/b_i

$\lambda_{ref.}$ saturation value of stationary phase concentration for reference component (eqns. 14 and 16)

μ_1 first moment of peak (Table I)

μ_2^* second central moment of peak (Table I)

μ_3^* third central moment of peak (Table I)

θ dimensionless time, t/t_0

θ_c dimensionless cycle time (eqn. 16)

σ_{gr}^2 peak variance in gradient linear elution

σ_{is}^2 peak variance in isocratic linear elution

τ dimensionless time for feed introduction, t_{inj}/t_0

REFERENCES

1 R. S. Alm, R. J. P. Williams and A. Tiselius, *Acta Chem. Scand.*, 6 (1952) 826.
2 Cs. Horváth and S. R. Lipsky, *Anal. Chem.*, 39 (1967) 1893.
3 B. Drake, *Ark. Kemi*, 8 (1955) 1.
4 E. C. Freiling, *J. Am. Chem. Soc.*, 77 (1955) 2067.
5 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
6 L. R. Snyder, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography—Advances and Perspectives*, Vol. 1, Academic Press, New York, 1980, pp. 208–316.
7 S. Yamamoto, K. Nakanishi, R. Matsuno and T. Kamikubo, *Biotechnol. Bioeng.*, 25 (1983) 1465.
8 P. Jandera and J. Churacek, *Gradient Elution in Column Liquid Chromatography* (*Journal of Chromatography Library*, Vol. 31), Elsevier, Amsterdam, 1985.
9 S. J. Gibbs and E. N. Lightfoot, *Ind. Eng. Chem. Fundam.*, 25 (1986) 490.
10 K. Kang and B. J. McCoy, *Biotechnol. Bioeng.*, 33 (1989) 786.
11 J. E. Eble, R. L. Grob, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 405 (1987) 51.
12 L. R. Snyder, G. B. Cox and P. E. Antle, *J. Chromatogr.*, 444 (1988) 303.
13 G. B. Cox, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 444 (1988) 325.
14 L. R. Snyder, J. W. Dolan and G. B. Cox, presented at *6th International Symposium on Preparative Chromatography, Washington, DC, May 8–10, 1989.*
15 F. D. Antia and Cs. Horváth, *Ann. NY Acad. Sci.*, (1989) in press.
16 I. Langmuir, *J. Am. Chem. Soc.*, 38 (1916) 2221.
17 G. M. Schwab, *Ergebnisse der exacten Naturwissenschaften*, Vol. 7, Julius Springer, Berlin, 1928, p. 276.
18 D. B. Broughton, *Ind. Eng. Chem.*, 40 (1948) 1506.
19 C. Kemball, E. K. Rideal and E. A. Guggenheim, *Trans. Faraday Soc.*, 44 (1948) 948.
20 J. Jacobson, J. Frenz and Cs. Horváth, *J. Chromatogr.*, 316 (1984) 53.
21 A. Velayudhan, *Ph.D. Thesis*, Yale University, New Haven, CT, 1989.
22 A. L. Lee, *Ph.D. Thesis*, Yale University, New Haven, CT, 1989.

23 A. Velayudhan and Cs. Horváth, *J. Chromatogr.*, 367 (1986) 160.
24 F. Helfferich, *Ion Exchange*, McGraw-Hill, New York, 1962.
25 A. Velayudhan and Cs. Horváth, *J. Chromatogr.*, 443 (1988) 13.
26 G. Guiochon and A. Katti, *Chromatographia*, 24 (1987) 165.
27 Cs. Horváth and H.-J. Lin, *J. Chromatogr.*, 149 (1978) 43.
28 A. L. Lee, A. Velayudhan and Cs. Horváth, in G. Durand, L. Bobichon and J. Florent (Editors), *8th International Biotechnology Symposium*, Société Française de Microbiologie, Paris, 1989, pp. 593–610.
29 N. S. Raghavan and D. M. Ruthven, *AIChE J.*, 29 (1983) 922.
30 Cs. Horváth and W. R. Melander, in E. Heftmann (Editor), *Chromatography—Fundamentals and Applications of Chromatographic and Electrophoretic Methods*, Part A, Elsevier, Amsterdam, 1983, pp. A27–A135.
31 L. R. Snyder and M. A. Stadalius, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography—Advances and Perspectives*, Vol. 4, Academic Press, New York, 1986, pp. 195–312.
32 M. T. W. Hearn and M. I. Aguilar, *J. Chromatogr.*, 392 (1987) 33.
33 L. R. Snyder, G. B. Cox and P. E. Antle, *Chromatographia*, 24 (1987) 82.
34 J. H. Knox and H. M. Pyper, *J. Chromatogr.*, 363 (1986) 1.
35 E. Glueckauf, *Discuss. Faraday Soc.*, 7 (1949) 12.
36 G. Guiochon and S. Ghodbane, *J. Phys. Chem.*, 92 (1988) 3682.
37 J. L. Wade and P. W. Carr, *J. Chromatogr.*, 449 (1988) 53.
38 P. C. Wankat, *Anal. Chem.*, 46 (1974) 1400.
39 F. D. Antia and Cs. Horváth, *Ber. Bunsenges. Phys. Chem.*, (1989) in press.
40 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 444 (1988) 275.
41 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 452 (1988) 209.
42 A. Katti and G. Guiochon, *Anal. Chem.*, 61 (1989) 982.
43 J. Frenz and Cs. Horváth, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography—Advances and Perspectives*, Vol. 5, Academic Press, New York, 1988, pp. 211–314.
44 G. B. Cox, B. J. Permar and L. R. Snyder, presented at *6th International Symposium on Preparative Chromatography, Washington, DC, May 8–10, 1989.*
45 D. D. Frey, *J. Chromatogr.*, 409 (1987) 1.
46 L. R. Snyder, M. A. Quarry and J. L. Glajch, *Chromatographia*, 24 (1987) 33.
47 B. F. D. Ghrist and L. R. Snyder, *J. Chromatogr.*, 459 (1988) 43.