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Analytic solution for volume-overloaded gradient elution chromatography

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ABSTRACT

An analytic solution to a partial differential equation model for gradient elution chromatography is obtained. The model is restricted to linear isotherms and treats mass transfer effects with the linear driving force approximation. The solution is obtained for periodic, rectangular feed pulses, with an arbitrary gradient shape and type, and is given in the form of a convergent series that allows a direct calculation of the effluent profile and of the average product concentration. Calculations for small feed pulses, show that the solution gives the retention time and peak spreading predicted by the linear solvent strength theory for reversed phase chromatography, in the limit of Gaussian peaks. For larger feed pulses the solution predicts asymmetric peaks with concentrations exceeding that of the feed sample. The theory developed is succesfully used to predict volume overload effects in gradient elution from isocratic elution data for an experimental system.

INTRODUCTION

Gradient elution chromatography is frequently used for both analytical and preparative applications, especially for the separation of mixtures whose components have a broad spectrum of retentivity [1,2] and for biopolymer separations [3]. While in analytical applications small feed pulses are used, in preparative applications the feed pulses are larger and may be have the shape of a rectangular slug. Often preparative gradient elution is carried out in industry with only slight concentration overloading during feed injection, but with considerable volume overloading, since such conditions tend to be the most reproducible to operate. There is a need, therefore, for simple solutions to the equations that describe linear gradient elution that can be used by the industrial practitioner for the purpose of interpretation of results and parameter optimization.

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Gradient elution chromatography has been studied theoretically by many authors with the assumption of linear adsorption equilibrium [3–12]. These analyses are restricted to solutions that are sufficiently dilute that adsorption is governed by Henry's law, or when a high initial solvent strength is used to maximize resolution [13]. Non-linear adsorption, on the other hand, must be considered for concentration overloading conditions, but this generally requires numerical simulation methods, such as Craig simulations [14,15], or orthogonal collocation [1,2, 16]. However, in gradient elution chromatography, the equilibrium distribution coefficient of each solute decreases rapidly as the solvent strength is increased. As a consequence, in many cases non-linear adsorption affects only the initial feed loading step and the initial phase of gradient elution. The linear approximation can then still provide an adequate representation of non-equilibrium spreading effects, if the equilibrium remains linear for most of the separation time [12,13].

The most comprehensive treatment of gradient elution chromatography appears to be the linear

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solvent strength (LSS) theory of Snyder and coworkers (see refs. 3 and 7). The theory is developed for infinitesimal feed pulses with a linear gradient of the modifier concentration, φ

$$\varphi = \varphi^0 + \beta t \tag{1}$$

and for solutes for which the dependence of the linear adsorption coefficient K on φ has the form

$$\ln K = \ln \kappa - S\varphi \tag{2}$$

Here κ and S are characteristic constants for each solute. Assuming that the modifier propagates through the bed as an ideal, undistorted wave, the retention time of a component is given by

$$t_R = \frac{\varepsilon z}{u} + \frac{1}{S\beta} \ln \left[1 + \frac{S\beta z}{u} K_0(1 - \varepsilon) \right] \tag{3}$$

K_0 is the distribution coefficient for the component at the start of the gradient, when $\varphi = \varphi^0$. The standard deviation of the component peak in time units is

$$\sigma = C \frac{z/u}{\sqrt{N}} \left[\varepsilon + \frac{K_0(1 - \varepsilon)}{K_0(1 - \varepsilon)S\beta z/u + 1} \right] \tag{4}$$

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

$$C = \frac{(1 + p + p^2/3)^{1/2}}{1 + p} \tag{5}$$

with

$$p = \frac{\varepsilon K_0(1 - \varepsilon)S\beta z/u}{\varepsilon + K_0(1 - \varepsilon)} \tag{6}$$

Frey [12] has recently generalized the treatment, providing asymptotic relations for preparative gradient elution chromatography, which converge to the results of the LSS theory for infinitesimally small feed pulses.

The objective of this paper is to provide an analytic solution in the form of explicit expressions, which are valid for both small and large (rectangular) feed pulses and which are applicable to any arbitrary gradient shape and type. The solution is restricted to linear isotherms and neglects the accumulation of solute in the mobile phase in the column. Thus, the solution is applicable only to systems in which the solutes are retained significantly by the stationary phase, but with a linear relationship between the concentration in the stationary phase and that in the mobile phase. However, the solution is general with respect to sample size, to the way in which the modifier concentration is varied, and to the functional relationship between the distribution coefficient and the modifier concentration. Thus, the solution applies to both narrow and wide feed pulses and to different branches of chromatography, such as reversed-phase and ion-exchange, since it can take into account the different characteristic dependencies of the distribution coefficient (or retention factor) on the modifier concentration.

MATHEMATICAL MODEL

We consider a fixed-bed which is supplied with a periodic, square wave feed of rectangular pulses, each of duration t_F, as shown in Fig. 1. For each period of duration t_P, after introduction of the feed slug, the solvent composition is changed in a prescribed way, either continuously or in a stepwise fashion. At the end of the period, the solvent composition is returned to the original value and a new feed slug is introduced. The corresponding variation of K for a solute is sketched in Fig. 1. The gradient ends at time $t_F + t_G$, after which K is held constant at a value K_G.

Neglecting axial dispersion and using a film model to describe mass transfer between the mobile phase

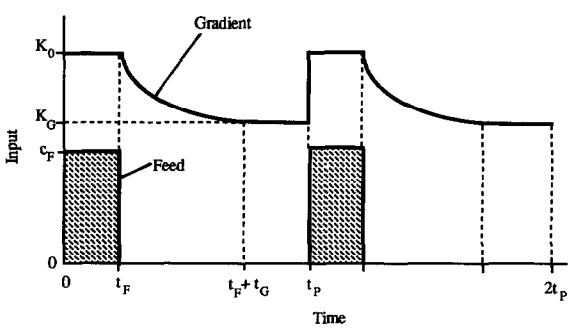

Fig. 1. Input for gradient elution chromatography. The gradient line shows the effect of the modifier concentration on K.

and the stationary phase, the conservation equations for a solute in the bed may be written as

$$\varepsilon \frac{\partial c}{\partial t} + (1 - \varepsilon) \frac{\partial q}{\partial t} + u \frac{\partial c}{\partial z} = 0 \tag{7}$$

$$(1 - \varepsilon) \frac{\partial q}{\partial t} = k_0 a \left(c - \frac{q}{K} \right) \tag{8}$$

with boundary conditions

$$\begin{aligned} c(0,t) &= c_F, \quad (j - 1)t_P \leqslant t < (j - 1)t_P + t_F \\ c(0,t) &= 0, \quad (j - 1)t_P + t_F \leqslant t < jt_p \\ & \qquad j = 1, 2, 3 \ldots \end{aligned} \tag{9}$$

Here we seek only the purely time-periodic solution, which is approached after a sufficiently large number of cycles. In this case, initial conditions are unimportant.

In the remainder of the development we neglect the mobile phase accumulation of solute; *i.e.*, we neglect the first term in eqn. 7. This is permissible when, throughout the gradient run, the retention factor k' satisfies the relationship

$$k' = \frac{K(1 - \varepsilon)}{\varepsilon} > 1 \tag{10}$$

We also assume that the modifier is not retained by the stationary phase. For these conditions, when eqn. 10 is valid, only the temporal variation of K needs to be accounted for. A similar assumption has been made by Gibbs and Lightfoot [10] in their treatment of gradient elution, and yields that everywhere in the bed

$$K = K(\varphi) = K(t) \tag{11}$$

We define the following dimensionless variables

$$X = \frac{K(t)}{K_0} \cdot \frac{c}{c_F} \tag{12}$$

$$Y = \frac{q}{K_0 c_F} \tag{13}$$

$$n = \frac{k_0 a z}{u} \tag{14}$$

$$\frac{d\vartheta}{dt} = \frac{k_0 a}{(1 - \varepsilon)K}, \quad \vartheta = 0 \quad \text{for} \quad t = 0 \tag{15}$$

X and Y are dimensionless mobile and stationary phase concentrations, n is the number of transfer units in the bed, and ϑ a dimensionless time. Since in gradient elution K decreases with time, the dimensionless time ϑ runs slowly at the beginning of the gradient run and faster towards the end. Eqns. 7–9 in dimensionless form are

$$\frac{\partial X}{\partial n} = -X + Y \tag{16}$$

$$\frac{\partial Y}{\partial \vartheta} = X - Y \tag{17}$$

$$\begin{aligned} X(0,\vartheta) &= 1, \quad 2\pi r(j - 1) \leqslant \vartheta < 2\pi r(j - 1) + \pi r_F \\ X(0,\vartheta) &= 0, \quad 2\pi r(j - 1) + \pi r_F \leqslant \vartheta < 2\pi rj \\ & \qquad j = 1, 2, 3 \ldots \end{aligned} \tag{18}$$

The quantities

$$2\pi r = \int_0^{t_P} \frac{k_0 a}{(1 - \varepsilon)K(t)} \, dt \tag{19}$$

$$\pi r_F = \int_0^{t_F} \frac{k_0 a}{(1 - \varepsilon)K(t)} \, dt = \frac{k_0 a t_F}{(1 - \varepsilon)K_0} \tag{20}$$

are the dimensionless durations of the total period and of each feed slug.

A time-periodic solution of eqns. 16–18 with period $2\pi r$ is easily found by applying the residue theorem to the general inversion integral of the Laplace transform solution of these equations [17]. We obtain eqn. 21. The solution consists of a series of terms with a sinusoidal component that determines the location of the peak, and an exponential decay that determines the spreading of the peak caused by mass transfer resistance. The convergence of this series has been discussed [17,18]. Four of five terms may be sufficient when mass transfer resistance is significant, but many more, perhaps 100 or more, if mass transfer is very effective. Note that this equation is totally general with respect to gradient shape and time and applies to both continuous and stepwise gradients. What varies is the definition of the dimensionless time, ϑ, that depends upon the integral of $1/K(t)$ per eqn. 15.

The time-average effluent concentration $\bar{c}$ between two times t_1 and t_2 is of interest in preparative applications to determine the purity of a product cut. This can be found directly from eqn. 21 as eqn. 22. Because of the $1/k^2$ term, this series is much more rapidly convergent than eqn. 21.

$$X = \frac{r_F}{2r} + \frac{2}{\pi}\sum_{k=1}^{\infty}\frac{1}{k}\exp\left(-\frac{k^2 n}{k^2+r^2}\right)\sin\left(\frac{k\pi r_F}{2r}\right)\cos\left(\frac{k\vartheta}{r} - \frac{k\pi r_F}{2r} - \frac{krn}{k^2+r^2}\right) \quad (21)$$

$$\bar{c} = \frac{1}{t_2 - t_1}\int_{t_1}^{t_2} c(z,t)\mathrm{d}t = \frac{c_F K_0(1-\varepsilon)}{k_0 a(t_2 - t_1)}\int_{\vartheta_1}^{\vartheta_2} X(n,\vartheta)\mathrm{d}\vartheta = \frac{c_F K_0(1-\varepsilon)}{k_0 a(t_2 - t_1)}\left\{\frac{r_F(\vartheta_2 - \vartheta_1)}{2r} + \right.$$

$$\left. + \frac{4r}{\pi}\sum_{k=1}^{\infty}\frac{1}{k^2}\exp\left(-\frac{k^2 n}{k^2+r^2}\right)\sin\left(\frac{k\pi r_F}{2r}\right)\cdot\sin\left[\frac{k(\vartheta_2 - \vartheta_1)}{2r}\right]\cos\left[\frac{k(\vartheta_2 + \vartheta_1)}{2r} - \frac{k\pi r_F}{2r} - \frac{krn}{k^2+r^2}\right]\right\} \quad (22)$$

These equations can be generalized to account for axial dispersion, external film mass transfer resistance, and intraparticle pore diffusion, by using the linear driving force approximation [19,20]. In this case eqns. 21 and 22 can be used as a good approximation if the mass transfer parameter $k_0 a$ is calculated from

$$\frac{1}{k_0 a} = \frac{\varepsilon D_L}{u^2} + \frac{1}{1-\varepsilon}\left(\frac{d_p}{6k_f} + \frac{d_p^2}{60\varepsilon_p D_p}\right) \quad (23)$$

where D_L is the axial dispersion coefficient, d_p the particle diameter, k_f the external film mass transfer coefficient, and D_p the pore diffusivity of the solute. The relationship between the number of transfer units, n, and the plate number, N, or the height equivalent to a theoretical plate, H, is also well known [21,22]

$$N = \frac{z}{H} \approx \frac{n}{2} \quad (24)$$

Thus, $k_0 a$ can be calculated directly from H obtained in isocratic experiments.

Use of eqns. 21 and 22 requires a knowledge of the temporal variation of K in the bed. For linear gradients we have

$$\varphi = \varphi^0 + \beta(t - t_F) \quad \text{for} \quad t_F < t \leqslant t_F + t_G$$
$$\varphi = \varphi^G \quad \text{for} \quad t_F + t_G < t \leqslant t_P \quad (25)$$

We consider here three examples of application of linear gradients: reversed-phase liquid chromatography (RPLC), ion-exchange chromatography (IEC), and stepwise elution (SE). In the first case the relationship between K and φ is given by eqn. 2. In the second case we assume

$$K = A\varphi^{-b} \quad (26)$$

which has been shown to be valid for biopolymers [23] as well as small ions [16]. Finally, for stepwise elution we assume an abrupt change of K from an initial value K_0 to a final value K_G, independent of the particular type of chromatography. The expressions for the transformed time ϑ and the peak profile c corresponding to these three cases are given in Table I. In each case, X is calculated from eqn. 21. Different gradient shapes and other relationships between K and φ can be readily handled via eqn. 15.

Calculation examples and discussion

The parameter values shown in Table II were used for calculations using eqns. 21 and 22, and with the appropriate definitions of ϑ given in Table I. The parameters were chosen arbitrarily to test the theory over a range of conditions. The parameter values, however, are similar to those obtained for an experimental system discussed below, and are representative of typical high-performance liquid chromatographic preparative separations. To insure accuracy, 100 terms were used in the series and a spreadsheet was used to carry out the computations.

Fig. 2 shows calculated peaks for gradient elution RPLC with small feed pulses and different gradient steepness ($\propto 1/t_G$). For these conditions the total period, t_P, chosen is sufficiently long that there is no interference from peaks generated by each injection on the next. The peaks become sharper as the

TABLE I

TRANSFORMED TIME FOR GRADIENT ELUTION

πr_F and πr_G are the values of ϑ at $t = t_F$ and $t = t_F + t_G$, respectively.

Linear gradients: $\varphi = \varphi^0 + \beta t$			Stepwise elution
Time	RPLC	IEC	
$0 < t \leqslant t_F$	$\vartheta = \dfrac{k_0 a}{(1-\varepsilon)K_0} t$	$\vartheta = \dfrac{k_0 a}{(1-\varepsilon)K_0} t$	$\vartheta = \dfrac{k_0 a}{(1-\varepsilon)K_0} t$
	$c = c_F X$	$c = c_F X$	$c = c_F X$
$t_F < t \leqslant t_F + t_G$	$\vartheta = \pi r_F + \dfrac{k_0 a[e^{S\beta(t-t_F)} - 1]}{(1-\varepsilon)K_0 S\beta}$	$\vartheta = \pi r_F + \dfrac{k_0 a\{[\varphi^0 + \beta(t-t_F)]^{b+1} - (\varphi^0)^{b+1}\}}{(1-\varepsilon)(b+1)A\beta}$	$\vartheta = \dfrac{k_0 a}{(1-\varepsilon)K_0} t$
	$c = c_F X e^{S\beta(t-t_F)}$	$c = c_F X K_0 [\varphi^0 + \beta(t-t_F)]^b / A$	$c = c_F X$
$t_F + t_G < t \leqslant t_P$	$\vartheta = \pi r_G + \dfrac{k_0 a(t - t_G - t_F)}{(1-\varepsilon)K_G}$	$\vartheta = \pi r_G + \dfrac{k_0 a(t - t_G - t_F)}{(1-\varepsilon)K_G}$	$\vartheta = \pi r_G + \dfrac{k_0 a(t - t_G - t_F)}{(1-\varepsilon)K_G}$
	$c = c_F X K_0 / K_G$	$c = c_F X K_0 / K_G$	$c = c_F X K_0 / K_G$

TABLE II

SIMULATED OPERATING CONDITIONS

Parameter	Value	Units
z	10	cm
u	0.1	cm s^{-1}
$k_0 a$	5	s^{-1}
K_0	10	–
K_G	2	–
ε	0.33	–
t_P	2000	s

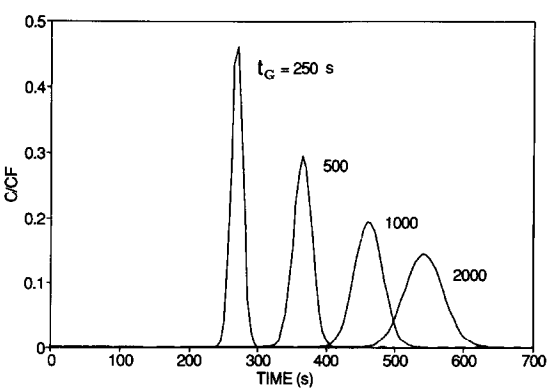

Fig. 2. Gradient elution chromatography peaks for RPLC obtained with eqn. 21 for different values of the gradient time t_G. Parameter values from Table II with t_F = 10 s. CF = feed concentration.

gradient steepness is increased. Note that for t_G = 250 s, a significant portion of the peak exists from the bed after K has reached its final constant value K_G. The first moment and the standard deviation of the computed peaks are given in Fig. 3, in comparison with the results of the LSS theory, eqns. 3 ($\mu \approx t_R$) and 4. The dotted lines show the results of the LSS theory if the same approximation that the mobile phase accumulation is negligible is made in eqns. 3 and 4; *i.e.*, taking $\varepsilon z/u \approx 0$. The error resulting from this approximation is small for the conditions simulated, and it would be much less for

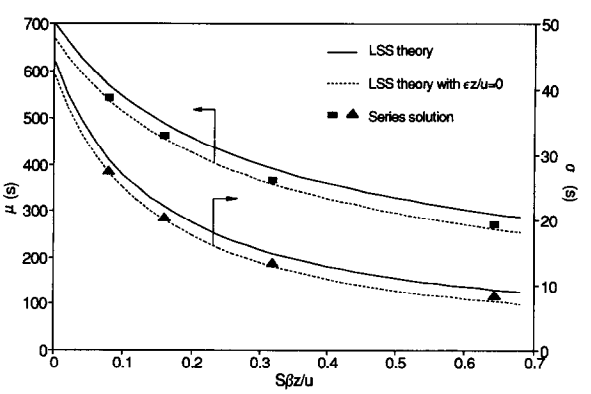

Fig. 3. Comparison of first moment and standard deviation for peaks in Fig. 2 with results of LSS theory.

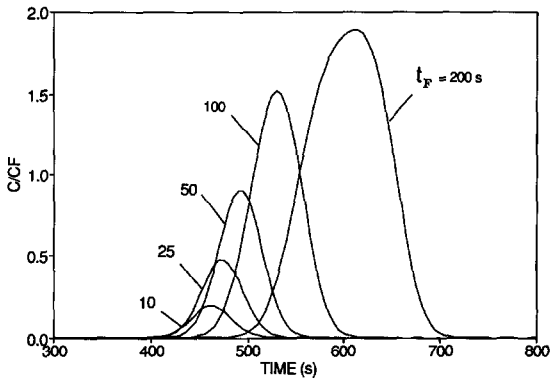

Fig. 4. Effect of feed slug size on gradient elution peaks for RPLC calculated from eqn. 21. Parameter values from Table II with t_G = 1000 s. CF = feed concentration.

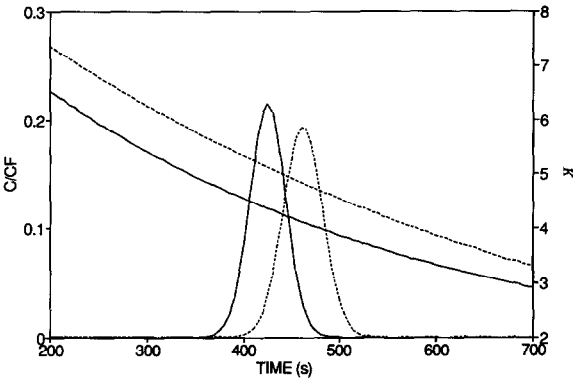

Fig. 5. Gradient shape and chromatographic peaks calculated from eqn. 21 for RPLC (– – –) and IEC (———). Parameter values from Table II with t_F = 10 s, t_G = 1000 s, and A = 10, b = 2, φ^0 = 1 for IEC. CF = feed concentration.

cases where K remains higher during the gradient run. The absolute deviation of the predicted peak position is equal to the quantity $\varepsilon z/u$ and can be easily estimated. The agreement between the series solution and the LSS theory is excellent for these small feed pulses.

The effects of volume overloading are shown in Fig. 4 which gives the calculated peaks obtained by varying the feed injection time t_F. The peaks are Gaussian for small t_F values, but they become increasingly asymmetrical as the loading is increased, reaching concentrations above the feed concentration. This occurs when solute accumulated in the stationary phase is desorbed as a result of the change in K induced by the gradient. Then, if the number of plates is sufficiently large, the component is concentrated into a band which may be narrower than the feed slug. It should be noted that the retention time of the peak also increases with volume loading, as a result of the delay in the gradient introduced by the finite size of the feed slug. The time-average effluent concentration between any two times t_1 and t_2 may be calculated directly from eqn. 22 for any of these peaks. Thus, the concentration and product recovery in any chosen fraction is easily obtained from the theoretical treatment. For example, with reference to the peak obtained with a feed injection time of 200 s (Fig. 4), eqn. 22 yields directly an average concentration $\bar{c}/c_F$ = 1.61 for a fraction between 550 and 650 s, corresponding to a recovery of 80.5% of the injected feed. This may be compared with an average concentration $\bar{c}/c_F$ = 0.741 obtained from eqn. 22 for a fraction between 450 and 720 s, corresponding to essentially 100% recovery.

Fig. 5 shows calculated peaks for gradient elution IEC for a narrow feed pulse with the operating conditions of Table II. Thus, the initial and final K values are the same as in the previous calculations for RPLC. The variation of K with time is, however, different. For these calculations we have chosen A = 10, b = 2 and ϑ^0 = 1, in consistent units. As shown in Fig. 5, for these parameter values, the effect of ϑ on K is more dramatic for IEC than for RPLC. Thus, the IEC peak is eluted faster and is sharper than the RPLC peak. Considerations on peak asymmetry and height similar to those made for RPLC can of course also be made for IEC, and are entirely predicted by the analytic solution.

Calculated peaks for stepwise gradient elution with the conditions of Table II are shown in Fig. 6, for small feed pulses. In the isocratic case, the peak is symmetrical and broad, since elution takes place with the initially large K value. For t_G = 625 s, however, we see that a portion of the peak elutes for isocratic conditions as the initial K value. When the step change in K occurs, the portion of the peak still within the column is sharpened and exits the bed as a narrow, more concentrated band. The abrupt transition in Fig. 6 is the result of having neglected the mobile phase accumulation term in eqn. 7. A smoother transition would, of course, be observed in practice. When t_G is reduced to values lower than

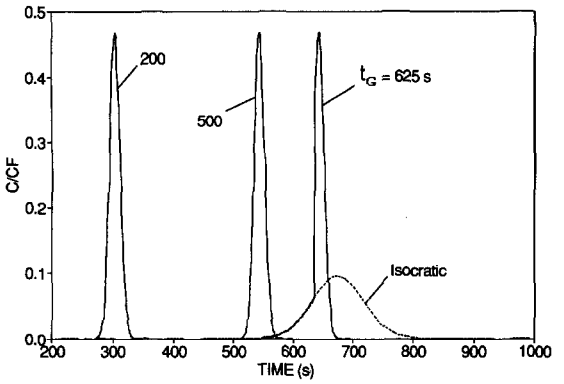

Fig. 6. Peaks calculated from eqn. 21 for stepwise elution chromatography. Parameters from Table II with t_F = 10 s. CF = feed concentration.

500 s, the solute band is well within the bed when the step change occurs. Since K is initially large, the solute band makes little headway through the bed, before the step change. As a result the peak shape and spreading is nearly independent of t_G.

EXPERIMENTAL

Equipment and materials

A Waters (Milford, MA, USA) liquid chromatograph with a Rheodyne Model 7010 injection valve was used. The injection valve was fitted with different loops with volumes of 0.02, 0.8, 2.2 and 4.1 cm^3, which were constructed of 0.039 in. I.D. stainless-steel tubing. The equipment dwell volume and gradient linearity were determined from blank methanol–water gradient runs, using 1% acetone in the methanol as a tracer. Measurements with flow-rates of 1–2.5 cm^3/min gave a value of the dwell volume of 2.1 ± 0.1 cm^3. Gradients with a maximum deviation from linearity of less than 2% were obtained with and without the column. The column packing was a bonded-phase octadecyl silica 55–105 μm irregular particles (Megabond; Waters), packed in a 25 × 0.46 cm I.D. stainless-steel column obtained from Biotage. This material is often used in large-scale preparative high-performance liquid chromatography equipment [24]. The void fraction of the packed column was estimated to be ε = 0.33 ± 0.06 from pressure drop measurements. The experiments were carried out at room temperature (23 ± 2°C) with UV detection at 254 nm.

Ethyl paraben (Sigma) was used as a test compound. Samples of ethyl paraben containing 0.008 g/l were prepared with various methanol–water mixtures to match the composition of the initial eluent used in gradient elution experiments. The linear adsorption coefficient of ethyl paraben, K, and H were determined from experimental peaks obtained isocratically for various eluent compositions, using the 0.02-cm^3 loop. K and H were calculated numerically from the first and second moments of the digitized experimental peaks. The results are shown in Fig. 7. The linear adsorption coefficient depends on the methanol volume fraction, φ, in the manner predicted by eqn. 2, with only a minor deviation at the highest φ value. The values of ln κ and S obtained from a regression of the data are 5.87 and 8.61, respectively. The linearity of the equilibrium was checked over the range of φ values from 0.3 to 0.7, by varying the amount of ethyl paraben injected with the various sample loops.

H was also found to vary with the methanol volume fraction in the eluent. This can be attributed to changes in viscosity and solute diffusivity that occur when the solvent composition is changed. The variation was, however, very small and limited to about ±7% of a mean value of 0.145 cm in the range of φ from 0.3 to 0.6. The corresponding value of the mass transfer parameter k_0a is obtained from eqns. 14 and 24, yielding a value of k_0a = 3.5 s^{-1}.

RESULTS AND COMPARISON WITH THEORY

Gradient elution experiments were carried out with the larger sample loops, in order to test the

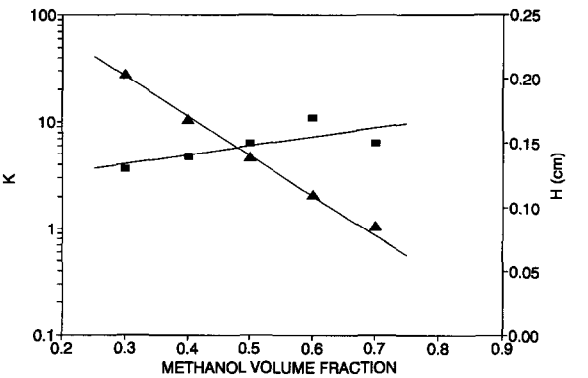

Fig. 7. Linear adsorption coefficient, K (▲) and H (■) for ethyl paraben obtained from isocratic elution experiments at 2.5 cm^3/min with different eluent compositions. K = exp (5.87 − 8.61 φ).

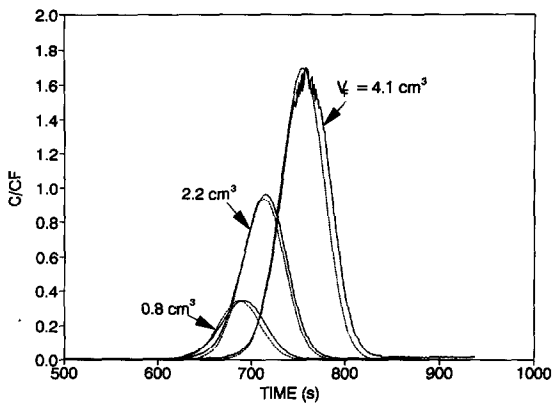

Fig. 8. Comparison of experimental (———) and predicted (· · · ·) gradient elution profiles for ethyl paraben. Gradient from 30 to 60% (v/v) methanol in water, in 15 min at 2.5 cm^3/min. V_F is the volume of the injected sample and CF the feed sample concentration (= 0.008 g/l).

ability of the theory to simulate gradient elution behavior from isocratic elution data, and to correctly predict volume overloading effects. A gradient of 30 to 60% methanol in 15 min was used at a flow-rate of 2.5 cm^3/min. After the start of the gradient, injection of the samples was delayed by an amount of time equal to the equipment dwell time, so that, the gradient started immediately after introduction of the feed slug. The results are shown in Fig. 8 in comparison with the peaks predicted by the theory. Since the fluid phase accumulation term has been neglected in the model, the theory would underestimate the elution time of the peak (see Fig. 3). Thus, in order to provide a clearer comparison of predicted and experimental peak shapes, the calculated peaks were shifted to the right by the quantity $\varepsilon z/u$ = 32.9 s. This correction is less then 5% of the mean retention time. Aside from this correction, the theory requires no adjustable parameter; *i.e.*, the values of K and H obtained from isocratic experiments are used to predict the gradient elution behavior. The volume overloading effects are well predicted by the theory. As the volume injected is increased, the peak becomes increasingly asymmetrical and reaches maximum concentrations that exceed the feed value. Only a small discrepancy between experimental and predicted peaks is observed. This may be due to the inability of the theory to provide an exact description of dispersion in gradient elution with the single lumped mass transfer parameter k_0a.

CONCLUSIONS

The analytic solution presented here is totally general with respect to gradient shape and type; *i.e.*, it applies to linear and non-linear gradients, to multiple step gradients, and to different relationships between K and the modifier concentration. The equations are limited to linear isotherms and to gradient conditions in which the linear adsorption coefficient remains large during the gradient run. The solution is explicit and allows a direct calculation of the effluent concentrations. The first moment and standard deviation of calculated peaks converge to the predictions of the LSS theory in the limit of small feed pulses and Gaussian peaks. Yet, the solution presented here is uniformly valid for small pulses and large, rectangular feed slugs. The series solution is also easy to use, although a programmable calculator or a personal computer may be needed to compute a sufficiently large number of terms to insure adequate precision. A significant advantage is that the solution can be formally integrated, so that the average concentration of any chosen product cut, may be calculated directly. This is convenient to select the product cuts that will provide a desired purity in preparative applications.

The theory was found to be able to correctly predict volume overload effects in gradient elution which were observed experimentally for a model system. The prediction was based solely on data obtained isocratically for k' and H. The close agreement between the theory and the experimental results would indicate that a single mass transfer parameter may be used to represent dispersion in gradient elution with reasonable accuracy.

Finally, it should be noted that the solution provides the time-periodic response obtained with periodic feed pulses. On the other hand, if the response to a single isolated pulse is desired, this is easily accomplished by selecting a sufficiently large total period to avoid interference from repeated injections, as we have done with the sample calculations reported here.

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SYMBOLS

A parameter in IEC, eqn. 26
b parameter in IEC, eqn. 26
c mobile phase concentration
$\bar{c}$ average value of c in a product cut
c_F feed concentration
C peak compression factor in LSS theory, eqn. 5
d_p particle diameter
D_L axial dispersion coefficient
D_p pore diffusivity
H height equivalent to a theoretical plate
k term number in series solution
k' retention factor
k_0a film mass transfer parameter
k_f external film mass transfer coefficient
K linear adsorption coefficient
K_0 initial value of K
K_G final value of K
n number of transfer units, eqn. 14
N plate number
p parameter defined by eqn. 6
q stationary phase concentration
r dimensionless period, eqn. 19
r_F dimensionless duration of feed injection, eqn. 20
r_G dimensionless gradient duration
S constant in eqn. 2
t time
t_F duration of feed injection
t_G duration of gradient
t_R retention time
t_P total period
u mobile phase superficial velocity
V_F feed volume
X dimensionless mobile phase concentration, eqn. 12
Y dimensionless stationary phase concentration, eqn. 13
z column length

Greek symbols

β gradient slope
ε bed void fraction
ε_p particle porosity
ϑ dimensionless time, eqn. 15
κ constant in eqn. 2
μ first moment of peak
σ standard deviation of peak
φ modifier concentration or volume fraction
φ^0 value of φ at the start of gradient run
φ^G value of φ at the end of the gradient run

REFERENCES

1 F. D. Antia and Cs. Horváth, *J. Chromatogr.*, 484 (1989) 65.
2 F. D. Antia and Cs. Horváth, in C. A. Costa and J. S. Cabral (Editors), *Chromatographic and Membrane Processes in Biotechnology*, Kluwer, Dordrecht, 1991, pp. 115–136.
3 L. R. Snyder and M. A. Stadalius, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography —Advances and Perspectives*, Vol. 1, Academic Press, New York, 1986, pp. 195–235.
4 B. Drake, *Arkiv. Kemi.*, 8 (1955) 1.
5 E. C. Freiling, *J. Am. Chem. Soc.*, 77 (1955) 2067.
6 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
7 L. R. Snyder, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography —Advances and Perspectives*, Vol. 1, Academic Press, New York, 1980, pp. 208–316.
8 S. Yamamoto, K. Nakanishi, R. Matsuno and T. Kamikubo, *Biotechnol. Bioeng.*, 25 (1983) 1465.
9 P. Jandera and J. Churáček, *Gradient Elution in Column Liquid Chromatography —Theory and Practice*, Elsevier, Amsterdam, New York, 1985.
10 S. J. Gibbs and E. N. Lightfoot, *Ind. Eng. Chem. Fundam.*, 25 (1986) 490.
11 K. Kang and B. J. McCoy, *Biotechnol. Bioeng.*, 33 (1989) 786.
12 D. D. Frey, *Biotechnol. Bioeng.*, 35 (1990) 1055.
13 S. Yamamoto, K. Nakanishi and R. Matsuno, *Ion Exchange Chromatography of Proteins*, Marcel Dekker, New York, 1988.
14 J. E. Eble, R. L. Grob, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 405 (1987) 51.
15 L. R. Snyder, G. B. Cox and P. E. Antle, *J. Chromatogr.*, 444 (1988) 303.
16 G. Carta, J. P. DeCarli, II, C. H. Byers and W. G. Sisson, *Chem. Eng. Commun.*, 79 (1989) 207.
17 G. Carta and R. L. Pigford, *Chem. Eng. Sci.*, 41 (1986) 511.
18 G. Carta, *Chem. Eng. Sci.*, 43 (1988) 2877.
19 E. Glueckauf, *Trans. Faraday Soc.*, 51 (1955) 1540.
20 D. M. Ruthven, *Principles of Adsorption and Adsorption Processes*, Wiley, New York, 1984, pp. 242–244.
21 P. Schneider and J. M. Smith, *AIChE J.*, 14 (1968) 762.
22 T. K. Sherwood, R. L. Pigford and C. R. Wilke, *Mass Transfer*, McGraw-Hill, New York, 1975, pp. 561–578.
23 A. Valeyudhan and Cs. Horváth, *J. Chromatogr.*, 367 (1986) 160.
24 *Technical Bulletin*, Biotage, Inc., Charlottesville, VA, 1991.