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NON-EQUILIBRIUM MODEL OF LIQUID COLUMN CHROMATOGRAPHY

I. EXACT EXPRESSIONS FOR ELUTION PROFILE MOMENTS AND RELATION TO PLATE HEIGHT THEORY

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SUMMARY

A model for column chromatography that includes non-equilibrated mass transfer, diffusion, and generalized boundary conditions at the top of the column is solved by the method of moments. The theory predicts that as the macroscopic velocity u becomes very small, the plate height approaches a constant (rather than diverging as u^{-1}). As u increases, the plate height drops to a minimum and then increases. We show that the past assumptions that mass transfer and various types of diffusion contribute additively to the plate height, hold only at or beyond the minimum. We show further that our expression fits observed data with only a *single* adjustable lumped parameter. The parameter contains the mass transfer rate k_{-1} for moving out of a bead. The data fit, as well as an analytical approximation that we derive for the position of the minimum, provides a relation between k_{-1} and bead particle size (d_p) and consequently between d_p and profile dispersion. Conditions under which the peak of the elution profile is a good approximation to the mean are described. For a suitably chosen flow-rate, the mass transfer rates can be estimated from the observed dispersion in the elution profile.

INTRODUCTION

The use of column chromatography as a quantitative tool for molecular weight determination¹ and chemical reaction characterization^{2,3} has increased continuously and rapidly during the past decade. However, the basis of its validity for quantitative thermodynamic (and perhaps kinetic⁴) studies is uncertain, resting largely on assumptions that local equilibrium is established instantaneously³, and that the contribution of chemical kinetics to elution profile broadening can be made to dominate the effects of diffusion and other none-equilibrium processes⁴. A general assessment of the range of validity of these assumptions has been difficult because of the formidable problems in obtaining analytic solutions for the elution profile, even when the mathematical

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system is linear. In this paper we extend a basic non-equilibrium model of chromatography and develop the experimental implications. This and the companion paper⁵, which includes heterogeneity and non-linear mass transfer, will provide a useful foundation for generalization to more complex systems involving chemical reactions^{6,7}.

Of the many important mathematical contributions to the theory of liquid column chromatography (*e.g.*, refs. 2 and 8, and references cited therein), those most similar to the model presented here are the non-equilibrium random walk model of Giddings and Eyring^{8,9} and the partial differential equations formulation of Thomas¹⁰. Our method of solution, involving explicit equations for the moments of the profile, is similar to that used by other authors (refs. 8 and 11–13, and references cited therein). Here we extend these methods by generalizing the boundary conditions at the top of the column and by pursuing the numerical consequences of the model.

After developing and solving the model, we study plate height (proportional to the dispersion in the profile divided by the square of the mean) as a function of the convection velocity u . At very low u , the plate height approaches a constant (rather than diverging as u^{-1}) which is explicitly given by the theory. As u increases, the plate height passes through a minimum in the usual way, and then increases. A good non-linear least-squares fit of our predicted equation to data is obtained with only one adjustable parameter. An analytic approximation is obtained for this minimum which relates the desorption mass transfer coefficient to bead diameter.

Criteria on the flow-rate are given which guarantee that the effects of diffusion on the means and variances can be neglected. A chromatography model which assumes local equilibration of mass transfer kinetics is described. When diffusion is negligible, this model yields a widely used formula for the peak of the elution profile. Identification of the assumptions required for its derivation provides a basis for assessing its range of validity.

Implications of our analysis are discussed. A complete procedure is outlined for estimating the equilibrium constant and the rate constants for the sorption-desorption kinetics from the mean and variance of the elution profile. Both the equilibrium constant and the rate constants can be estimated from the mean and variance, but *only* the equilibrium constant can be estimated from the mean.

A CHROMATOGRAPHY MODEL WITH DIFFUSION

We formulate an initial-boundary value problem which models a small-zone chromatography experiment with a single type of molecule in the sample. We model only non-gradient, elution chromatography with a constant temperature in the column and a constant pressure on the solvent. Consider a column where x measures the distance up from the bottom of the bed; x is h at the top of the bed and x is f at the top of the solvent above the bed. Let V_0 be the void volume, *i.e.*, the volume exterior to the beads, and let V_p be the volume interior to the beads that can be penetrated by the macromolecules. Define the void cross-sectional area as $A_0 = V_0/h$ and the penetrable cross-sectional area as $A_p = V_p/h$. The concentration (over A_0) of solute molecules in the mobile phase is $C(x,t)$ and the concentration (over A_p) of molecules in the stationary phase is $B(x,t)$.

Using a conservation of mass approach on $0 < x < h$, we have derived a system of reaction-diffusion-convection partial differential equations for the density

A_0C (in units of molecules per unit column length) of solute molecules in the mobile phase and the density A_pB of solute molecules in the stationary phase. By dividing these equations by the initial number of molecules I , the following partial differential equations for the probability density $p(x,t)$ for the mobile phase and $q(x,t)$ for the stationary phase are obtained:

$$\frac{\partial p}{\partial t} = u \frac{\partial p}{\partial x} + D \frac{\partial^2 p}{\partial x^2} - k_1 p + k_{-1} q \quad (1)$$

$$\frac{\partial q}{\partial t} = k_1 p - k_{-1} q \quad (2)$$

In eqn. 1 the first term on the right corresponds to mobile-phase flow (convection) with velocity u ($u = F/A_0$ where F is the flow-rate), and the second term corresponds to diffusion with diffusion constant D . The movement of solute molecules back and forth between the flowing solvent (mobile phase) and the inside of the beads (stationary phase) is described by the sorption and desorption rate constants, k_1 and k_{-1} . At sorption-desorption equilibrium the concentrations C and B are equal so that eqn. 2 implies that the equilibrium constant $K = k_1/k_{-1}$ satisfies $K = A_p/A_0$ or $K = V_p/V_0$. The equilibrium constant K is not equal to the partition or distribution coefficient defined as $K_d = V_p/V_i$, where V_i is the volume inside the beads that is not gel matrix; however, $K_d = KV_0/V_i$. The retention ratio R used by Giddings⁸ satisfies $R = 1/(1 + K)$.

The initial layer at the top of the bed containing sample molecules is assumed to be sufficiently small so that it can be considered an instantaneous source (small zone). Thus, the initial conditions on $0 \leq x \leq f$ are

$$\begin{aligned} p(x,0) &= \delta(x - h) \\ q(x,0) &= 0 \quad x \neq h \end{aligned} \quad (3)$$

where $\delta(x - h)$ is a Dirac delta function. We remark that alternatively the instantaneous source could have been included as a term $\delta(x - h) \delta(t)$ in eqn. 1.

Diffusion of molecules into the solvent above the top of the bed is possible; however, if the flow-rate is positive, then these molecules would soon be moved into the bed by the solvent flow. The partial differential equation for the mobile phase molecule density A_1C above the top of the bed ($h < x < f$) involves flow and diffusion (A_1 is the cross-sectional area of the column). By dividing this equation by the initial number of molecules I , we obtain the following differential equation for the probability density $p(x,t)$:

$$\frac{\partial p}{\partial t} = u_1 \frac{\partial p}{\partial x} + D_1 \frac{\partial^2 p}{\partial x^2} \quad (4)$$

Above the bed the velocity u_1 of the solvent is F/A_1 and the diffusion constant of the molecules is D_1 . Since no molecules can move above the top of the solvent, the molecular current is zero there; that is,

$$u_1 p(f, t) + D_1 \frac{\partial p}{\partial x}(f, t) = 0 \quad (5)$$

for $t \geq 0$.

The number of molecules and the current must be continuous at the top of the bed so that

$$p(h^-, t) = p(h^+, t)$$

$$u_1 p(h^-, t) + D_1 \frac{\partial p}{\partial x}(h^+, t) = u p(h^-, t) + D \frac{\partial p}{\partial x}(h^-, t) \quad (6)$$

for $t > 0$. Precise incorporation of diffusion below the bottom of the bed would require a differential equation like eqn. 4 for the solute molecules below the bed and matching conditions similar to eqn. 6 at the bottom of the bed. It is more convenient to assume that the elution profile is measured at the bottom of the bed ($x = 0$) and that there is an absorber (sink) there. This corresponds to $A_0 C(0, t) = 0$ or

$$p(0, t) = 0 \quad (7)$$

for $t \geq 0$. This condition is reasonable since the rate of movement by flow of solute molecules in the solution below the bed is large compared with the rate of movement of these molecules in the bed. This model is also applicable to affinity chromatography in which ligands, covalently bound to the surfaces of impenetrable beads, interact monovalently with solute molecules. In that case the equilibrium and rate constants describe the binding reaction.

THE MOMENTS OF THE ELUTION PROFILE

Rather than attempting to solve the initial-boundary value problem (eqns. 1-7), we show how the model can be used to obtain ordinary differential equations with boundary conditions for the mean and variance of the passage time. The mean passage time $T_1(x)$ is defined to be the mean time for molecules starting in the initial layer to move past a position x ^{6,14,15}. The mean elution time $M_e = T_1(0)$ (also called the mean residence time or the mean retention time) is the mean time for molecules to move out of the bottom of the bed. Definitions of higher moments are similar. The fraction of molecules per unit time moving by position x in the bed at time t is the current $u p(x, t) + D \frac{\partial p}{\partial x}(x, t)$. Thus, the j th moment of the passage time at position x is defined to be

$$T_j(x) = \int_0^{\infty} t^j \left[u p(x, t) + D \frac{\partial p}{\partial x}(x, t) \right] dt \quad (8)$$

Mean passage time

Direct integration with respect to t on the interval $(0, \infty)$ of 1 times and t times the partial differential equations, and use of the boundary conditions, leads to the following boundary value problem for $T_1(x)$:

$$D_1 \frac{d^2 T_1}{dx^2} + u_1 \frac{dT_1}{dx} = 0 \quad h < x < f \tag{9}$$

$$D \frac{d^2 T_1}{dx^2} + u \frac{dT_1}{dx} = -(1 + K) \quad 0 < x < h$$

The boundary conditions in eqns. 5-7 convert to

$$T_1(f) = 0, \quad \frac{dT_1}{dx}(0) = 0 \tag{10}$$

$$T_1(h^+) = T_1(h^-), \quad (1 + K) \frac{dT_1}{dx}(h^+) = \frac{dT_1}{dx}(h^-)$$

The solution for $0 \leq x \leq h$ of the boundary value problem (eqns. 9 and 10)

$$T_1(x) = (1 + K) \left[\frac{h - x}{u} - \frac{D}{u^2} (e^{-ux/D} - e^{-uh/D}) \right] + \frac{D_1}{u_1 u} (1 - e^{-uh/D}) \times [1 - e^{-u_1(f - h)/D_1}] \tag{11}$$

so that the mean elution time is

$$M_e = T_1(0) = \frac{h}{u} \left\{ (1 + K) \left[1 - \frac{(1 - e^{-r})}{r} \right] + \frac{(1 - e^{-r})}{r_1} (1 - e^{r_1 - r_2}) \right\} \tag{12}$$

where $r = uh/D$, $r_1 = u_1 h/D_1$ and $r_2 = u_1 f/D_1$.

Variance of the passage time

If the differential equations 4, 1 and 2 are multiplied by t^2 and integrated with respect to t on $(0, \infty)$, then the boundary value problem for the variance $S(x) = T_2(x) - T_1(x)^2$ is

$$D_1 \frac{d^2 S}{dx^2} + u_1 \frac{dS}{dx} = -2D_1 \left(\frac{dT_1}{dx} \right)^2 \quad h < x < f \tag{13}$$

$$D \frac{d^2 S}{dx^2} + u \frac{dS}{dx} = -2D \left(\frac{dT_1}{dx} \right)^2 - \frac{2K}{k-1} \quad 0 < x < h$$

$$S(f) = 0, S(h^+) = S(h^-), \quad \frac{dS}{dx}(0) = 0 \quad (14)$$

$$\frac{dS}{dx}(h^-) = (1 + K) \frac{dS}{dx}(h^+) + \frac{2K}{k_{-1}} \frac{dM}{dx}(h^+)$$

From the solution $S(x)$ the variance of the elution time is

$$\begin{aligned} S_e = S(0) &= \frac{2Kh}{k_{-1}u} \left[1 - (1 - e^{-r})/r \right] \\ &+ \frac{2(1 + K)^2 h^2}{r u^2} \left[1 + 2e^{-r} - 2 \frac{(1 - e^{-r})}{r} - \frac{(1 - e^{-2r})}{2r} \right] \\ &+ \frac{2(1 + K) h^2}{r_1 u^2} \left[1 - e^{r_1 - r_2} \right] \left[\frac{(1 - e^{-r})}{r} - 2e^{-r} \right] \\ &+ \frac{h^2}{r_1^2 u^2} (1 - e^{-r})^2 (1 - e^{r_1 - r_2})^2 \end{aligned} \quad (15)$$

Although the mean elution time (eqn. 12) depends only on the sorption-desorption equilibrium constant K , the variance S_e in eqn. 15 depends on both K and the desorption rate constant k_{-1} . Special cases of some of the equations in this section were obtained by Weiss¹⁶. When diffusion is ignored ($D = 0$, $D_1 = 0$), then the mean and variance of the elution time are

$$M_e = (1 + K) h/u, S_e = 2Kh/(k_{-1}u) \quad (16)$$

Moments of the elution volume

An elution profile is a graph of the number of molecules eluted from the bottom of the bed as a function of time or of eluted solvent volume. For positive flow F , the mean elution volume V_e is defined to be the total volume of solvent eluted up to the mean elution time M_e . Since $K = V_p/V_0$, and the volume eluted up to time t is $V = Ft = V_0 ut/h$, the following mean elution volume expression can be found from eqn. 16 when diffusion is neglected:

$$V_e = V_0 + V_p \quad (17)$$

Similarly the variance found from eqn. 16 when diffusion is neglected is

$$W_2 = F^2 S_e = 2FV_0 K/k_{-1} \quad (18)$$

Note that the mean elution volume V_e does not depend on the flow-rate F , but the variance increases as the flow-rate increases. Eqn. 17 is formally identical to a stan-

standard chromatography equation; however, the V_e in the standard equation is the peak of the elution profile instead of the mean. (See later for further comparison with the local equilibration and elution profile peak approach).

Thick sample layer

If the effects of diffusion can be neglected and T is the thickness of the initial layer of sample, then the means and variances given by eqns. 16–18 become

$$M_e = (1 + K)h/u + T/(2u) \quad (19)$$

$$S_e = 2Kh/(k_{-1}u) + T^2/(12u^2)$$

$$V_e = V_0 + V_p + V_0T/(2h) \quad (20)$$

$$W_e = 2FV_0K/k_{-1} + V_0^2T^2/(12h^2)$$

Third central moment

The methods above can be used to calculate higher moments, but the results are complicated. *Neglecting diffusion* we find that the third central moment (*i.e.*, around the mean) of the elution profile as a function of time is

$$N_e = 6Kh/(k_{-1}^2u) \quad (21)$$

and as a function of volume is

$$U_e = 6F^2V_0K/k_{-1}^2 \quad (22)$$

Since the third central moment of the time for a layer of thickness T to enter the bead is zero, eqns. 21 and 22 hold for both thin and thick sample layers. When the flow-rate is such that diffusion is negligible, the moment coefficient of skewness for a thin sample layer is

$$G_1 = U_e/W_e^{3/2} = 3(F/2k_{-1}V_0)^{1/2} \quad (23)$$

Thus, the skewness increases as the flow-rate F increases, as noted in numerical solutions¹⁷.

RELATION TO PLATE HEIGHT THEORY

Since plate height terminology is still widely used, we now present and discuss our results in that notation. The spreading of solute molecules from a thin initial layer to a bell-shaped distribution along the bed at later times is called dispersion or zone spreading or band broadening. The causes of dispersion are the sorption-desorption kinetics (also called non-equilibrium effects) and diffusion in the mobile phase. Here we use diffusion (in the mobile phase) as a general term which includes longitudinal diffusion (molecular diffusion) and diffusion-related flow phenomena such as eddy diffusion and velocity profile heterogeneity.

The relative dispersion about the mean of the elution profile is the variance S_e divided by the square of the mean M_e . If diffusion above the bed is negligibly small, then from eqns. 12 and 15 we obtain

$$\frac{S_e}{M_e^2} = \frac{\alpha r}{1 - (1 - e^{-r})/r} + \frac{2[1 + 2e^{-r} - 2(1 - e^{-r})/r - (1 - e^{-2r})/2r]}{r[1 - (1 - e^{-r})/r]^2} \quad (24)$$

where $r = uh/D$ and

$$\alpha = 2KD_i/[h^2k_{-1}(1 + K)^2] \quad (25)$$

The two terms in eqn. 24 correspond to the two causes of dispersion described above.

The height equivalent to a theoretical plate has been shown^{1,9,18} to satisfy $H = hS_e/M_e^2$. Division by the bead diameter d_p leads to the reduced plate height

$$\tilde{h} = hS_e/(d_p M_e^2) \quad (26)$$

which is dimensionless. Using eqn. 24, it can be shown that \tilde{h} equals $2(\alpha + 1/3)h/d_p$ when $r = 0$, that it decreases to a minimum when $r \approx \sqrt{2/\alpha}$ and that it then increases. Near and beyond the minimum, a good approximation is

$$\tilde{h} = 2/v + \alpha h/d_p + \alpha(h/d_p)^2 v \quad (27a)$$

where $v = rd_p/h = ud_p/D$ is a dimensionless quantity called the reduced velocity. For typical parameter values and typical reduced velocities ($v > 0.01$), the second term in eqn. 27a is entirely negligible so that

$$\tilde{h} \approx 2/v + \alpha(h/d_p)^2 v \quad (27b)$$

Eqn. 27a is similar to the van Deemter equation^{1,19} given by

$$\tilde{h} = B/v + A + Cv \quad (28)$$

where the constants B , A and C are associated with axial molecular diffusion, eddy diffusion, and non-equilibrated mass transfer. However, eqn. 28 involves three undetermined parameters, B , A and C , whereas eqn. 27a has only one undetermined parameter, α . Many equations similar to eqn. 28 have been derived for the reduced plate height²⁰⁻²³. For example, the Knox equation²³ is

$$\tilde{h} = B/v + Av^{1/3} + Cv \quad (29)$$

Although eqn. 24 is valid for $v \geq 0$, eqns. 27-29 are only reasonable near and beyond the minimum, since the plate height should be a constant when $v = 0$ and should not be infinite.

Data fitting

Plots of $\log \tilde{h}$ against $\log v$ have been obtained experimentally^{1,21,23}. Using a

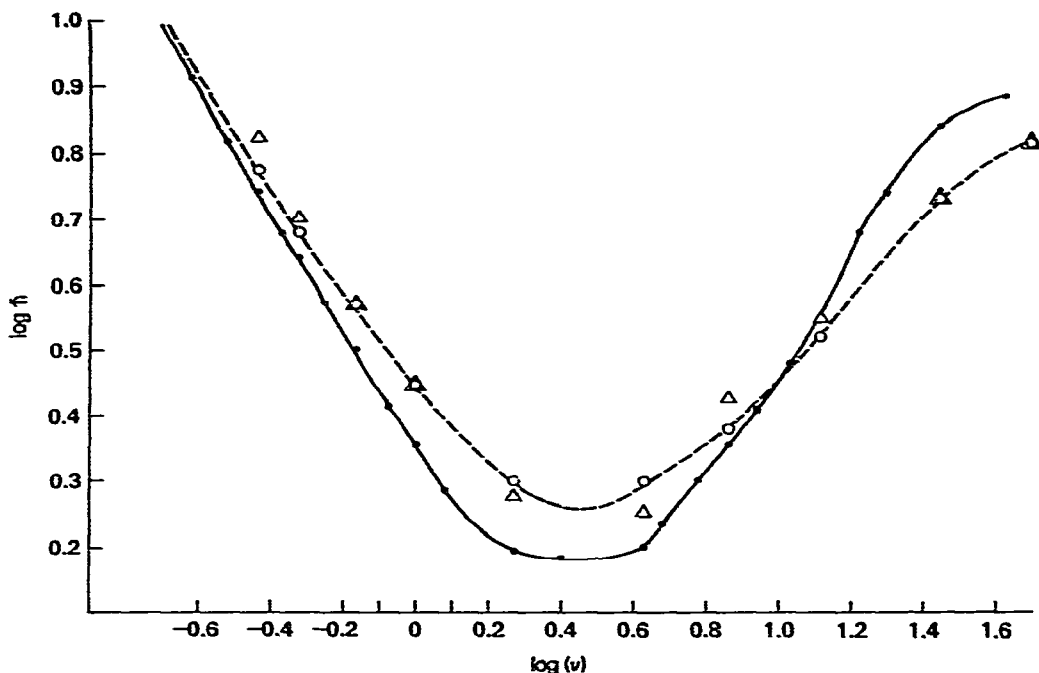


Fig. 1. Log-Log plot of reduced plate height h as a function of reduced velocity v . (Δ) are the experimental data points (ref. 21). The RMS error of the fits using the van Deemter equation (eqn. 28) and the Knox equation (eqn. 29) are virtually identical (dashed line). The best least-square parameter sets (A, B, C) are (0.83, 1.86, 0.17) for eqn. 28 and (0.62, 2.03, 0.13) for eqn. 29. The best least-square fit of eqn. 27b is shown by the solid line. The curve was generated using the experimental value $h/d_p = 3837$ and determining $\alpha(h/d_p)^2 = 0.26$ by a least-squares fit.

data set from Fig. 7 in ref. 21, we have determined the best fits of eqns. 27b, 28 and 29. The best least-square fits of the two empirical equations (28 and 29) are virtually identical in terms of root mean square error, although the parameter sets (A, B, C) that produce the fits are of course different for the two equations. Our eqn. 27b, derived from a basic chromatography model, gives a somewhat poorer fit, but it has only one adjustable parameter.

Interestingly, the best least-square parameter B in the Knox equation turns out to be 2, as predicted by our result. The strikingly close agreement is probably to some extent fortuitous. Nevertheless, the agreement suggests that the main reason for the difference between the data and the prediction based on eqn. 27b is the absence of velocity profile effects. As a check on this, we added a term $Av^{1/3}$ onto eqn. 27b, thus producing a two-parameter equation, and obtained a fit that was slightly better (in terms of RMS error) than that based on the Knox equation. One way of looking at these results is to regard eqn. 27b as providing a partial theoretical basis for the Knox equation, pinning down two of the three adjustable parameters in that equation to values determined by a molecular model.

Desorption rate constant related to bead diameter

Let v_m be the reduced velocity at which the minimum of h (or $\log h$) occurs.

Since the minimum of eqn. 24 occurs when $r \approx \sqrt{2/\alpha}$, where α is given by eqn. 25, we obtain

$$k_{-1} \approx \frac{KDv_m^2}{(1+K)^2 d_p^2} \quad (30)$$

From various plots of $\log h$ against $\log v$, it has been observed²³ that v_m is consistently *ca.* 3. Thus, eqn. 30 predicts that the desorption rate constant k_{-1} is inversely proportional to the square of the bead diameter d_p .

THE SIGNIFICANCE OF DIFFUSION

From eqns. 24 and 27 we see that diffusion contributes more to dispersion for low flow-rates and that non-equilibration of mass transfer contributes more for high flow-rates. For many chromatography systems an intermediate flow-rate is chosen so that the axial dispersion is minimized²¹⁻²³. Here we choose a flow-rate (higher than the dispersion minimizing flow-rate) so that the effects of diffusion are negligible. This procedure allows us to use the information contained in the observed dispersion to estimate the sorption and desorption rate constants.

The expressions for the mean and variance of the elution time are particularly simple when the contribution of terms corresponding to diffusion are sufficiently small so that they can be neglected. From eqn. 12 we find the following bound for large flow-rate on the relative difference between the mean elution times with and without diffusion

$$\left| \frac{M_c - (1+K)h/u}{(1+K)h/u} \right| \leq \frac{1}{r} + \frac{1}{r_1(1+K)} \quad (31)$$

For large flow-rate F the relative magnitude of the terms corresponding to diffusion in eqn. 15 for the variance are given in the following inequality:

$$\left| \frac{S_c - 2Kh/(k_{-1}u)}{2Kh/(k_{-1}u)} \right| \leq \frac{1}{r} + \frac{k_{-1}h}{Ku} \left[\frac{(1+K)^2}{r} + \frac{(1+K)}{rr_1} + \frac{1}{2r_1^2} \right] \quad (32)$$

For positive K it is clear that the contributions to the mean elution time and to the variance of the diffusion-related terms can be neglected if the flow-rate (and hence u, u_1, r and r_1) is chosen large enough so that the right sides of eqns. 31 and 32 are small. Note that the above inequalities are also measures of the relative contributions due to diffusion for the mean and variance of the elution volume. Calculations using typical parameter values reveal that the contribution of diffusion above the bed is much less than the contribution of diffusion in the bed and that diffusion terms have a larger influence on the variance than on the mean.

Local equilibration and the elution profile peak

The widely used equation for the elution profile peak can be obtained by assuming local equilibration and negligible diffusion. If it is assumed that the mobile

phase and stationary phase molecules equilibrate locally for each fixed x , then $B(x,t) = C(x,t)$ so that $q(x,t) = Kp(x,t)$. In this case the sum of eqns. 1 and 2 reduces to

$$\frac{\partial p}{\partial t} = \frac{u}{1+K} \frac{\partial p}{\partial x} + \frac{D}{1+K} \frac{\partial^2 p}{\partial x^2} \quad (33)$$

The solution of the initial value problem for $-\infty < x < \infty$ consisting of eqn. 33 and $p(x,0) = \delta(x-h)$ is

$$p(x,t) = [4\pi Dt(1+K)]^{-1/2} \exp\left[\frac{-[h-x-ut/(1+K)]^2}{4Dt/(1+K)}\right] \quad (34)$$

This solution has the form of a Gaussian distribution function which moves and spreads simultaneously. The elution profile $up(0,t) + D \frac{\partial p}{\partial t}(0,t)$ is not a Gaussian distribution as a function of t , and it is not symmetric around the peak which occurs at

$$T(\text{peak}) = \frac{(1+K)h}{u} \left(1 - \frac{7}{3r} + \frac{91}{54r^2} + \dots\right) \quad (35)$$

When diffusion is negligible ($1/r \ll 1$), the elution profile is approximately $\delta[h-x-ut/(1+K)]$ and the peak is approximately equal to the mean (eqn. 16). Hence the corresponding formula $V(\text{peak}) = V_0 + V_p$ assumes both local equilibration and negligible diffusion while the formula $V_e = V_0 + V_p$ for the mean elution volume assumes only that diffusion is negligible. The local equilibration assumption is usually unreasonable since it implies that the variance is approximately zero. Differences between the peak and the mean are discussed further in ref. 5.

DISCUSSION

We outline a procedure for determining the sorption-desorption equilibrium constant K , the sorption rate constant k_1 and the desorption rate constant k_{-1} by liquid column chromatography. This procedure works for both small-zone and large-zone chromatography.

(1) Use eqns. 31 and 32 with estimates for the parameter values to choose the flow-rate F large enough so that the contributions of diffusion are negligible.

(2) Find the void volume V_0 experimentally by using the mean elution volume as V_0 when a weaker solvent or a non-retained larger molecule is run through the column¹.

(3) Find values of the mean volume V_e and the variance W_e of the elution profile by running the sample through the column.

(4) Calculate the equilibrium constant K from

$$K = V_p/V_0 = [V_e - V_0 - V_0 T/(2h)]/V_0 \quad (36)$$

by using the values determined in steps 2 and 3. The equations in this step are

obtained from eqn. 20. Eqns. 19 would be used if the elution profile were given as a function of time. The term in eqn. 36 involving the sample thickness T can be omitted if the volume measurement is started when half of the sample has entered the column. Now calculate the desorption rate constant from

$$k_{-1} = 2F V_0 K / [W_c - V_0^2 T^2 / (12h^2)] \quad (37)$$

The term involving the sample thickness T is always included but may be negligibly small if T is small. Of course, the sorption rate constant can now be found from $k_1 = k_{-1} K$.

(5) Use the values calculated above in eqns. 31 and 32 to verify that the contributions of diffusion were indeed negligible.

We emphasize that the sorption and desorption rates can be found by using the first and second moments, but only the equilibrium constant can be found from the first moment. The third central moment does not yield any new information; however, the observed third central moment can be used in eqn. 22 to check the estimates of K and k_{-1} obtained from the first two moments.

If the only constant of interest is the equilibrium constant (and not the rate constants), then the most accurate method of determining this value might be to choose a chromatography system so that the dispersion (band width) is minimized^{21,23}. The dispersion can be made quite small by using optimum bed particle sizes, column diameters and lengths, flow-rates, pressures, sample dilutions and sample sizes in high-performance liquid chromatography²³. In this case the mean and peak of the elution profile would essentially coincide so that the equilibrium constant could be easily estimated. We emphasize that if the goal is to also estimate the rate constants for movement in and out of the beads, then the procedure outlined at the beginning of this section should be followed. Estimates of the sorption and desorption rate constants are necessary for the quantitative analysis of more complicated systems that include chemical reaction^{6,7}.

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