NON-EQUILIBRIUM AND DIFFUSION: A COMMON BASIS FOR THEORIES OF CHROMATOGRAPHY*

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INTRODUCTION

There are three theories commonly used to describe the position and structure of bands in chromatography (we limit our discussion to cases involving linear sorption isotherms; *i.e.*, nearly all partition chromatography). These are the theoretical-plate model[1,2], the conservation of material approach[3,4], and the stochastic theory[5,6,7]. The material-conservation approach and the stochastic theory are very closely related. For this reason they have been collectively termed as "rate" theories[8]. With a given set of kinetic parameters describing transitions between phases (mobile and stationary), it is the scope of these theories to predict the structure of the elution curve. The relative advantage of one theory over the other depends upon the ease of application to specific examples.

The theoretical-plate model ("plate" theory) is of a different nature. The parameter (HETP) for this model must be measured in a given experiment. The model then describes the development of a chromatogram in terms of this parameter and the R value**.

These two areas of approach can be compared to the relationship between statistical mechanics and thermodynamics. The former depends on specific information concerning microscopic events in order to derive macroscopic results, while the latter concerns general rules, equally valid for simple and complex underlying microscopic behavior.

This relationship between the rate and plate theories tells us beforehand the limitations of a treatment comparing them. Since no kinetic parameters enter the plate theory, the results of this theory cannot be directly checked against those of the rate theories. However, a comparison of the results yields a relationship between (HETP) and the kinetic parameters. The treatment presented here is more fundamental than this. Instead of starting with the results (elution or band structure) of plate theory, we have gone back to the basic concept that the chromatogram can be divided into discrete cells, and that the length of the cell (HETP) is related to an equilibrium condition between the content of the cell and its effluent. The latter relationship,

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** We will hereafter use R as the equilibrium ratio of the amount of solute in the mobile phase to the total amount of solute. We may also interpret R as (1) the probability that a particular molecule at a given instant is in the mobile phase, or (2) the ratio of the velocity of the center of the band of solute, u, to the average velocity in the mobile phase, v. For one-dimensional flow (*i.e.* not circular chromatography) R may usually be equated to R_F.

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since it concerns non-equilibrium kinetics, can be directly compared to the results of the kinetic relaxation-time model[9]. This method not only yields the desired relationship between (HETP) and the kinetic parameters, but illustrates the nature and limitations of the theoretical-plate model as applied to chromatography and related procedures.

The relationship between the rate theories is different, since they can be directly checked against one another. There are two ways to effect this comparison. Following an obvious method, we could stipulate a given set of boundary conditions relating to column input, obtain solutions by the two methods, and directly compare them. This method will be used in later publication. We have found a method that gives far better illustration of chromatography as a non-equilibrium phenomenon. One restriction is necessary, but this is a very practical one. Our treatment is restricted to those cases where the departure from equilibrium is not large. Once this is allowed, the treatment becomes very general in that it does not depend upon a particular set of boundary (input) conditions. This is done simply by relating the structure of the band to an effective diffusion coefficient, given in terms of the kinetic parameters. It is suggested that the spreading of bands due to this process be termed chromatodiffusion following an analogous term of MYSELS[10] (Electrodiffusion).

Other effects are operative in chromatography that cause spreading of component bands[5]. Ordinary molecular diffusion in the longitudinal direction is always occurring, both in the mobile and stationary phases, though the latter may be negligible. The flow of solvent through a porous media always adds an additional diffuseness (the so-called eddy diffusion) to an included solute band[11]. Both of these two effects can be computed in theory just as chromatodiffusion can. It is then necessary to add the individual diffusion coefficients together to obtain the overall coefficient for diffusion in the chromatogram. In working with the theoretical plate model, it has been shown that (HETP) is the sum of three terms, each stemming from a single one of the above sources of diffusion[8]. In the treatment here we will be concerned with chromatodiffusion, and thus the diffusion coefficient D and (HETP) that we discuss will simply be the contribution due to kinetic effects.

Any of the above effects can be important in chromatographic separations, although in those procedures where the flow velocity is unduly increased for rapid completion of the process, the kinetic effects dominate. For gas chromatography, the relative values have been experimentally determined[8, 12].

It would be clearly advantageous, in many cases, to consider chromatography, from the beginning, as a diffusion process. The voluminous literature concerned with the solutions of the diffusion equation can be immediately applied to a multitude of boundary conditions for chromatography.

THE STOCHASTIC THEORY

The stochastic theory of chromatography is concerned with the movement of a single molecule through a chromatographic column. The successive sorptions and desorptions are comparable to random walk processes, and mark a fruitful field for the

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application of probability theory. It is to be noted that the problem of equilibrium within the chromatogram arises only in a very limited sense from the point of view of the stochastic theory. This results since the theory, for the most part, is concerned only with the time-dependent behavior of single molecules. However, if a molecule is applied to the column in the mobile phase, then the probability that the molecule will be found in the mobile phase is unity at the instant of application, and asymptotically approaches R as time elapses. This probability is related to equilibrium; the relaxation time for the approach of the probability to its asymptotic value is identical to the relaxation time for the approach of a large collection of molecules to their equilibrium values.

The point of real interest in chromatography, however, concerns equilibrium only in a local sense. In any useful chromatogram, the total concentration of solute in the mobile phase divided by the total concentration, for any calculational purpose, equals R. However, at a given point on the chromatogram, local equilibrium does not obtain, and a deficiency of concentration in the mobile phase at one point is balanced by a surplus at another point. We will expand on these concepts in our discussion of the material-conservation approach, since there we will find it necessary to quantitatively evaluate the local non-equilibrium effects.

In a previous publication, chromatography was considered as a diffusion process[13]. Through expanding the probability density function in a Taylor series along the lengthwise coordinate, the diffusion concept was found to be valid whenever the average number of sorptions is large. The validity of the diffusion concepts can be more fundamentally shown by the methods used by EINSTEIN concerning Brownian motion[14]. It is shown in that treatment that diffusion results from a large number of independent, random displacements. The conditions are the same, since each sorption is independent of the previous one, and the large number of them is stipulated.

When the diffusion conditions are fulfilled, the diffusion coefficient for a band on the column, due to kinetic effects (chromatodiffusion), is

$$D = \frac{k_1 k_2 v^2}{(k_1 + k_2)^3} \tag{1}$$

where v is the longitudinal component of flow velocity, and k_1 and k_2 are the average number of sorptions and desorptions, respectively, in unit time.

THE MATERIAL-CONSERVATION APPROACH

Since the following treatment is concerned with general systems involving reaction kinetics, it is necessary to outline chromatography as a kinetic system. It has been shown that the kinetics of chromatography can be reduced to that of simple kinetic analogs. The simplest one, adequate in most cases, is

$$\mathrm{A} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{B} \tag{2}$$

where A represents a molecule in the mobile phase, and B a molecule in the stationary phase. The arrows indicate the continuous transition between the two "configurations". The transition rates, k_1 and k_2, have been defined.

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In order to discuss both the material-conservation approach and the theoretical-plate model, it is necessary to use a method for treating the kinetics of chemical systems near equilibrium. The relaxation-time model for chemical kinetics[9] has been devised for this purpose, and is easily adapted to the treatment of chromatographic processes.

If the concentration, c_1, of a chemical species, A, is perturbed slightly from its equilibrium value (more generally, from its quasi-equilibrium value), it will return to the equilibrium in the manner of exponential relaxation. The relaxation time, t_r, for this process can be easily obtained[9].

$$t_r = \frac{c_1 - c_1^*}{-\mathrm{d}c_1/\mathrm{d}t} \tag{3}$$

where c_1^* is the equilibrium concentration of A.

Referring, now, to the kinetic model of this section, A represents a molecule in the mobile phase, and its equilibrium concentration is

$$c_1^* = \frac{k_2 c_2^*}{k_1} \tag{4}$$

where c_2^* is the concentration of B, and the stars denote the equilibrium. All concentrations are referred to a unit volume of the overall chromatogram. For this model, the relaxation time is found to be

$$t_r = 1/(k_1 + k_2) \tag{5}$$

Let us examine a small volume of carrier fluid as it passes along the chromatogram. We will assume that the concentration, c_1, in this element is near the equilibrium value, c_1^*. However, c_1^* varies as the volume element moves along the column. The concentration, c_1, lags behind c_1^*, since equilibration is not instantaneous. It is shown elsewhere[9] that the time lag is just the relaxation time for equilibrium, t_r. This is described by the equation

$$c_1(z,t) = c_1^*(z, t - t_r) \tag{6}$$

where $c_1(z,t)$ is the concentration in the moving volume element at the point z and the time t. In the discussion of the theoretical plate model, it will be shown that the concentration $c_1(z,t)$ may be considered equal to the equilibrium concentration, either at a time t_r past, as above, or at the same time but a distance $vt_r(1 - R)$ upstream.

$$c_1(z,t) = c_1^*(z - vt_r(1 - R), t) \tag{7}$$

From this equation it is necessary to find the equilibrium departure in the mobile phase, defined by $\Delta c_1 = c_1 - c_1^*$. (This is the negative of the equilibrium departure in the stationary phase, since $\Delta c_1 + \Delta c_2 = 0$.)

$$\begin{aligned} \Delta c_1 &= c_1^*(z - vt_r(1 - R), t) - c_1^*(z,t) \\ &= -\frac{\delta c_1}{\delta z} vt_r(1 - R) \end{aligned} \tag{8}$$

Both Δc_1 and $\delta c_1^*/\delta z$, of course, are evaluated at the distance z and time t. The latter relation holds for values of t_r, and hence Δc_1, that are small.

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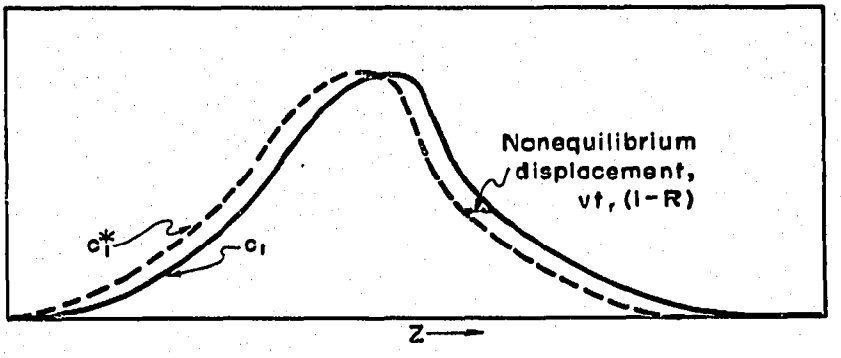

Fig. 1. Schematic diagram showing the shift of the actual mobile phase concentration, c_1, downstream from the equilibrium concentration, c_1^*.

Fig. 1 illustrates the application of this equation to a solute on a chromatographic column. The mobile phase moves in the direction, z, along the column. It is seen that the actual concentration, c_1, is displaced downstream from the equilibrium concentration, c_1^*, a distance, $vt_r(1-R)$, equivalent to a time, t_r. The figure shows how the departure from local equilibrium varies from one point to another on the column.

The mathematical expression of the equilibrium departure is particularly simple when the concentration profile is "Gaussian" or "normal". If we divide each side of equation (8) by c_1^* we get the fractional departure

$$\frac{\Delta c_1}{c_1^*} = -\frac{\delta \ln c_1^*}{\delta z} vt_r (1-R) \tag{9}$$

Since c_1^* is a definite fraction of the total concentration, it too is Gaussian.

$$c_1^* = \text{const. } e^{-\frac{(z-\bar{z})^2}{2\sigma^2}} \tag{10}$$

Whenever such a peak results from diffusion, the standard deviation and the diffusion coefficient are related by $\sigma^2 = 2Dt$. Using this and taking the derivative $\delta \ln c_1^*/\delta z$ we have

$$\frac{\Delta c_1}{c_1^*} = vt_r \frac{(1-R)(z-\bar{z})}{2Dt} \tag{11}$$

For the A $\rightleftarrows$ B model we may use D from equation (1), t_r from equation (5), and as the fraction of time spent in the mobile phase, $R = k_2/(k_1 + k_2)$. The resulting expression is

$$\frac{\Delta c_1}{c^*_1} = \frac{z-\bar{z}}{2Rvt} \tag{12}$$

the term Rv is the average velocity of the zone and this multiplied by the time t is the displacement of the zones center, $\bar{z}$. The final simple result is

$$\frac{\Delta c_1}{c_1^*} = \frac{z-\bar{z}}{2\bar{z}} \tag{13}$$

This shows the departure from equilibrium to be a function of the distance z along the chromatogram. At the center of the zone it approaches zero. It can also be shown that at a distance σ from the center of the zone, $z - \bar{z} = \sigma$,

$$\frac{\Delta c_1}{c_1^*} = \frac{1}{2\sqrt{N}} \tag{14}$$

where N is the theoretical plate number occupied by the center of the zone. The results given in the last two equations are valid with any kinetics as long as a t_r can be found for the system.

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It can now be easily shown why the spreading (diffusion) of a band is related to non-equilibrium values. At the center of the band, the departure from equilibrium is negligible. This portion of the band is displaced down the column with a velocity, $\bar{u} = Rv$. On the upstream (trailing) side of the band, the concentration is less than equilibrium. This deficiency in the mobile phase causes this part of the band to move more slowly than the center. On the other hand, the surplus concentration on the downstream (leading) side of the band causes this part to move more rapidly than the center. The net result is the spreading of the two edges away from the center.

For a quantitative formulation, we will refer our calculations to a point moving downstream with the average velocity of the band, $\bar{u} = Rv$. The flux of material through a unit area of a plane that is perpendicular to the flow direction and includes this point consists of contributions from all possible phases containing the solute,

$$q = \Sigma c_i u_i, \tag{15}$$

where c_i is the concentration in phase i, and u_i is the average velocity of this phase with respect to the reference point. For most cases it is ample to consider just a single mobile phase (phase 1), and a single stationary phase (phase 2). For the velocities in the last equation, we have $u_1 = v - \bar{u} = (1 - R)\, v$, and $u_2 = -\bar{u} = -Rv$. The flux, then, is

$$q = v\,[c_1\,(1 - R) - c_2 R] \tag{16}$$

and since $(c_1 + c_2)$ equals the total concentration, c

$$q = v\,(c_1 - Rc). \tag{17}$$

Using the definition of Δc_1 and the equilibrium expression, $c_1^* = Rc$, we have

$$q = v\Delta c_1 \tag{18}$$

At equilibrium, of course, $\Delta c_1 = 0$ and the net flux vanishes.

The value of Δc_1 in this equation for the net flux can be obtained from equation (8). Substitution yields

$$q = -\frac{\delta c_1^*}{\delta z}\, v^2 t_r\,(1 - R), \tag{19}$$

and since $c_1^* = Rc$, then

$$q = -\frac{\delta c}{\delta z}\, v^2\, t_r R\,(1 - R) = -D\,\frac{\delta c}{\delta z} \tag{20}$$

the latter relation results since D can be so defined in terms of the net flux, q. Hence a very general expression for the effective diffusion coefficient for this process is

$$D = v^2\, t_r R\,(1 - R) \tag{21}$$

This equation can be more specifically written in terms of the kinetic analog, (2). For this particular analog, $t_r = 1/(k_1 + k_2)$, and $R = k_2/(k_1 + k_2)$. With these substitutions, (21) becomes

$$D = \frac{k_1 k_2 v^2}{(k_1 + k_2)^3} \tag{22}$$

a result identical to that derived from the stochastic theory.

An approach similar to the above has been used in the description of boundary spreading in electrophoresis[15]. The mathematics of electrophoretic and chromatographic spreading are the same[13]. In either case an interconversion of species exists with the various species moving at different velocities. The interconversion reaction

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may be as simple as A $\rightleftarrows$ B or much more complicated for either electrophoresis or chromatography. When this simple reaction occurs in electrophoresis the effective D is the same as in equation (22) except that v_2 must be replaced by $(v_1 - v_2)^2$. The latter are the respective velocities of the ionic species. This result is not the same as that obtained in the work of FIELD AND OGSTON[15].

THE THEORETICAL-PLATE MODEL

The theoretical-plate model serves to relate chromatography to classical distillation procedures. For a given experiment under given conditions, the development of a chromatogram is related to an empirical parameter, (HETP), or the height equivalent to a theoretical plate. With a given column length, L, the number of theoretical plates, N, is simply obtained as $L/(\mathrm{HETP})$.

We will relate the parameters, (HETP) and N, directly to the kinetic parameters involved in the other theories. This, of course, is exceedingly useful, since the theoretical-plate model has had the disadvantage of not being related to fundamental physical quantities, such that, for instance, one could not find an expression for the temperature dependence of N even though it is known for the kinetic rates of the underlying processes.

The theoretical-plate model is obtained by dividing the chromatogram into discrete, adjacent cells. The length of a cell, (HETP), is determined by the condition that the mean concentration in the cell is in equilibrium with its own effluent. For small (HETP), the concentration at the midpoint of the cell is approximately equal to the mean concentration. Thus (HETP) is determined by the condition that the midpoint concentration is in equilibrium with the small volume element leaving the cell. However, the small volume element, according to equation (6), is in equilibrium with the point on the column crossed a time, t_r, previously. Since the flow velocity within the column is v, this equilibrium point is the distance vt_r upstream, and this must be the distance between the end of the cell and its midpoint

$$vt_r = \frac{(\mathrm{HETP})}{2} \tag{23}$$

Thus we have

$$(\mathrm{HETP}) = 2vt_r, \qquad N = t_1/2t_r \tag{24}$$

Where L/v is t_1, the passage time of the carrier through the chromatogram. This is the most general expression of the theoretical plate parameters in terms of kinetic processes. For the A $\rightleftarrows$ B kinetic model, equation (2), we have (see equation (5))

$$(\mathrm{HETP}) = 2v/(k_1 + k_2), \qquad N = (k_1 + k_2)\, t_1/2 \tag{25}$$

These equations can be easily verified. This is done simply by comparing the band half-width obtained by MAYER AND TOMPKINS[2], expressed in terms of N, to the half-width obtained in the use of the stochastic theory. For the A $\rightleftarrows$ B model, the result is identical to (25).

It has been tacitly assumed in the foregoing treatment that the volume element leaving a cell is in equilibrium with the midpoint cell concentration, not as it has been modified by incoming fluid, but as if it had remained constant as the volume

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element moved through the cell. Thus we have treated the MAYER AND TOMPKINS[2] discontinuous flow model of chromatography. The continuous-flow model, introduced by MARTIN AND SYNGE[1] in the original theoretical plate treatment of chromatography, is a better physical model. Our method for relating N to the kinetic rates involves, in this case, the additional concept of concentration displacement.

For a small downstream displacement of the mobile phase, say a distance vt_r, a given segment of the overall concentration profile will move downstream a distance $c_1 vt_r/(c_1 + c_2)$. Since the concentrations c_1 and c_2 are near their equilibrium values, the concentration displacement is approximately Rvt_r. The relaxation-time model states that the concentration of a given volume element is the equilibrium concentration of that volume element a time t_r previously. At that time the volume element was a distance vt_r upstream. However, as the volume element moved downstream from this point a distance vt_r, the overall concentration, which determines the equilibrium, moved the above quoted distance of Rvt_r. The distance between these displacements, $vt_r(1 - R)$, becomes, in this formulation, the distance from the midpoint to the end of the cell.

$$\frac{(\mathrm{HETP})}{2} = vt_r(1 - R) \tag{26}$$

In this case, then

$$(\mathrm{HETP}) = 2vt_r(1 - R), \qquad N = t_1/2t_r(1 - R) \tag{27}$$

and for the A $\rightleftarrows$ B model, with $t_r = 1/(k_1 + k_2)$ and $R = k_2/(k_1 + k_2)$, we have

$$(\mathrm{HETP}) = 2k_1 v/(k_1 + k_2)^2, \qquad N = (k_1 + k_2)^2 t_1/2k_1 \tag{28}$$

The latter expression results when the half-width for the continuous-flow model is compared to that from stochastic theory. We have used an expression derived by SAID[16] for this comparison.

DISCUSSION

The foregoing treatment relates both an effective diffusion coefficient and the number of theoretical plates to the kinetics of sorption and desorption. For practical purpose, it is necessary that these parameters be related to experimental procedure. This matter has been extensively discussed in the case of the number of theoretical plates[17].

It is common practice to inject a sample into a chromatogram with the least possible spread. In the limit we may consider all molecules started simultaneously in the column. When, in addition, near-equilibrium conditions prevail, the concentration profile becomes Gaussian, both when the component is spread as a band on the column, and in the column effluent. A measure of the diffusion effect is the standard deviation of this concentration profile. In general a procedure will become more valuable as the ratio of the standard deviation to the displacement from the origin (referred to the center of the band) becomes smaller[8]. With a component still on the column, the standard deviation is $\sigma = \sqrt{2Dt}$ (for any diffusion process), and the distance of the band from the origin is $\bar{z} = \bar{u}t = Rvt$. Substituting in the appropriate values from the kinetic analog, (2), we find that the dimensionless ratio, $\lambda = \sigma/\bar{z}$, is

$$\lambda = \left(\frac{2k_1}{k_2(k_1 + k_2)\,t}\right)^{1/2} \tag{29}$$

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In the case of elution, the standard deviation is usually measured in terms of time. The displacement from the origin, the elution time, t, is of the same dimensionality. For the kinetic analog, (2), the former is[5]

$$\sigma = (2k_1Rt)^{1/2}/k_2 = [2k_1t/k_2(k_1 + k_2)]^{1/2} \qquad (30)$$

and the ratio, $\lambda = \sigma/t$, is identical to (29).

The value of λ can be easily obtained for the theoretical-plate model. Substituting $t_1 = Rt$ into (28) and comparing with (29), we find, $\lambda = 1/\sqrt{N}$.

With the foregoing results, and the experimental values for both λ and R, it is easy to determine the kinetic rates of transition, k_1 and k_2. It is then possible to study the dependence of the transition rates upon temperature, solvent and sorbent properties, etc. Such a study shows promise of additional control over the movement of chromatographic zones.

SUMMARY

It has been the object of this presentation to show connecting links between the various theories of chromatography. The stochastic theory, the material-conservation approach, and the theoretical-plate model are treated individually and in relation to one another. The last two are treated as problems in non-equilibrium kinetics, exemplifying the concept that it is the lack of equilibrium between the mobile and stationary phases that causes the smearing of individual solute bands. The source of the non-equilibrium, as well as the smearing effect due to non-equilibrium, are discussed both qualitatively and quantitatively. The quantitative treatment of these cases depends on the use of the kinetic relaxation-time model, originally devised for the study of non-equilibrium kinetics in the flame front.

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