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NOTE

Can One Measure Rate Constants Using Chromatographic Methods?

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Abstract

It has recently been suggested that affinity chromotography can be used to measure rate constants from parameters determined from the elution curve. The analysis suggesting this possibility assumes that there is one type of reaction occurring and that steric factors can be ignored. In this paper a model is analyzed in which there can be a number of different binding sites. If one waits long enough, the resulting concentration profile tends toward a Gaussian form. This suggests that it would be experimentally very difficult to distinguish between single site and multiple site systems. Since steric factors undoubtedly operate, they would change the single site to the multiple site system. The implication of the analysis is that the proposed experiment does not necessarily measure the rate constants of interest.

Giddings and Eyring appear to have been the first to analyze a stochastic model for elution chromatography (*1*). Subsequent studies of equivalent or similar models were made by MacQuarrie (*2*) and Weiss (*3*). Continuum models somewhat similar to the stochastic theories were analyzed by Bak (*4*), van Holde (*5*), Cann, Kirkwood, and Brown (*6*), and undoubtedly many others. More recently Denizot and Delaage have given results for the original Giddings-Eyring model for the purpose of deriving kinetic parameters using affinity chromatography (*7*). Most of the authors cited above work with the two-state model in which the molecule being analyzed is assumed to be in one of two phases: mobile or stationary. MacQuarrie (*2*), Weiss (*3*), and Giddings (*8–10*), have also presented results where $n > 1$ different kinds of binding can take place. The experiment suggested by Denizot and Delaage suggests that kinetic parameters can be deter-

mined from the elution profile. An alternative technique which would be more accurate for analytic purposes is that of the scanning experiment rather than the elution method. Although scanning techniques have not been applied to affinity chromatography, they have been successfully developed for gel chromatography by Ackers and his collaborators (*11*).

Ideally, one would like to take steric factors into account in describing the motion of a molecule through the chromatographic column. This is clearly impossible in detail. Therefore, in order to take some account of these factors, a model will be adopted in which there are n different types of binding sites. In addition to allowing variations in the rate constants, the order of the reaction at each binding site will be allowed to be arbitrary, in contrast with earlier models that assume only first-order reactions. The variations in model parameters give a phenomenological representation of the effects of steric factors on the chromatographic kinetics. The customary assumption of first-order kinetics would seem to ignore steric factors related to gel geometry. A further assumption in the analysis is that molecules in the mobile phase do not necessarily travel at constant speed. Under these circumstances, if it is further assumed that successive sojourns in the mobile and stationary phases are for a finite average time, then when the number of binding events is large in the traversal of a column, the concentration profile for a single species tends toward the Gaussian form independent of the sojourn time distributions. With this simplification it becomes relatively easy to calculate the parameters determining the observed concentration profile and its development in time. It is assumed that the column has uniform properties, and that there are $n > 1$ different types of binding sites. As in the analyses cited above, it is assumed that the course of motion of a molecule through the column can be described as a succession of sojourns in the mobile and stationary phases, the time of each sojourn being a random variable. Let the probability that a single sojourn in the stationary phase will be between t and $t + dt$ and that termination occurs by binding to a site of type j be $\alpha_j(t)\,dt$. For example, if the binding events are first-order reactions with rate constants $k_1, k_2, \ldots, k_n$, then

$$\alpha_j(t) = k_j \exp\left[-(k_1 + k_2 + \cdots + k_n)t\right] \tag{1}$$

Notice that

$$\int_0^\infty \alpha_j(t)\,dt = f_j \tag{2}$$

where f_j is the probability of binding to a j-site. The probability density for a single sojourn on a j-site will be denoted by $\beta_j(t)$. For first-order processes with rate constants $k_1', k_2', \ldots, k_n'$, $\beta_j(t) = k_j' \exp(-k_j' t)$. The

probability that the displacement of a molecule in a single sojourn in the mobile phase lasting for a time t will be between x and $x + dx$ will be denoted by $g(x, t)\,dx$. When a molecule in the mobile phase is assumed to move at uniform speed v, as is the case in the Giddings-Eyring model, then

$$g(x, t) = \delta(x - vt) \tag{3}$$

where $\delta(u)$ is the Dirac delta function. When the displacement occurs by means of a combination of constant convection and diffusion,

$$g(x, t) = \frac{1}{\sqrt{4\pi Dt}} \exp\left[-\frac{(x - vt)^2}{4Dt}\right] \tag{4}$$

More generally, it will be assumed that the average displacement in time t will be proportional to time, i.e.,

$$\int_{-\infty}^{\infty} xg(x, t)\,dx = vt \tag{5}$$

and that the variance of displacement is also proportional to time,

$$\sigma_x^2(t) = \int_{-\infty}^{\infty} x^2 g(x, t)\,dx - v^2t^2 = 2Dt \tag{6}$$

The form of g used in Eq. (3) leads to $\sigma^2(t) = 0$ while that in Eq. (4) allows us to interpret D as a diffusion constant.

The idea behind the following calculation is straightforward. The total displacement at time t can be written

$$x(t) = x_1 + x_2 + \cdots + x_{j(t)} + y(t) \tag{7}$$

where x_m is the displacement in the mth completed sojourn in the mobile phase (which starts at the time at which the molecule enters the mobile phase for the mth time, and ends at the immediately following trapping), $j(t)$ is the total number of complete cycles (a sojourn in the mobile phase followed by a sojourn in a stationary phase), and $y(t)$ is the displacement in any uncompleted cycle. Let us assume that the sojourn times in both the mobile phase and in the stationary phases have finite first and second moments. This is a plausible assumption in the context of chromatography, but is not necessarily valid for hopping transport in solids (*12–15*). The process defined in Eq. (7) is a cumulative process in the sense of Smith (*16–18*), so that we may adapt results that have been proved by him and others (*19*). Let t denote the average time spent in a complete cycle, let $\sigma^2(t)$ denote the variance of this time, let x be the average displacement in a single cycle, let $\sigma^2(x)$ be the variance of that displacement, and let cov (x, t) be the covariance of displacement and time. These quantities

will be calculated from the underlying model parameters after the results implied by Smith's analysis have been stated.

The results to be given are valid in the limit of a large number of trappings. More precisely, they are valid when $t/\bar{t} \gg 1$ where t is the average cycle time. Smith has shown that the first two moments of $x(t)$ are, asymptotically,

$$\overline{x(t)} \sim \frac{\bar{x}}{\bar{t}} t$$

$$\sigma^2(x(t)) \sim \left\{\sigma^2(x) + \sigma^2(t)\frac{\bar{x}^2}{\bar{t}^2} - 2\frac{\bar{x}}{\bar{t}}\operatorname{cov}(x, t)\right\}\frac{t}{\bar{t}} \tag{8}$$

plus terms that are small with respect to $t/\bar{t}$. Furthermore, $x(t)$ has an asymptotic Gaussian distribution with parameters given in this last equation. We therefore know what to expect on a macroscopic level. Next we must translate the parameters of the microscopic model into those appearing in Eq. (8). If the probability density for the time in a complete cycle is denoted by $h(t)$, then, by our definitions,

$$h(t) = \sum_{j=1}^{n} \int_0^t \alpha_j(\tau)\beta_j(t - \tau)\, d\tau \tag{9}$$

The joint density for the displacement in a single cycle, x, and the cycle time is

$$f(x, t) = \sum_{j=1}^{n} \int_0^t g(x, \tau)\alpha_j(\tau)\beta_j(t - \tau)\, d\tau \tag{10}$$

since motion only occurs in the mobile phase. These last two formulas enable us to calculate the parameters that appear in Eq. (8). If we define moments of the components of cycle time as

$$\int_0^\infty t^n\alpha_j(t)\, dt = f_j\overline{T_j^n}, \qquad \int_0^\infty t^n\beta_j(t)\, dt = \overline{(T_j')^n} \tag{11}$$

then

$$\bar{t} = \sum_{j=1}^{n} f_j(\overline{T_j} + \overline{T_j'}), \qquad \sigma^2(t) = \sum_{j=1}^{n} f_j(\overline{T_j} + \overline{T_j'})^2 - \bar{t}^2 \tag{12}$$

It is convenient to define the following moments averaged over all types of cycles:

$$\overline{T^n} = \sum_j f_j\overline{T_j^n}, \qquad \overline{(T')^n} = \sum_j f_j\overline{(T_j')^n}, \qquad \overline{TT'} = \sum_j f_j\overline{T_j}\,\overline{T_j'} \tag{13}$$

in terms of which Eq. (12) can be rewritten

$$\bar{t} = \overline{T} + \overline{T'}, \qquad \sigma^2(t) = \sigma^2(T) + \sigma^2(T') \tag{14}$$

The calculation of moments of displacement is just slightly more

complicated. Let us, for example, consider the evaluation of the expression for x:

$$\bar{x} = \int_0^\infty dt \int_0^\infty xf(x, t)\, dx = v \sum_{j=1}^{n} \int_0^\infty dt \int_0^t \tau\alpha_j(\tau)\beta_j(t - \tau)\, d\tau \quad (15)$$

This latter expression can be written in terms of Laplace transforms evaluated at $s = 0$. It we set

$$\alpha_j^*(s) = \int_0^\infty e^{-st}\alpha_j(t)\, dt, \qquad \beta_j^*(s) = \int_0^\infty e^{-st}\beta_j(t)\, dt \quad (16)$$

then $\bar{x}$ takes the form

$$\bar{x} = -v \sum_{j=1}^{n} \left(\beta_j^*(s) \frac{d\alpha_j^*}{ds}\right)\Bigg|_{s=0} = v\overline{T} \quad (17)$$

since $\beta_j^*(0) = 1$. This result together with Eq. (15) implies

$$\bar{x}(t) \sim \frac{v\overline{T}}{\overline{T} + \overline{T'}}\, t = \theta v t \quad (18)$$

where $0 \leq \theta \leq 1$. This intuitively reasonable result indicates that the motion of the peak is slowed up by time spent in the trapped state. The calculation of $\sigma^2(x(t))$ is only slightly more complicated and leads to the expression

$$\sigma^2(x(t)) \sim \{2D\overline{T} + v^2\overline{[(1 - \theta)T - \theta T']^2}\}(t/\bar{t}) \quad (19)$$

where θ is defined in Eq. (18). We see that there are two contributions to the variance, the first being that due to diffusion and the second being that due to trapping processes that occur as the column is being traversed. Furthermore, the parameters appear only in the form of lumped moments, $\overline{T}$, $\overline{T'}$, $\overline{T^2}$, $\overline{(T')^2}$ and $\overline{T'T}$, so that contributions from the different sites cannot be distinguished in the Gaussian limit.

As is the case in elution chromatography, it would be experimentally impossible to distinguish between single and multiple site trapping without starting from a detailed theory of the transient processes. Thus it would appear that unless one could demonstrate independently of the chromatographic experiment that the single site model is an adequate description of what is going on in the column, and that steric factors are negligible, it would be unwise to use affinity chromatography to measure rate constants as suggested by Denizot and Delaage. The quantities measured in this way would be a complicated combination of many factors involving both local geometry and the rate constants.

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