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George H. Weiss[a]; Menachem Dishon[b]
[a] PHYSICAL SCIENCES LABORATORY DIVISION OF COMPUTER RESEARCH AND TECHNOLOGY NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND [b] DEPARTMENT OF APPLIED MATHEMATICS, WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL

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Resolution in Nonuniform Chromatographic Systems

GEORGE H. WEISS

PHYSICAL SCIENCES LABORATORY
DIVISION OF COMPUTER RESEARCH AND TECHNOLOGY
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

MENACHEM DISHON

DEPARTMENT OF APPLIED MATHEMATICS
WEIZMANN INSTITUTE OF SCIENCE
REHOVOTH, ISRAEL

Abstract

The resolving power of a chromatographic column is commonly expressed in terms of the peak positions and standard deviations of the two peaks to be separated. Calculation of the resolution for Gaussian peaks is straightforward. When the column properties vary with position, the peaks are no longer generally Gaussian. We use a recently developed theory to calculate approximations to moments of the peak when diffusion is a relatively small effect, and thereby calculate an expression for the resolving power of the system.

We have recently presented a technique for calculating concentration profiles in nonuniform chemical separation systems, exemplified by pore gradient electrophoresis, density gradient centrifugation, GPC with varying gel density, and isoelectric focusing (*1–3*). In general, the concentration profile is not Gaussian for these systems, and the form of the moments defined by these profiles is not readily evident from the results that have been presented in Ref. *1*. The moments are of some general interest because they appear in the expression for resolution. It is the purpose of this note to calculate approximate expressions for the moments and for the resolving power of a nonuniform separation column. As in our earlier work, the basic assumptions are (a) that diffusion is a secondary effect relative to convection; more specifically, the peak width of an initial pulse is small compared to column length, and (b) that neither the diffusion function or the convection function are concentration dependent.

Let the column length be L; let $x = Ly$ be the spatial coordinate, so that y is dimensionless with $0 \leqslant y \leqslant 1$, let the diffusion function be expressed as $D_1 f(y)$, where $f(y)$ is dimensionless; and let the convective term be denoted $v_1 g(y)$, where $g(y)$ is likewise dimensionless. By convention we can choose v_1 and D_1 so that $f(0) = g(0) = 1$. Then a transport equation can be written for the concentration, $c(y, t)$

$$\frac{\partial c}{\partial t} = \frac{D_1}{L^2}\frac{\partial}{\partial y}\left[f(y)\frac{\partial c}{\partial y}\right] - \frac{v_1}{L}\frac{\partial}{\partial y}[g(y)c] \tag{1}$$

or, using the dimensionless variables,

$$\varepsilon = D_1/(v_1 L), \qquad \tau = v_1 t/L \tag{2}$$

we have

$$\frac{\partial c}{\partial \tau} = \varepsilon\frac{\partial}{\partial y}\left[f(y)\frac{\partial c}{\partial y}\right] - \frac{\partial}{\partial y}[g(y)c] \tag{3}$$

The parameter ε is assumed to be small in what follows; for most of the systems mentioned in the first paragraph ε is 10^{-2} or smaller. The functions $f(y)$ and $g(y)$ are assumed to be of the order of 1, insuring that $\varepsilon f(y) \ll |g(y)|$ for systems that do not come to equilibrium within the column. Although y lies between 0 and 1, we neglect boundary effects, a procedure that is allowable on physical grounds for most analytic techniques, but not necessarily for elution techniques. The assumption simplifies the mathematics considerably.

Define a running space coordinate ζ by

$$\zeta = \int_0^y \frac{du}{g(u)} - \tau \tag{4}$$

In the absence of diffusion an initial pulse at $y = 0$ will propagate along $\zeta = 0$. When diffusion is a small effect, only small $|\zeta|$ will be important. Let the solution to Eq. (4) for y in terms of ζ and τ be $y = H(\zeta + \tau)$ so that when $\varepsilon = 0$ the trajectory of a peak initially at $y = 0$ is $y = H(\tau)$. If $\psi(y,\tau)$ is defined to be $g(y)c(y,\tau)$, then it has been shown in Ref. 1 that $\psi(y,\tau)$ can be expanded as

$$\psi(y,\tau) = \psi_0(y,\tau) + \varepsilon^{1/2}\psi_1(y,\tau) + \varepsilon\psi_2(y,\tau) + \cdots \tag{5}$$

and the first two terms of this series were calculated explicitly for a pulse initially at $y = 0$. Moments of concentration are defined by

$$\mu_n(\tau) = \int_{-\infty}^{\infty} \frac{y^n}{g(y)}\,\psi(y,\tau)\,dy \bigg/ \int_{-\infty}^{\infty} \psi(y,\tau)\frac{dy}{g(y)} \tag{6}$$

where, in accordance with the premise that boundary effects are negligible, the limits of integration have been extended to $\pm\infty$. Noticing that

$$dy/g(y) = d\zeta \tag{7}$$

from Eq. (4), we can rewrite Eq. (6) as

$$\mu_n(\tau) = \int_{-\infty}^{\infty} H^n(\zeta + \tau)\psi(\zeta,\tau)\,d\zeta \tag{8}$$

in which we have chosen the units of concentration so that

$$\int_{-\infty}^{\infty} c(x,t)\,dx = 1$$

The calculation proceeds by expanding $H(\zeta + \tau)$ in a Taylor series around $\zeta = 0$, so that, for example

$$\mu_1(\tau) = \int_{-\infty}^{\infty} \left[H(\tau) + \zeta H'(\tau) + \frac{\zeta^2}{2} H''(\tau) + \cdots\right] \cdot [\psi_0(\zeta,\tau) + \varepsilon^{1/2}\psi_1(\zeta,\tau) + \varepsilon\psi_2(\zeta,\tau) + \cdots]\,d\zeta \tag{9}$$

We retain all terms up to those proportional to ε and neglect those proportional to higher powers of ε. This allows us to truncate the series for ψ at the term $\varepsilon\psi_2$, and the series for $H(\zeta + \tau)$ at $\zeta^2 H''(\tau)/2$. For an initial loading symmetric around $\zeta = 0$ we can eliminate a certain number of the remaining terms by symmetry. If we define the functions $F(u)$ and $G(u)$ in terms of $f(u)$ and $g(u)$ by

$$F(u) = f[H(u)], \quad G(u) = g[H(u)] \tag{10}$$

and further define

$$
\begin{aligned}
A(u) &= F(u)/G^2(u) \\
B(u) &= A'(u) - [A(u)/G(u)]G'(u) \\
C(u) &= -\frac{d}{du}\left[\frac{A(u)}{G(u)}\frac{dG}{du}\right]
\end{aligned}
\tag{11}
$$

then it has been shown that the equations for ψ_0, ψ_1, and ψ_2 are

$$\frac{\partial\psi_0}{\partial\tau} - \varepsilon A(\tau)\frac{\partial^2\psi_0}{\partial\zeta^2} = 0 \tag{12a}$$

$$\frac{\partial\psi_1}{\partial\tau} - \varepsilon A(\tau)\frac{\partial^2\psi_1}{\partial\zeta^2} = \varepsilon^{1/2}\zeta A'(\tau)\frac{\partial^2\psi_0}{\partial\zeta^2} + \varepsilon^{1/2}B(\tau)\frac{\partial\psi_0}{\partial\zeta} \tag{12b}$$

$$
\begin{aligned}
\frac{\partial\psi_2}{\partial\tau} - \varepsilon A(\tau)\frac{\partial^2\psi_2}{\partial\zeta^2} &= \frac{\zeta^2}{2}A''(\tau)\frac{\partial^2\psi_0}{\partial\zeta^2} + \zeta B'(\tau)\frac{\partial\psi_0}{\partial\zeta} + C(\tau)\psi_0 \\
&\quad + \varepsilon^{1/2}A'(\tau)\zeta\frac{\partial^2\psi_1}{\partial\zeta^2} + \varepsilon^{1/2}B(\tau)\frac{\partial\psi_1}{\partial\zeta}
\end{aligned}
\tag{12c}
$$

Let us consider the case of an initial pulse loading, $c(x, 0) = \delta(x)$, where $\delta(x)$ is a Dirac delta function. The solution for $\psi_0(\zeta,\tau)$ has been shown to be

$$\psi_0(\zeta,\tau) = [4\pi\varepsilon\Delta(\tau)]^{-1/2}\exp\left[-\frac{\zeta^2}{4\varepsilon\Delta(\tau)}\right]$$

where

$$\Delta(\tau) = \int_0^\tau A(u)\,du,$$

that is, $\psi_0(\zeta,\tau)$ is symmetric around $\zeta = 0$ and

$$\psi_0(\pm\infty,\tau) = \frac{\partial}{\partial\zeta}\psi_0(\zeta,\tau)\bigg|_{\zeta=\pm\infty} = 0 \tag{13}$$

It is easy to verify from Eq. (12) that these facts imply that $\psi_1(\zeta, \tau)$ is an odd function and $\psi_2(\zeta, \tau)$ is even, and that the property of Eq. (13) holds for these functions as well. Thus we can write for $\mu_1(\tau)$:

$$\mu_1(\tau) = H(\tau) + H''(\tau)\frac{a(\tau)}{2} + \varepsilon^{1/2}H'(\tau)b(\tau) + \varepsilon H(\tau)\rho(\tau) + \cdots \tag{14}$$

in which

$$\begin{aligned} a(\tau) &= \int_{-\infty}^{\infty} \zeta^2 \psi_0(\zeta,\tau)\, d\zeta \\ b(\tau) &= \int_{-\infty}^{\infty} \zeta \psi_1(\zeta,\tau)\, d\zeta \\ \rho(\tau) &= \int_{-\infty}^{\infty} \psi_2(\zeta,\tau)\, d\zeta \end{aligned} \tag{15}$$

While it is possible to calculate explicit expressions for the ψ's, a simpler procedure for calculating the quantities $a(\tau)$, $b(\tau)$, and $\rho(\tau)$ is to multiply Eq. (12) by the appropriate power of ζ and perform the integrations. If we follow this prescription for $a(\tau)$, for example, we find, by integrating by parts, using Eq. (12a), that

$$\begin{aligned} a'(\tau) = \varepsilon A(\tau) \int_{-\infty}^{\infty} \frac{\zeta^2 \partial^2 \psi_0(\zeta,\tau)}{\partial \zeta^2}\, d\zeta &= 2\varepsilon A(\tau) \int_{-\infty}^{\infty} \psi_0(\zeta,\tau)\, d\zeta \\ &= 2\varepsilon A(\tau) \end{aligned} \tag{16}$$

or since $a(0) = 0$

$$a(\tau) = 2\varepsilon \Delta(\tau) \tag{17}$$

In similar fashion we find

$$\begin{aligned} b(\tau) &= 2\varepsilon^{1/2}[A(\tau) - A(0)] - \sqrt{\varepsilon} \int_0^\tau B(u)\, du \\ \rho(\tau) &= 0 \end{aligned} \tag{18}$$

so that

$$\begin{aligned} \mu_1(\tau) = H(\tau) + \Big\{ \Delta(\tau)H''(\tau) &+ 2[A(\tau) - A(0)]H'(\tau) \\ &- \int_0^\tau B(u)\, du\, H'(\tau) \Big\} \varepsilon + \cdots \end{aligned} \tag{19}$$

The remaining terms in the series are proportional to ε^2 or higher powers of ε. In a similar fashion we can calculate the variance σ^2 as

$$\begin{aligned} \sigma^2(\tau) = \mu_2(\tau) - \mu_1{}^2(\tau) &= 2\varepsilon\Delta(\tau)[H'(\tau)]^2 + \cdots \\ &= 2\varepsilon\Delta(\tau)G^2(\tau) + \cdots \end{aligned} \tag{20}$$

In general the expansion of $\mu_r(\tau)$ up to terms proportional to ε can be shown by the same methods to be

$$\mu_r(\tau) = H^r(\tau) + \left\{ rH^{r-1}(\tau)H'(\tau)\left[2A(\tau) - 2A(0) - \int_0^\tau B(u)\,du\right] \right.$$
$$\left. + r\Delta(\tau)[(r-1)H^{r-2}(\tau)(H'(\tau))^2 + H''(\tau)H^{r-1}(\tau)] \right\} \varepsilon + \cdots \qquad (21)$$

For example, in pore gradient electrophoresis the experimental data (4) indicate that to a good approximation we can choose

$$f(y) = \exp(-\lambda\alpha y); \qquad g(y) = \exp(-\lambda y) \qquad (22)$$

in which λ is a dimensionless constant and for which

$$H(\tau) = \frac{1}{\lambda}\ln(1 + \lambda\tau)$$
$$\Delta(\tau) = \frac{1}{\lambda(3-\alpha)}[(1 + \lambda\tau)^{3-\alpha} - 1] \qquad (23)$$

Thus, in the limit of large time, $\sigma^2(\tau)$ increases monotonically for $\alpha < 1$, approaches a limit for $\alpha = 1$, and tends to zero for $\alpha > 1$. The detailed calculations of concentration profiles given in Ref. 2 for $\alpha = 1$ do appear to indicate that the width of the peaks remain constant after the initial broadening.

The moments μ_1 and σ are the parameters required to study resolution, conventionally defined as

$$R = \frac{1}{2}\left(\frac{\mu_2 - \mu_1}{\sigma_2 + \sigma_1}\right) \qquad (24)$$

where the subscripts refer to the different proteins. We have calculated values of the resolution for a nonuniform column, using the model of pore gradient electrophoresis for which the relevant functions are enumerated in the last paragraph. We will specifically assume, in what follows, that the mobilities of the two species differ, but that the two diffusion constants are equal:

$$v_2 = \theta v_1, \qquad D_1 = D_2 = D, \qquad \lambda_1 = \lambda_2 \qquad (25)$$

so that if we define $\varepsilon = D/(v_1 L)$, then $\varepsilon_1 = \varepsilon$ and $\varepsilon_2 = \varepsilon/\theta$. The expression for resolution now becomes

$$R = \frac{1}{(8\varepsilon)^{1/2}} \frac{\ln\left(\dfrac{1 + \theta\lambda\tau}{1 + \lambda\tau}\right)}{\dfrac{[\Delta(\tau)]^{1/2}}{1 + \lambda\tau} + \left(\dfrac{\Delta(\theta\tau)}{\theta}\right)^{1/2} \dfrac{1}{1 + \theta\lambda\tau}} \qquad (26)$$

neglecting terms proportional to $\varepsilon^{1/2}$ (this amounts to ignoring terms proportional to ε relative to 1). The resolution was calculated as a function of τ for $\lambda = 0.4$, a typical value, and for different values of α. We compared the results to

$$R = \frac{1}{(8\varepsilon)^{1/2}} \frac{(\theta - 1)}{2} \left(\frac{y}{\theta}\right)^{1/2} \tag{27}$$

obtained for a uniform column. For $\alpha = 0.5$, 1, and 2, the results obtained indicate that the resolution with the nonuniform column would be experimentally indistinguishable from that of the uniform column. For $\alpha = 0.5$, the ratio of resolution obtained with a nonuniform column to that obtained with a uniform column lies between 0.95 (at $y = 1$) to 1 at $y = 0$. When $\alpha = 2$ the ratio lies between 1.10 and 1. If the resolution is calculated as a function of time rather than of space, the uniform column tends to give better values of resolution for comparable sets of parameters. These conclusions are valid for the spatial dependence characteristic of pore gradient electrophoresis and for the separation of two macromolecules. When one wishes to separate more than two macromolecules, these conclusions are not necessarily valid.

Other quantities that are of some possible interest and can be expressed in terms of moments relate to skewness (*5*). Our expressions for the moments in Eqs. (19)–(21) presuppose an initial pulse loading. The calculations have to be redone *ab initio* for any alternate initial condition. The present theory and that of Ref. *1* cannot be applied directly to elution chromatography because it neglects boundary effects, but a parallel theory is presently under development for that case.

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