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Resolution in Time of Two Electrophoretic Peaks

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ABSTRACT

The question of whether a gel gradient can improve the resolvability of two peaks by means of gel electrophoresis is studied. The criterion used is the time to a specific resolution, defined as the time to the appearance of a minimum in the concentration profile between the peaks. Although the analysis is phrased in terms of gel electrophoresis, it is more generally applicable to other separation techniques.

INTRODUCTION

Most measures of the degree of resolution of two peaks in chromatographic or electrophoretic separation systems combine the effects of mobility and band spreading in the definition of a single resolution parameter. One commonly used parameter is

$$R = \Delta X/(\sigma_1 + \sigma_2) \tag{1}$$

where ΔX is the peak separation, and σ_i the width of peak i, $i = 1, 2$. The resolution parameter can be used to optimize the design of columns for separating components of a given mixture by presetting the desired value of R. The use of R as just defined is deficient in not accounting for the possibility of unequal peak heights.

A particularly important set of applications of electrophoretic analysis is to the separation of DNA fragments with different numbers of base pairs. In a recent paper Aldroubi and Garner suggested a resolution parameter for such applications which essentially measures the time to the appearance of a minimum between two peaks in the observed concentration profile (1). The theory in their analysis requires that the polymer or gel concentration be uniform. In the absence of possible nonlinear effects, this implies that the shape of each peak remains Gaussian at all times. This assumption permits one to calculate a resolution parameter, R_p, which depends on the two mobilities, the bandwidths and relative concentrations of the two species. As well as answering an important design question related to a widely used separation technique, the Aldroubi–Garner approach also overcomes the difficulty inherent in Eq. (1) in not including the concentrations.

In the present paper we extend the Aldroubi–Garner analysis to deal with the time to resolve two peaks in a nonuniform gel, in which the diffusion coefficient of species $i(= 1, 2)$ is $D_i(x)$ and the velocity parameter is $v_i(x)$. While it is not usually possible to find an exact solution to the equations describing peak spreading and migration in such gels, it is possible to find quite accurate approximations to the solutions when diffusive effects are much smaller than those of migration. This is generally the case in electrophoretic systems, and especially so in gel electrophoresis.

DERIVATION OF AN APPROXIMATE SOLUTION

We make explicit the assumption of small diffusive effects by expressing the diffusion coefficient as $\epsilon D_i(x)$ where $D_i(x)$ gives the analytic form of the diffusion coefficient and ϵ is a small dimensionless parameter. A basic assumption in our analysis is that $D_i(x)$ and $v_i(x)$ are approximately of the same order of magnitude and that ϵ sets the scale for a perturbation analysis. Let $c_i(x,t)$ denote the concentration of species i at x at time t. These functions satisfy the equations

$$\frac{\partial c_i}{\partial t} = \epsilon \frac{\partial}{\partial x}\left[D_i(x)\frac{\partial c_i}{\partial x}\right] - \frac{\partial}{\partial x}[v_i(x)c_i], \qquad i = 1, 2 \tag{2}$$

In electrophoretic measurements the velocity $v_i(x)$ is the product of the mobility and the applied electrical field. An approximate solution to Eq. (2) can be found when the initial conditions are

$$c_1(x,0) = c_1\delta(x),\ c_2(x,0) = c_2\delta(x) \tag{3}$$

in which c_1 and c_2 are constants which measure the relative amount of each species. The set of initial conditions in Eq. (3) is equivalent to the one used in the analysis by Aldroubi and Garner in Ref. 1.

In practical systems the order of magnitude of the dimensionless parameter ϵ is of the order of 10^{-3} or less. This permits the use of a singular perturbation expansion for the solution, of which only the lowest order term will be considered. The perturbation expansion to be used will be based on one developed by van Kampen (2–4), although there are other approaches available as mentioned in Ref. 4. In any electrophoretic measurement of a two-peak system, the observed concentration at a point is equal to $c(x,t)$, which is the sum

$$c(x,t) = c_1(x,t) + c_2(x,t) \tag{4}$$

We seek an estimate of the time at which a minimum first appears between the two peaks. Because of the difficulty in solving Eq. (2), this can only be done approximately.

The method suggested by van Kampen for approximating to the solution of Eq. (2) is to change the spatial variable to a new one based on the solution to the diffusion-free approximation to that equation, i.e., one sets $\epsilon = 0$. In following this approach we let $X_i(t)$ be the solution to the equation

$$\dot{X} = v_i(X), \qquad i = 1, 2 \tag{5}$$

and define rescaled spatial variables y_i by

$$y_i = \frac{x - X_i(t)}{\sqrt{\epsilon}}, \qquad i = 1, 2 \tag{6}$$

The introduction of these variables has the effect of eliminating the parameter ϵ from the first term on the right-hand side of Eq. (2). The lowest order of approximation to Eq. (2) is then found to be (2)

$$\frac{\partial c_i}{\partial t} = \mathcal{D}_i(t)\frac{\partial^2 c_i}{\partial y_i^2} - w_i(t)\frac{\partial(y_i c_i)}{\partial y_i}, \qquad i = 1, 2 \tag{7}$$

in which

$$\mathcal{D}_i(t) = D[X_i(t)],\ w_i(t) = \left.\frac{dv_i}{dx}\right|_{x = X_i(t)} \tag{8}$$

Equation (7) is an Ornstein–Uhlenbeck equation which can be solved by taking a Fourier transform with respect to y_i. A derivation of the solution to Eq. (7) is given in Ref. 4. The result is

$$c_i(x,t) = \frac{c_i}{\sigma_i(t)\sqrt{2\pi\epsilon}} \exp\left[-\frac{(x - X_i(t))^2}{2\epsilon\sigma_i^2(t)}\right] \tag{9}$$

which indicates that the broadening around the diffusion-free solution at sufficiently early times is approximately described by a Gaussian. In the lowest order the maximum concentration is located at a position found by neglecting diffusive effects, i.e., it is at $X_i(t)$. The time-dependent function that describes the development of peak broadening is found from

$$\sigma_i^2(t) = 2\int_0^t \mathcal{D}_i(\tau) \exp\left(2\int_\tau^t w_i(\xi)d\xi\right) d\tau \tag{10}$$

Thus we see that the basic shape of the peak is Gaussian, at least at sufficiently short times. At longer times the peak shape will change to a form which is specific to the form of the gel gradient. A more involved analysis is required to calculate correction terms to Eq. (9) but we here deal only with consequences implied by the Gaussian term in Eq. (9). The important feature that emerges from the analysis is that both the position of the peak, $X_i(t)$, and $\sigma_i^2(t)$ are, in general, no longer proportional to t, as is the case for a uniform gel. This allows for the possibility that a slowing in the motion of the peak due to the gel gradient might be compensated for by a decrease in the peak width. It is this issue which will be addressed in the following section, in the context of a particular example.

IMPLICATIONS OF THE SOLUTION FOR RESOLVING POWER

A generic figure indicating the form of the total concentration profile at a given time is shown in Fig. 1, with $h_{\min}(t)$ being the concentration at the interpeak minimum and $H_{\max}(t)$ the concentration of the lower of the two peaks. At this point we describe our generalization of the analysis of Aldroubi and Garner which is made possible by the fact that Eq. (9) is approximately a Gaussian, just as in the absence of a gradient. The first step in the analysis is to define the function $\rho(t) = H_{\max}(t)/h_{\min}(t)$ which measures the extent of resolution. Two peaks will be said to be resolved at time t if $\rho(t) > 1$. We further define $\sigma_{\max}(t)$ to be the larger of $\sigma_1(t)$ and $\sigma_2(t)$, and the function α as the smaller of the two ratios c_1/c_2 and c_2/c_1. Finally, we define the resolution function $R(t)$ by

$$R(t) = \frac{\alpha}{2} \exp\left[\frac{1}{8}\left(\frac{X_1(t) - X_2(t)}{\sigma_{\max}(t)}\right)^2\right] \tag{11}$$

Aldroubi and Garner have shown that $R(t)$ is a lower bound for $\rho(t)$, which

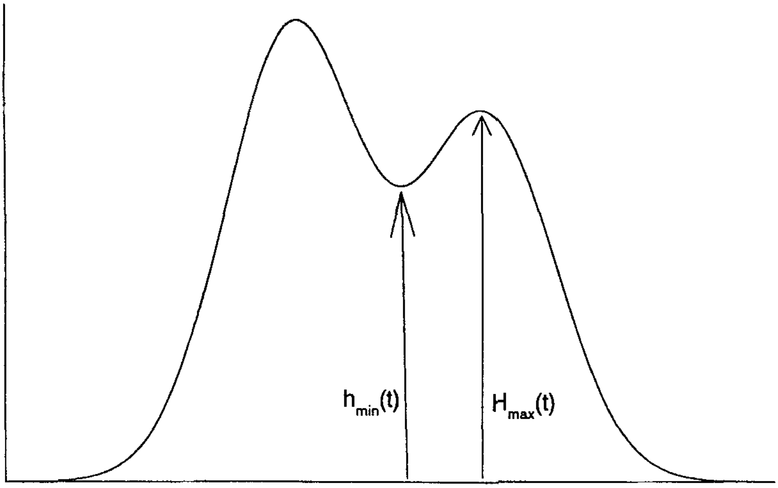

FIG. 1 A concentration profile with two peaks, showing the definition of $h_{min}(t)$ and $H_{max}(t)$.

is to say that

$$\rho(t) \geq R(t) \tag{12}$$

The desired resolution occurs at a time t_r which is found as the solution to the equation

$$R(t_r) = 1 \tag{13}$$

The results found by Aldroubi and Garner correspond to setting $X_i(x) = \mu_i Et$, where μ_i is a constant mobility, E is the field strength, and at the same time setting $\sigma^2_{max}(t) = 2D_{max}t$, where D_{max} is the greater of the two diffusion constants D_1 and D_2.

As an example of our formalism applied to a case which is not otherwise generally solvable, we examine the implications of a linear gel gradient for which the analytic forms for the diffusion and velocity functions are

$$D_i(x) = D_i \exp(-\lambda_i x) \quad \text{and} \quad v_i(x) = \mu_i E \exp(-\eta_i x), \quad i = 1, 2 \tag{14}$$

where D_i and μ_i are the diffusion constants and mobilities at zero gel concentration, respectively, E is the applied field, and λ_i and η_i characterize the effect of the gradient. The results of Aldroubi and Garner are recovered by setting $\lambda_i = \eta_i = 0$. The model defined by Eq. (14) generalizes an *ansatz* made by Rodbard and Chrambach (5), although an additional assumption was made that $\lambda_i = \eta_i$. An exact solution to Eq. (2) can be found for that special case (6).

Two steps are required in the application of our formalism. The first is to calculate the functions $X_1(t)$ and $X_2(t)$ that appear in Eq. (11), and the second is to calculate $\sigma_{\max}(t)$. Equation (5) for the choice of $v_i(x)$ in Eq. (14) is

$$\dot{X} = \mu_i E \exp(-\eta_i X) \tag{15}$$

This is readily solved for the $X_i(t)$:

$$X_i(t) = \frac{1}{\eta_i} \ln(1 + \mu_i E \eta_i t) \tag{16}$$

The next step is to calculate the quantities $\mathscr{D}_i(\tau)$ and $w_i(t)$ that appear in the integrand of the expression for $\sigma_i^2(t)$ as indicated in Eq. (10). In the present model these are found to have the forms

$$\mathscr{D}_i(\tau) = \frac{D_i}{(1 + \mu_i E \eta_i t)^{\lambda_i/\eta_i}}, \qquad w_i(\xi) = -\frac{\mu_i E \eta_i}{1 + \mu_i E \eta_i t} \tag{17}$$

These are sufficiently simple so that the integral in Eq. (10) can be evaluated in closed form. Let $m_i = \lambda_i/\eta_i$. The expression for $\sigma_i^2(t)$ is then found to be

$$\sigma_i^2(t) = \frac{2D_i}{(m_i - 1)\mu_i E \eta_i (1 + \mu_i E \eta_i t)^2} \left\{ 1 - \frac{1}{(1 + \mu_i E \eta_i t)^{m_i - 1}} \right\} \tag{18}$$

It is easily verified that in the limit $\mu_i E \eta_i t \to 0$, this reduces to the standard result $\sigma_i^2(t) = 2D_i t$.

The results in Eqs. (16) and (18) are useful in suggesting that there are choices of parameters for which the use of a gel gradient for the model defined by Eq. (14) can yield a better resolution than that found in the absence of a gradient. For this purpose we must compare values of the resolution function $R(t)$ both with and without the gradient, since, as is shown in Ref. 1, the separation time is proportional to the logarithm of the resolution factor. A further consideration is that one can only assume that the theoretical results will be valid at short times (in dimensionless units) since the van Kampen approximation is only accurate at short times (4). Consequently we first examine the behavior of the exponent that appears in Eq. (11) in the short-time limit. For definiteness we will take $\sigma_{\max}(t) = \sigma_1(t)$ and define a dimensionless time τ by

$$\tau = \mu_1 E \eta_1 t \tag{19}$$

so that by short times we will mean that τ is small in comparison to 1. Hence the term that appears in the numerator of the exponent in Eq. (11) is

$$[X_1(t) - X_2(t)]^2 \approx \left(1 - \frac{\mu_2}{\mu_1}\right)^2 \frac{\tau^2}{\eta_1^2}\left[1 - \frac{\mu_1^2\eta_1 - \mu_1^2\eta_2}{\mu_1\eta_1(\mu_1 - \mu_2)}\tau + \cdots\right] \tag{20}$$

to lowest order in τ. The term outside of the brackets is the diffusion-free result, and the bracketed terms are corrections to this result due to the nonuniform gel gradient. The analogous expansion of $\sigma_{\max}^2(t)$ as obtained from Eq. (18) is

$$\sigma_{\max}^2(t) \approx 2\,\frac{D_1\tau}{\mu_1 E\eta_1}\left[1 - \left(1 + \frac{\lambda_1}{2\eta_1}\right)\tau + \cdots\right] \tag{21}$$

It therefore follows that at short times the exponent in Eq. (11), which for convenience we denote by $U(t)$, can be expanded around the value found in the absence of a gradient, $U_0(t)$, as

$$U(t) \approx U_0(t)\left[1 + \left\{\frac{\lambda_1}{2\eta_1} + \frac{\mu_2^2\eta_2 - \mu_1^2\eta_1}{\mu_1\eta_1(\mu_1 - \mu_2)}\right\}\tau + \cdots\right] \tag{22}$$

where

$$U_0(t) = \frac{(\mu_1 - \mu_2)^2E^2}{16D}\,t \tag{23}$$

As a result, the resolution function will initially increase over its gradient-free value whenever the expression in braces in Eq. (22) is positive. This will certainly be true when $\eta_2 > (\mu_1^2/\mu_2^2)\eta_1$, which is to say that whenever the width of the slower peak decreases sufficiently quickly relative to the faster one the resolution function will increase over the zero-gradient value.

We have demonstrated the possibility of increasing the resolution, thereby decreasing the time to peak separation through the introduction of a gel gradient. If the van Kampen approximation is taken seriously at long times ($\tau \gg 1$), then the resolution function will also exceed its gradient-free value, which is to say, when the effect of the gradient in lessening the amount of peak spreading is greater than its effect in slowing the velocity. However, this cannot true in general since the accuracy of the van Kampen approximation almost always decreases as a function of time.

In conclusion, we have demonstrated that when one measures resolution in terms of time it is possible that a proper choice of gel gradient can enhance resolving power. While our mathematical development assumed a linear Ferguson plot (Eq. 14) for both diffusion and mobility, it is clear that the application of an approximate solution to the generalized diffusion equation is not restricted to such systems. Rather, the essential ingredient in the analysis is that band spreading is small in comparison to convection in an appropriately defined sense.

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