Analytical and Preparative Electrophoresis in a Nonuniform Electric Field

A nonuniform electric field is proposed as the basis for the batch and continuous electrophoretic separation of biomolecules. A theoretical analysis shows the advantages of these techniques over conventional separation processes that use a uniform electric field. The mathematical modeling of such processes and its implications for the experimental design of a nonuniform field separator are presented.

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Introduction

Over the past 20 years the development of effective analytical-scale separation techniques has had a major impact on biotechnology. The development of genetically engineered organisms, DNA and protein sequencing technology, and the ability to isolate and purify bioactive chemicals for fundamental research are just a few of the achievements made possible with the development and use of current analytical separation methods.

While the improvement of existing analytical separation techniques will continue to aid our understanding of fundamental biochemical phenomena, there is also a need to develop preparative or large-scale separation methods for industry. Here, the promise of many new commerical products from genetically engineered organisms is moving the field of biochemical separations into the domain of chemical engineering. Depending upon the quantity of the product to be purified, a direct scale-up of an appropriate analytical technique may be sufficient to process the product of interest, but in many cases a continuous process will be required.

This work is aimed at the development of both analytical and preparative scale separation methods. We first examine one of the more useful biochemical separation methods, gel electrophoresis. Here, we show how a relatively simple change in the design of the conventional technique can be used to improve the resolution of this analytical separation method. We then look at a combination of the concepts employed in this method with an imposed flow field to provide the advantages of a continuous separation.

Gel Electrophoresis

One of the most often employed analytical methods for separating molecules involves electrophoresis, the transport of a charged species in an electric field. Many electrophoretic separation processes, such as polyacrylamide gel electrophoresis (PAGE) and gel electrophoresis in the presence of detergents such as sodium dodecyl sulfate (SDS-PAGE) have been developed. These methods have been invaluable analytical tools in science and medicine for determining inhomogeneties among cell populations, subcellular organelles, and macromolecules such as proteins and DNA. Reuss first documented his observations on electrophoresis in 1809. Since then, moving boundary electrophoresis was described by Lodge in 1886, and was improved upon by Tiselius in 1937, and Longsworth in 1946. Picton and Linder (1942) were the first to study the electrophoresis of proteins. They showed that hemoglobin migrates to the anode in alkaline solutions and to the cathode in acidic solutions. In the 1950's, electrophoresis on paper supports was found to be more efficient and convenient than that in free solution. Since then, supporting gels such as starch, agarose, and polyacrylamide have proven to be even more effective for fractionating small quantities of charged material.

Analytical gel electrophoresis is almost always operated in a batch mode. Typically, a sample comprised of N species is placed within a solvent-filled porous medium. When a uniform electric field is applied across the medium, each species migrates toward the anode or cathode with a characteristic velocity, Figure 1. In this technique, the buffer pH is adjusted such that all the components in the sample migrate in the same direction. The velocity of each species is dependent upon the particle's charge-to-mass ratio, the pH and ionic strength of the solvating buffer, and the magnitude of the applied electric field. The conventional way to characterize this velocity is in terms of the electrophoretic mobility μ, defined as the velocity of that particle or molecular species when the electric field acting upon it is 1 V/cm (Cann, 1970). The mobility has dimensions of $cm^2/V \cdot s$ and has the same sign as the particle's net charge.

Much of the success of this technique is due to the gel, which prevents thermal convection. If a liquid film of electrolyte is

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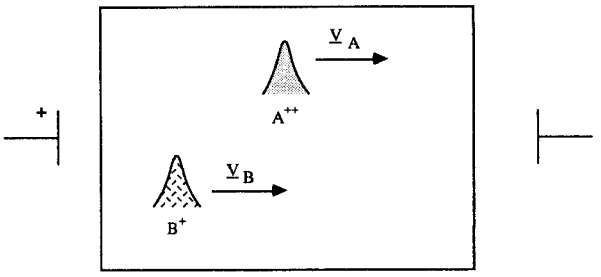

Figure 1. Gel electrophoresis.
Peaks represent concentration distribution of *A* and *B* within gel

used in place of the saturated porous medium, the Joule heating generated by the presence of the electric field can induce a buoyant flow that will overwhelm the separation process. Often, the gel also serves as a molecular sieve (Andrews, 1981), retarding the motion of larger species. In the case of agarose and polyacrylamide gels, where the pore size of the gel matrix can be controlled easily, the sieving effect can be used to improve the separation.

Prior work on gel electrophoresis in a nonuniform electric field

A feature common to most electrokinetic processes is the use of a uniform electric field to facilitate the separation. This type of homogeneous electric field provides a constant driving force that causes electrophoresis to occur at a constant rate. Because of this fact, the separation between two species originally located at the same starting position occurs at a steady rate. Techniques that employ a nonuniform electric field in place of the uniform field, however, are infrequently cited in the literature (Carle et al., 1986; Pflug, 1985; Schwartz and Cantor; 1984, Dennison et al., 1982, 1983; Carreira et al., 1980; Souther, 1979).

One of the first reports of an apparatus that uses a nonuniform electric field was by E. M. Southern in 1979. In this design, the gel is cast in the form of an annulus, with one electrode located at the outer radius of the gel and a second at the center of the annulus. The electrode at the center is surrounded by a membrane impermeable to the proteins or nucleic acids to be separated. The sample is placed along the outer periphery of the gel and is collected at the inner radius. In this case, the use of the radial electric field to improve separations was not the major purpose of the design. Instead, the geometry of this apparatus was designed for efficient heat removal. Since the field is radially distributed, most of the heat is dissipated at the electrode in the center of the annulus. At this point, however, the semipermeable cellulose membrane that surrounds the electrode allows a rapid flow of buffer to be pumped along the electrode, carrying away the heat and products of electrolysis.

The apparatus designed by Carreira et al. (1980) is a modified version of that designed by Southern. The major variation is an improved, more sophisticated collection scheme, which is completely automated for efficient recovery and concentration of the sample fractions. Here, as in the device proposed by Southern, the effects that the nonuniform electric field would have on the separation of nucleic acids or proteins are not addressed.

In 1982, Dennison et al. proposed the use of wedge-shaped gels for the separation of proteins and nucleic acids. This design is based upon the experimental observation that under ideal conditions the steric and sieving resistances experienced by molecules in a gel matrix are not linearly related to their size. The result of this nonlinearity is a poorer separation of larger molecular species. Attempting to linearize the approximately logrithmic distribution of the protein bands that is normally seen, these investigators substituted a nonlinear electric field in place of a uniform field. The pH of the buffer was adjusted, and the sample applied, such that electrophoresis occurred from high to low electric field intensity. While their experimental results showed better resolution of the higher molecular weight proteins and have led to the development of a number of commercial wedge-shaped electrophoresis units, their mathematical model used to characterize the system was quantitatively incorrect. Sample distribution was not linearized as predicted by the model. The use of wedge-shaped gels has also been successfully extended to isoelectric focusing methods (Pflug, 1985).

Another relatively new form of nonuniform field electrophoresis, used for separating DNA and RNA molecules with a molecular weight greater than 10^7 Dalton (1 Dalton = 1 atomic mass unit [AMU] $\simeq 1.66 \times 10^{-27}$ kg) or a length greater than 20 kilobase (kb), uses electric fields that are spatially and/or temporally inhomogeneous. The major problem that exists when attempting to separate very large nucleotide chains is that they exhibit nearly size-independent mobilities (Fangman, 1978). This independence is attributed to a reptile-like mode of migration called reptation (Lumpkin and Zimm, 1982; Jamil and Lerman, 1985; Lerman and Firsch, 1982). Schwartz and Cantor (1984) attempted to solve this problem by alternately applying two perpendicular nonlinear electric fields to agarose gels. They report separating DNA molecules from 30 to 2,000 kb. Since then, Carle et al. (1986) have used a method in which a single uniform electric field is applied in a single direction, but the polarity of the field is periodically reversed. The time that the field was reversed ranged from 25 to 50% of the total time, and a single cycle ranged from 1 to 3 s. In summary, these investigators were able to separate DNA of up to 700 kb, but the quality of the separations were not as good as the separations reported by Schwartz and Cantor. Finally, Chu et al. (1986) were able to separate DNA of up to 2,000 kb with an apparatus similar to the one developed by Schwartz. The major difference between these two designs is that Chu's contour-clamped electrode system is more sophisticated.

Present work with gel electrophoresis in nonuniform electric fields

The advantages of nonuniform electric fields in electrophoretic separations have stimulated us to examine their effects more carefully. In particular, we have been exploring the effects of replacing the conventional uniform electric field with a nonuniform electric field that increases in intensity in the direction of the electrophoretic motion. It should be noted that this arrangement is opposite to that of Dennison. Consider two species initially located at the same position. As the two species begin to separate, the species with the higher mobility will be located in a more intense field. The leading species will therefore experience a greater electrophoretic force than the trailing species and tend to accelerate away from the slower moving species. Relative to a process using a uniform electric field of the same average voltage drop per unit length of gel, the separation

between the two species will be enhanced because of the increasing electric field gradient. This discussion is quantified with the following mathematical models.

Mathematical modeling of uniform and nonuniform field systems

The electrophoretic transport of a charged species can be modeled using the differential equations for conservation of mass, charge, and energy. These equations are mathematically and physically coupled in a number of ways. The electrokinetic transport of the charged species depends directly on the electric field distribution. At the same time, the electric field depends upon the distribution of the charged species within the system. The conservation of energy equations are also coupled to the conservation of mass equations since the electrophoretic mobility and the diffusion coefficients are temperature-dependent.

As a lowest order approximation, however, a number of assumptions can be applied to unlink these equations. Since the sample is typically dilute and carries no appreciable current relative to that carried by the supporting electrolyte within the system, the electric field can be assumed to be independent of the sample location. Also, since most gel electrophoresis systems are designed to be efficiently cooled, only small temperature gradients are produced. It is therefore appropriate to assume that the diffusion coefficient and electrophoretic mobility are constant, permitting the energy and mass equations to be uncoupled. After these assumptions have been invoked, the differential equation describing the transport of a charged species in a gel matrix takes the following form:

$$\frac{\partial C_j}{\partial t} = -\nabla \cdot (N_{diff} + N_{electrophor}) \tag{1}$$

where the individual fluxes N are given by $N_{electrophor} = \mu_j C_j \underline{E}$ and $N_{diff} = -D_j \nabla C_j$. Here, C_j, D_j, and μ_j are the concentration, diffusion coefficient, and electrophoretic mobility of species j, respectively, and $\underline{E}$ is the electric field. The effects of the void fraction available for species transport have been factored out of this equation. In the following discussion the species subscript j will be dropped for simplicity.

Using Gauss's law, we can derive the form of the electric field $\underline{E}$ for a given physical system. Two different fields will be analyzed: a uniform electric field, and a nonuniform electric field in an annular geometry. These fields are generated with the geometries shown in Figure 2. The mathematical form of the uniform electric field equation is:

$$\underline{E} = \frac{V}{W}\underline{e}_x$$

and the form for the nonuniform field is:

$$\underline{E} = \frac{V}{\ln\left(\frac{r_o}{r_i}\right)}\frac{1}{\bar{r}}\underline{e}_r$$

where V is the voltage applied across each of the two systems ($V = V_1 - V_2$). Given the form of the nonuniform electric field shown in Figure 2, the electrophoretic and diffusive transport within the system are modeled by the partial differential equation

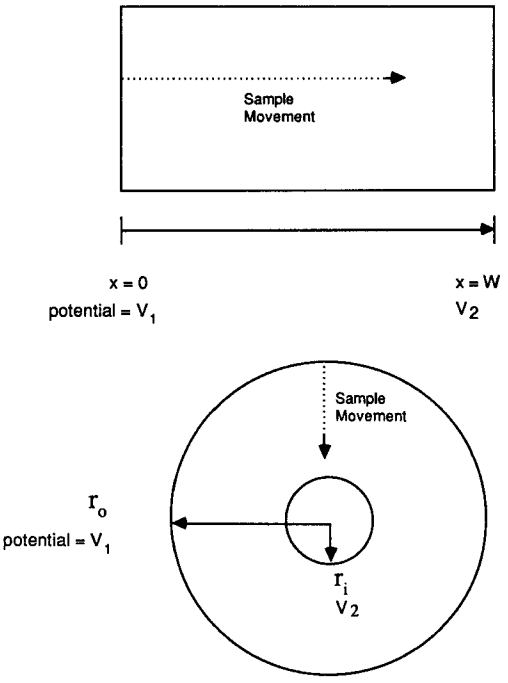

Figure 2. Coordinate systems used in modeling one-dimensional gel electrophoresis systems.
Top, uniform system
Bottom, nonuniform system

$$\frac{D}{\bar{r}}\frac{\partial}{\partial \bar{r}}\left(\bar{r}\frac{\partial \bar{C}}{\partial \bar{r}}\right) + \frac{\mu V}{\ln\left(\frac{r_o}{r_i}\right)}\frac{1}{\bar{r}}\frac{\partial \bar{C}}{\partial \bar{r}} = \frac{\partial \bar{C}}{\partial t} \tag{2}$$

with the initial condition

$$\bar{C}(\bar{r}, 0) = N_o \frac{\delta(\bar{r} - r_o)}{2\pi \Delta h \bar{r}}$$

where r_o and r_i are the outer and inner radii of the annular system, respectively, N_o is the mass placed into the system, Δh is the thickness of the gel matrix, and δ is the Dirac delta function. By defining the dimensionless variables,

$$r = \frac{\bar{r}}{r_o}, \quad R = \frac{r_i}{r_o}, \quad \gamma = \ln\left(\frac{1}{R}\right),$$

$$C = \frac{\bar{C}}{M_o}, \quad \tau = \frac{t}{\frac{r_o^2 \gamma}{\mu M}}, \quad El = \frac{\mu V}{D}$$

where El is the electrophoretic Peclet number and R is the dimensionless inner radius, we can rewrite the mass transport equation in dimensionless form as

$$\frac{1}{r}\frac{\gamma}{El}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) + \frac{1}{r}\frac{\partial C}{\partial r} = \frac{\partial C}{\partial \tau} \tag{3}$$

with the initial condition $C(r, 0) = \delta(r - 1)/r$. Since this equation is linear, the exact solution can be obtained using a conven-

tional separation-of-variables technique. A difficulty in evaluating the solution occurs, however, because of the presence of the inverse electrophoretic Peclet number multiplying the diffusion terms. Values of El^{-1} are on the order of 10^{-3} and smaller, implying that relative to the electrophoretic transport, diffusion is negligible. At first glance it seems reasonable to neglect the first term on these grounds; however, the separation between two independent species will be on the order of the diffusion length scale. Since the goal of this analysis is to evaluate the potential separation capacity of this system and compare it to that of the uniform field design, diffusion must be included.

To include the effects of diffusion, we can apply a singular perturbation approach. The frame of reference of the equation is transformed such that the observer is always located at the center of mass of the pulse. In addition, the length scale is redefined to be on the order of the diffusion length. The form of this transformation is

$$X = \frac{r - r_c(\tau)}{\sqrt{\epsilon}}$$

where $\epsilon = El^{-1}$ and $r_c(\tau)$ is the radial position of the pulse as a function of dimensionless time. If we now expand the concentration C in an asymptotic series with gauge function $(\epsilon^{1/2})^n$, the lowest order ($n = 0$) equation describing diffusion is

$$\gamma \frac{\partial^2 C_o}{\partial X^2} - \frac{X}{1 - 2\tau} \frac{\partial C_o}{\partial X} = \frac{\partial C_o}{\partial \tau} \tag{4}$$

with the initial condition $C_o(X, 0) = \delta(X)$. This equation is solved using a finite-difference scheme and is valid until the pulse is within one diffusion length of the inner radius. The additional constraint,

$$\int_{-\infty}^{\infty} C_o(X)\, dX = 1$$

is imposed on the numerical integration scheme to insure that all the mass is conserved and round-off error is minimized.

A similar approach is used to model the uniform field system; however, the solution to the lowest order equation in this case can be generated in a closed form. With the solutions to both uniform and nonuniform systems at hand (Rolchigo, 1988), we can analyze the separation of any two arbitrary species. The simulation of the separation of two species, each with different electrophoretic Peclet numbers, can be described by again changing the reference frame to one of constant position and observing the unsteady state concentration profile as each species elutes from the end of the system ($x = W$ in the uniform system and $r = r_{inner}$ in the nonuniform system). To characterize the quality of the separation, we can use the classical chromatography term "resolution":

$$\text{Resolution} = \frac{t_B - t_A}{\Delta t_B + \Delta t_A}$$

where t_B, t_A, Δt_B, and Δt_A are defined in Figure 3. The terms Δt_B, and Δt_A are defined such that a resolution greater than 1 implies that the separation is complete to within 95% of the mass in each of the original pulse inputs.

Figure 4 represents the physical situation where the diffusion

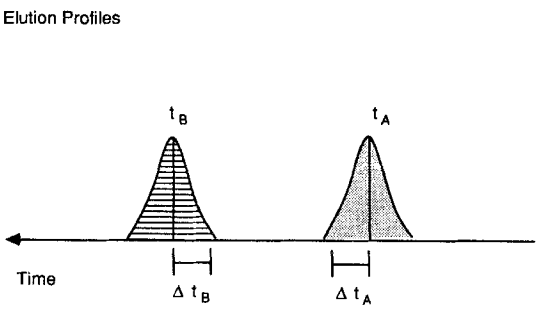

Figure 3. Terms used to determine resolution.
Resolution = $(t_B - t_A)/(\Delta t_B + \Delta t_A)$

coefficient is approximately one order of magnitude smaller than the electrophoretic mobility of each species (i.e., $\mu/D \sim 10$ V^{-1}) and the applied potential difference is 200 V. This represents physically a worst case low-mobility, high-diffusivity/dispersion system. In Figure 4a, resolution is plotted against the dimensionless inner radius of the annular system for two species whose mobilities differ by 10, 20, and 30%. The first trend to note is that resolution increases as the dimensionless inner radius R is decreased. Our physical explanation for this occurrence is that as R is decreased, the electric field gradients become steeper, accenting the differences between the mobilities of the two species being separated. As R is decreased below 0.05 (not shown), however, the resolution under these operating conditions levels off. Here we find that the increase in resolving capacity of the nonuniform system is offset by an increase in dispersion due to the asymmetry of the electric field. That is, the peak widths are large enough that their leading and trailing edges move at significantly different velocities.

To compare the effectiveness of this system to that of the uni-

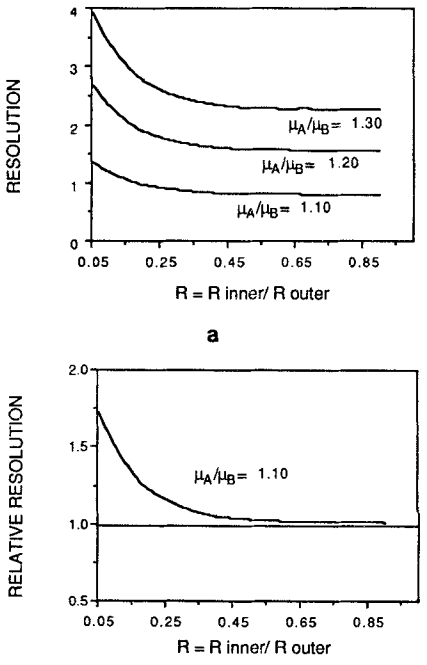

Figure 4. $El_B = 2{,}000$; $El_A = El_B(\mu_A/\mu_B)$

form field system, we can define the term "relative resolution," a ratio of the resolution in the nonuniform field system to that in the uniform field system. A relative resolution greater than 1 shows that the annular system achieves better separation than the constant field case. A plot of this parameter is shown in Figure 4b under the same conditions as stated for Figure 4a. From this figure we see that as R approaches 1, the relative resolution also approaches 1. This is the appropriate physical limit. As R approaches 1, the geometry of the annular system loses its curvature and simulates a uniform field system. The most important result of this analysis is that as R is decreased, the quality of the separation in the nonuniform system can be enhanced by over 50% relative to that in a uniform system under identical conditions before the band spreading effect sets in. With the nonuniform field system, we now have an added degree of freedom to improve separations.

In Figure 5, the resolution of a two-component sample whose diffusion coefficients are two orders of magnitude smaller than the electrophoretic mobilities of their constituents (i.e., $\mu/D \sim 100\ V^{-1}$) is analyzed. These conditions are more realistic than the conditions used in Figure 4. Again the trends shown in Figure 4 are observed; however, they are exaggerated. Figure 5a shows that resolution increases with decreasing R, but now two species whose mobilities differ by 5% can be resolved. The relative resolution also shows similar trends. Here, however, the quality of the separation in the nonuniform field system is significantly greater than that in the uniform field system. With a mobility ratio of $\mu_A/\mu_B = 1.05$ and equal dispersion coefficients ($D_A = D_B$), enhancement of resolution up to 350% is predicted for small values of R. A value of $R = 0.01$ is expected to be the practical experimental limitation of the design due to dispersion in the nonuniform field.

The results from this analysis for the one-dimensional separation process confirm the intuitive notion that a nonuniform electric field in place of a uniform electric field can be beneficial for enhancing electrophoretic separations. We will now extend the concept to a consideration of continuous-flow systems.

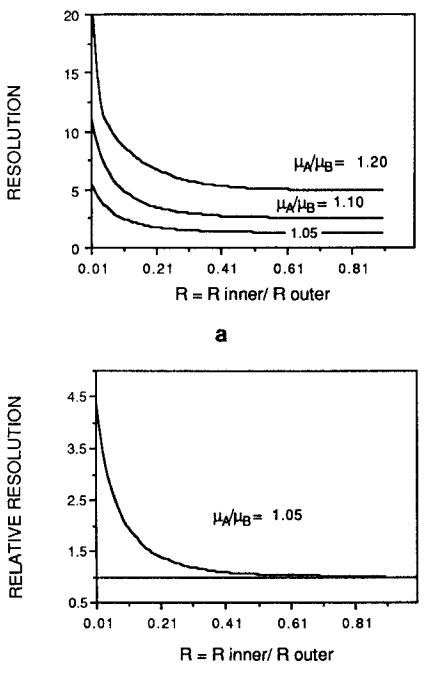

Figure 5. $El_B = 20{,}000$; $El_s = (\mu_A/\mu_B)$

Continuous-Flow Electrophoresis

Prior work

Batch electrophoresis can be transformed into a continuous process by simultaneously inducing each species of a heterogeneous sample to move in a second dimension, normal to that of the electrophoretic motion. The additional velocity component forces the N species (each with a different mobility) present in a sample to migrate along N unique pathways if the sample is introduced at a stationary feed point. If a sample is continuously introduced at this feed point, the different fractions of the sample can be collected continuously at unique exit sites. This concept is illustrated in Figure 6. Although a number of continuous electrokinetic processes using this principle have been designed previously, all have been subject to similar limitations and difficulties. The major problems are loss of resolution due to electroosmotic flows, low throughput limited by the onset of convective instabilities arising from Joule heating, and product dilution. In general, these designs can be divided into two major categories: moving-bed electrophoresis, and continuous-flow electrophoresis.

In moving-bed electrophoresis (Lammel, 1981), a second velocity component is added to the electrophoretic velocity of each species in the sample by moving the bed, or support medium, relative to the direction of electrophoresis, Figure 7a. This form of continuous electrophoresis is advantageous since the supporting medium stabilizes against thermal convection and can also be used to produce a molecular sieving effect. These advantages, however, are offset by the operational and maintenance problems encountered in the mechanically complicated design.

Hannig et al. (1975) and Hannig (1978) are responsible for much of the initial development of continuous-flow electrophoresis. In this apparatus, fluid flows between two flat plates and a uniform electric field is applied normal to the direction of flow, Figure 7b. Theoretical modeling of this type of system shows that the separation between two species is proportional to the magnitude of the applied voltage and inversely proportional to the average linear velocity of the fluid between the plates. Typically, since large flow rates are used to process the sample fractions at high rates, very large voltage drops are required to

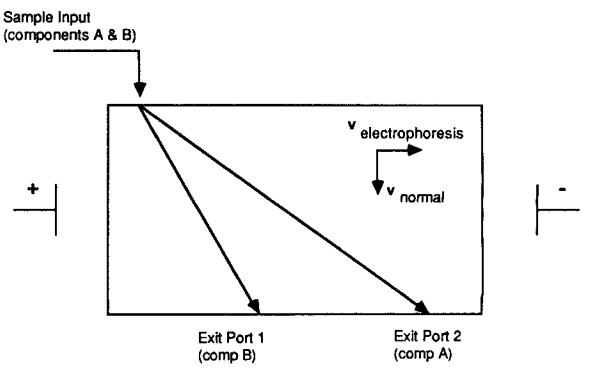

Figure 6. Continuous-flow electrophoresis, sample collection.

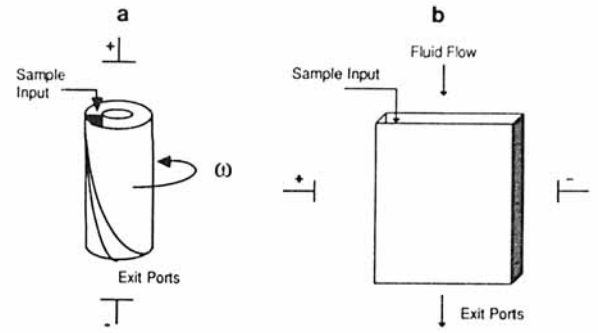

Figure 7a. Moving-bed electrophoresis.
b. Continuous-flow electrophoresis, flow between flat plates.

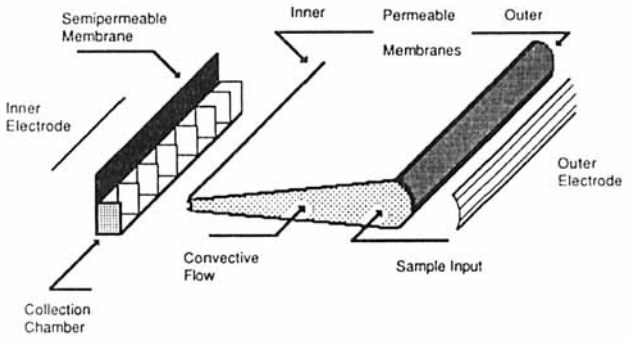

Figure 8. Proposed nonuniform field continuous-flow electrophoresis system.
Exploded view; permeable inner membrane is obscured by the angle of the diagram

achieve a worthwhile fractionation of the sample. The thermal stability of the flow profile, however, is dependent on the power dissipated by Joule heating. Since the power dissipated is proportional to the square of the voltage drop, and the separation increases linearly with voltage, the separation capacity is often limited by the onset of thermal convection. A horizontal orientation of the apparatus does help to relax the convective instability problem. In this orientation, however, cell and protein sedimentation from the sample stream can become a problem. Models of the fluid mechanics and separations in this type of system have been posed by a number of authors (Ivory, 1980, 1981; Ostrach, 1977; Saville, 1979, 1980).

Stabilization of the flow profile with regard to thermal convection has also been attempted by Mattock (1980). In his design the separation process is performed in an annulus. The electric field is applied radially across the annular gap and facilitates the electrophoretic motion of the sample in the radial direction from the inner radius to the outer radius. An axial flow induces the sample to move in a second dimension. The annular gap width is narrow enough that the electric field is essentially uniform. In addition to the axial flow, an angular velocity is added to the fluid flow by rotating the outer wall of the annulus relative to the inner wall. The presence of the angular velocity helps to stabilize the laminar flow profile in the presence of Joule heating.

Vermuelen et al. (1971) and Hybarger et al. (1963) attempted to help stabilize the flow profile by packing an annular column with nonporous monodisperse spherical particles. The major difficulty with the use of a packing material as an anticonvective agent is the resultant increase in dispersion for linear flow velocities greater than 10^{-2} cm/s. As in Mattock's design, the electric field is applied in the radial direction and sample migration is from the inner radius to the outer radius. In this device, however, the gap is sufficiently large such that the electric field is nonuniform.

Continuous-flow electrophoresis in a nonuniform electric field

In light of the results from the modeling of the one-dimensional system, and the problems encountered by some of the existing continuous electrophoretic processes, a new process is proposed. Limitations caused by electroosmosis and thermal convection are expected to be similar to the processes discussed above. It is our hope, however, that the use of a nonuniform electric field can improve separations and offset some of these problems. This hypothetical process is illustrated in Figure 8. Separation of N species is accomplished in a nonuniform electric field generated in an annular wedge-shaped column. The electric field is oriented and the pH of the solution adjusted such that each species in the sample will migrate from the outer to the inner radius of the column. The separation process is transformed into a continuous process by introducing a convective flow normal to the direction of electrophoresis. When each species reaches the inner radius of the column, it elutes electrophoretically through the permeable membrane located there and is found at a unique axial position along the inner radius. At this point, each species is electrophoretically collected in one or more chambers against the protein-impermeable membrane adjacent to the inner electrode. Since this membrane is permeable to the ions in the buffer solution and impermeable to the components in the sample, each of the separated species will concentrate against this membrane. The dense protein solution will then sediment into the collection chambers illustrated. This phenomenon, which is called electrodecantation (Posner, 1976), is known but is infrequently applied. With the recent introduction of a commercial electrodecanter, this situation may change. The chambers are purged at regular intervals to recover the sample fractions. This arrangement is especially interesting since the separation and the product concentration occur simultaneously. To help stabilize the flow against thermal convection, the column is mounted horizontally and is cooled on both sides of the wedge and at the outer electrode surface.

Before the mathematical modeling of the system shown in Figure 8 is presented, a physical discussion of the transport within the system will be given. Consider the situation that exists when a pulse of sample mixture is placed into the system at the outer radius. As each species migrates at its electrophoretic and convective velocities, the initial pulse separates into several pulses each having its own center of mass. As time progresses this separation is partially nullified as the biomolecules in each pulse begin to diffuse away from this center of mass. Because of this diffusional spreading, the resolution achieved is dependent on the distance between the leading edge of a slow species and the trailing edge of a faster species. However, we must first define where these edges are positioned relative to the location of each pulse's center of mass. At any instant during the separation process, a circular boundary with radius ρ, the diffusion length, can be constructed about the center of mass of each pulse, Figure 9a. This boundary is used to define the position of the leading and trailing edges of each species. Since diffusion

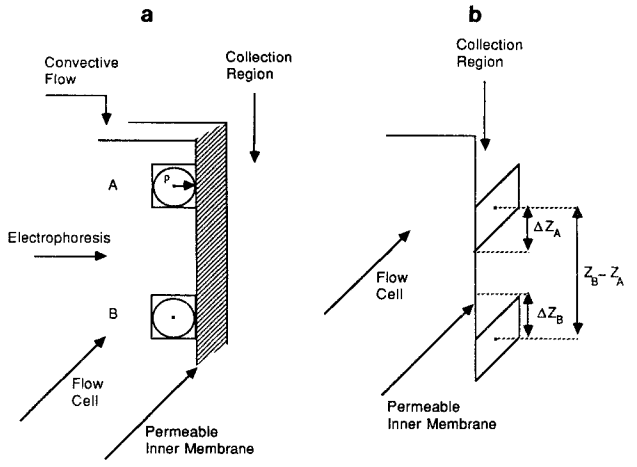

Figure 9a. Circular boundary around mass of each pulse.
b. Distortion of pulse boundary.
$z_B - z_A$ = separation of species A and B
Δz_A, Δz_B are net axial dispersions

gives rise to a diffuse boundary, ρ is arbitrarily defined as that distance which encompasses a given fraction of the total mass of each species. As the diffusion length ρ is increased, more of the mass from the original pulse is contained within this boundary.

Figure 9b illustrates how the pulse boundary distorts as it migrates across the porous membrane at the inner radius. Since the convective axial velocity exists only within the annular wedge-shaped column, the foremost portion of the pulse moves only in the direction of the electric field after it reaches the inner permeable membrane, while the trailing portion is still being convected in the axial direction. As shown in Figure 9b, the shear at the inner membrane causes an effective broadening of the sample boundaries. After each of the pulses has moved through the inner membrane, the resolution can be defined in a manner analogous to that used previously as:

$$\text{Resolution} = \frac{z_B - z_A}{\Delta z_B + \Delta z_A}$$

where z_B, z_A, Δz_B, and Δz_A are shown in Figure 9b. ρ is defined to include 95% of the mass of each species originally introduced into the system.

Mathematical modeling of nonuniform field system with fluid flow

The overall approach to modeling the proposed system is to separate the transport process into two stages:

1. Electrophoretic, convective, and diffusive transport within the annular column (region 1)
2. Electrophoretic transport beyond the permeable membrane at the inner radius (region 2)

The differential conservation of mass equations are used to model transport within the first region, while an integrated form of the conservation of mass equations is used to model the second region.

Equation 5 is the partial differential equation used to model the transport of species j within region 1,

$$\frac{D}{\bar{r}}\frac{\partial}{\partial \bar{r}}\left(\bar{r}\frac{\partial \bar{C}}{\partial \bar{r}}\right) + \frac{\mu V}{\ln\left(\frac{r_o}{r_i}\right)}\frac{1}{\bar{r}}\frac{\partial \bar{C}}{\partial \bar{r}} + D\frac{\partial^2 \bar{C}}{\partial \bar{z}^2} - v\frac{\partial \bar{C}}{\partial \bar{z}} = \frac{\partial \bar{C}}{\partial t} \quad (5)$$

It is solved with an initial condition described by a Dirac pulse of mass located at $r = r_o$ and $z = 0$. For simplicity, the subscript j to denote species again has been dropped. The coordinate system employed is shown in Figure 10. Here, V is the applied voltage, and D and μ are the diffusion coefficient and the electrophoretic mobility of species j, respectively. As a simplification of the column fluid mechanics, a plug flow velocity profile with a linear velocity v is used in the model. Additionally, we have assumed that the process is isothermal and the flow due to electroosmosis is negligible. It should be noted that in real systems, either or both of these conditions may be difficult to achieve. Our intention, however, was to compare uniform and nonuniform systems independent of such problems. More rigorous modeling than that presented here will be needed to compare experimental results in our system quantitatively with theoretical predictions.

By choosing the following dimensionless variables,

$$r = \frac{\bar{r}}{r_o}, \quad z = \frac{\bar{z}}{r_o}, \quad \tau = \frac{t}{\frac{r_o^2 \ln(r_o/r_i)}{\mu V}}, \quad C = \frac{\bar{C}}{M_o}$$

and defining the Peclet number Pe, the electrophoretic Peclet number El, the dimensionless inner radius R, and the log of its reciprocal γ:

$$Pe = \frac{v(r_o - r_i)}{D}, \quad El = \frac{\mu V}{D}, \quad R = \frac{r_i}{r_o}, \quad \gamma = \ln\left(\frac{1}{R}\right)$$

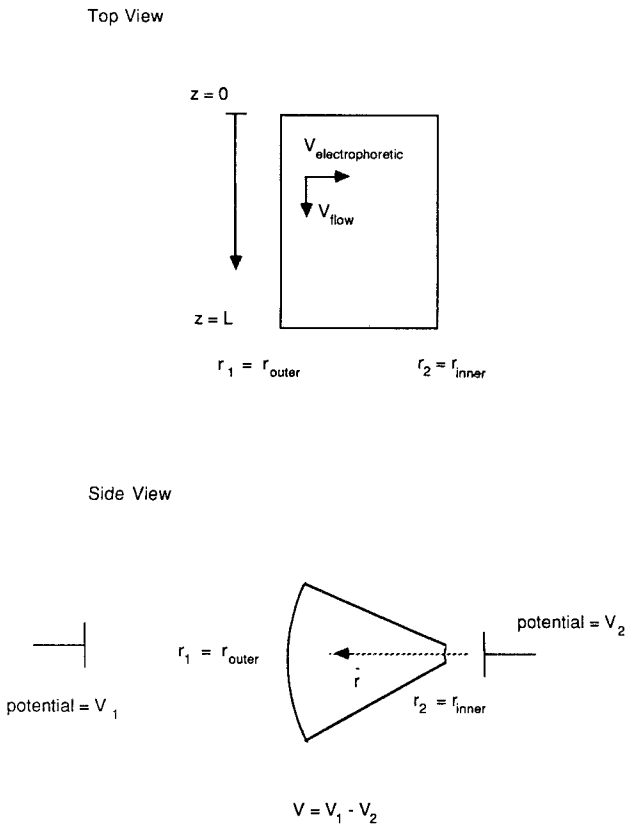

Figure 10. Coordinate system used in modeling continuous-flow nonuniform field electrophoresis system.

we can rewrite Eq. 5 as

$$\frac{\gamma}{El}\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right)+\frac{1}{r}\frac{\partial C}{\partial r}+\frac{\gamma}{El}\frac{\partial^2 C}{\partial z^2}-\frac{R}{1-R}\frac{Pe}{El}\gamma\frac{\partial C}{\partial z}=\frac{\partial C}{\partial \tau} \quad (6)$$

As discussed in the one-dimensional case, values for the inverse electrophoretic Peclet number are small and analytical or numerical integration of the above equation in its present form will be difficult. To obtain an approximate solution to this equation and include the effects of diffusion, a singular perturbation technique is again applied. The frame of reference of the governing mass transport equation is transformed to move at the center of mass of the pulse at the electrophoretic and convective velocities. The length scales for r and z are then rescaled to be on the order of the diffusion length. After expanding the concentration C in terms of $\epsilon^{1/2}$, the lowest order equation representing dispersion about $X = Y = 0$ is

$$\gamma\frac{\partial^2 C_o}{\partial X^2}-\frac{X}{1-2\tau}\frac{\partial C_o}{\partial X}+\gamma\frac{\partial^2 C_o}{\partial Y^2}=\frac{\partial C_o}{\partial \tau} \quad (7)$$

with the initial conditions $C_o = \delta(X)\delta(Y)$, where X and Y are the r and z coordinates transformed and rescaled, respectively. Equation 7 has been solved numerically using a finite-difference algorithm. Using the solution to this equation, a relationship defining the diffusion length ρ as a function of position, time, and the percentage of the original mass accounted for can be derived.

The second part of the analysis involves the transport across the permeable boundary at the inner radius of the column (region 2). The conservation of mass equation neglecting dispersion, and the initial conditions, represented by the spatial distribution derived from the analysis of region 1, are used to model this transport. Resolution can now be shown to be a function of the Peclet number and the electrophoretic Peclet number for each pair of species being separated and the dimensionless inner radius R of the nonuniform system. Recall that the Peclet number is defined as $Pe_j = v(r_{outer} - r_{inner})/D_j$ and the electrophoretic Peclet number is defined as $El_j = \mu_j V/D_j$.

In order to compare the performance of this hypothetical system to that of a similar system employing a uniform electric field, we have also solved the model equations for a continuous-flow uniform field system similar to the annular system just described. As in the nonuniform field process, idealizations such as isothermal operation and no electroosmosis have again been assumed to insure an equal basis of comparison. Although the model for this uniform field case is not presented here (Rolchigo, 1988), the results of calculations carried out with this model are shown in Figures 11 and 12.

The following discussion highlights the results of the mathematical modeling. The maximal effects of diffusion on resolution were examined by assuming that the sample consists of two species with a 10% difference between their values of El (El_A = 1,100 and El_B = 1,000). These are worst case values derived using a high estimate for the diffusion coefficient (10^{-6} cm^2/s), very similar values for their electrophoretic mobilities of 1.0×10^{-5} and 1.1×10^{-5} cm^2/V · s, and an applied potential difference of 100 V. In this case Figure 11a shows that neither the annular nor the uniform system can adequately resolve these components. However, an increasing resolution with a decreasing dimensionless inner radius is observed, as was the case with

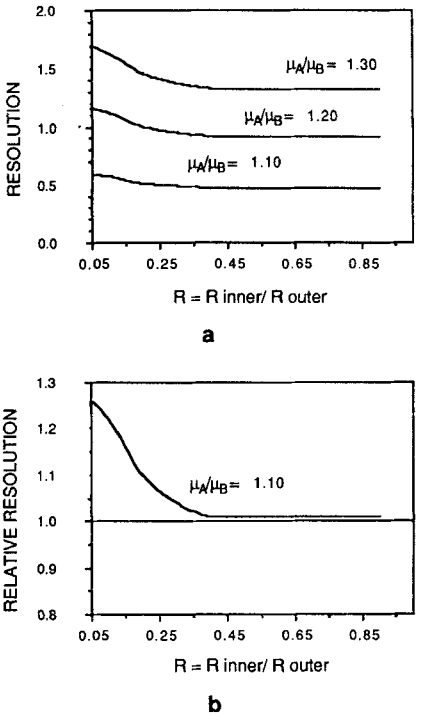

Figure 11. Model calculations for continuous-flow uniform field system.

$Pe_A = Pe_B = 30{,}000$
$El_B = 1{,}000$; $El_A = El_B(\mu_A/\mu_B)$

the one-dimensional process. For these experimental conditions, the curves for resolution level off when R is decreased below 0.05. The explanation for this observation is the same as the one offered for the one-dimensional nonuniform system.

To compare the effects of the nonuniform electric field on the separation to those of a uniform field, we have previously defined in Figure 9b the relative resolution as the ratio of resolution in the nonuniform field process to that of the uniform process. A value greater than one shows an enhancement of separation produced by the nonuniform field relative to that of the uniform field. A significant enhancement in resolving power is demonstrated in Figure 11b. The appropriate limiting value is also observed as R approaches 1.

Figure 12a illustrates the resolving capacity of the nonuniform field system when the parameters are evaluated with more practical values of the diffusion coefficient and mobility. Here we see a significant improvement in the resolving capacity of the annular system. As the dimensionless inner radius R is decreased to below 0.05, two species whose mobilities differ by 5% theoretically can be separated. We can compare this result to the effectiveness of the uniform field system by examining the plot of relative resolution shown in Figure 12b. By decreasing R we observe a relative increase in the resolving capacity of the nonuniform system over the uniform system of up to 130%.

Discussion and Conclusions

The use of a nonuniform electric field as the basis for both a batch and a continuous electrophoretic separation process appears to have several benefits over conventional methods. The strongest conclusions can be drawn from our analysis of the one-

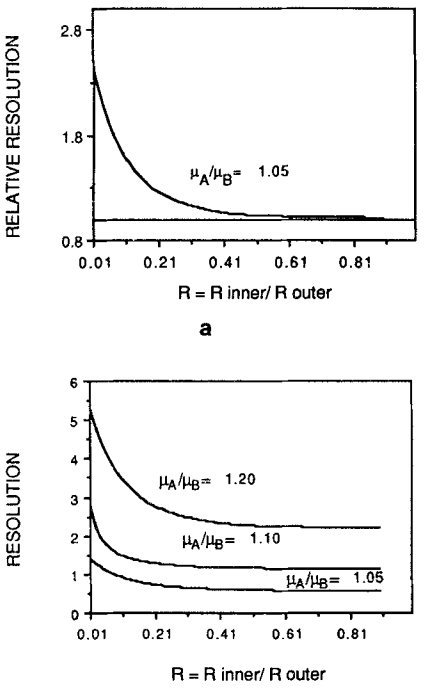

Figure 12. Model calculations for continuous-flow uniform field system.

$Pe_A = Pe_B = 30{,}000$
$El_B = 10{,}000$; $El_A = El_B(\mu A/\mu_B)$

dimensional batch gel electrophoresis system. Here, the assumptions used in the modeling are quite good, and it is expected that the theoretical predictions of resolution and experiment will agree well. An experimental investigation of this system is now under way. The potential for this system to be used as a preparative-scale batch technique is also of interest, and the possibilities of scale-up are also being addressed.

Strong conclusions, however, cannot be drawn from the modeling of the flow systems. Only in the idealized case of no electroosmosis and isothermal operation can we conclude that the separations can be significantly improved with the use of the nonuniform electric field. In general, the design should be subject to the same limitations experienced by existing continuous-flow designs. All of these limitations being equal, however, the use of the nonuniform field may improve resolution. We do not expect, however, quantitative agreement between the theory and experiments in this case. A more sophisticated model, which includes the true axial flow profile, the effects of electroosmosis, and nonisothermal operation is needed to further explore the possible benefits to be achieved with the use of the nonuniform electric field in a flow system. An experimental investigation of some of these concepts is also under way.

Acknowledgment

This work was partially supported by the National Science Foundation, Grant No. CBT-8419140.

Notation

$\bar{C}, C$ = dimensional, nondimensional concentration
C_o = lowest order nondimensional concentration
D = diffusion or dispersion coefficient
E = electric field
$\bar{El}$ = electrophoretic Peclet number
M_o = initial concentration
N_o = mass
Pe = Peclet number
$\bar{r}, r$ = dimensional, nondimensional radial coordinate
$r_c(\tau)$ = dimensionless radial position of mass pulse as a function of dimensionless time τ
r_i = inner radius of annular system
r_o = outer radius of annular system
R = dimensionless inner radius, r_{inner}/r_{outer}
t = time
V = applied voltage
v = linear velocity of convective flow
W = length of uniform field gel
X = transformed, rescaled radial coordinate
Y = transformed, rescaled z coordinate
$\bar{z}, z$ = dimensional, nondimensional axial coordinate

Greek letters

$\gamma = \ln(1/R)$
Δh = thickness of uniform and nonuniform gel systems
δ = Dirac delta function
$\epsilon = El^{-1}$
μ = electrophoretic mobility
ρ = diffusion length
τ = nondimensional time

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Manuscript received June 18, 1987 and revision received Oct. 27, 1987.