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Continuous Free Flow Electrophoresis in an Alternating Electric Field with a Variable Buffer Flow

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Abstract

A novel method of continuous free flow electrophoresis in an alternating electric field with a variable buffer flow is proposed as a basis for continuous separation of biomolecules. A model is developed to evaluate the migration of charged particles as they move with the buffer flow. Numerical calculations on the trajectory and dispersion are presented for particles with different electrophoretic mobilities.

INTRODUCTION

Continuous free flow electrophoresis is an efficient way to fractionate on a large scale cells, cell organelles, viruses, and other solutes that are subject to irreversible adsorption, sedimentation, or denaturation. Most often continuous free flow electrophoresis is done in a "thin-film" apparatus where a narrow streak of the feed mixture is fractionated by flowing it with a thin film of buffer between two parallel electrodes (*1*). This method is limited by relatively small flow rates, typically 5–10 mL/h. Larger scale separations are achieved with the rotating free flow electrophoretic system where the separation takes place in an annulus between two rotating outer and stationary inner cylinders which house the electrodes (*2*). Both of these methods use a uniform electric field. Preparative free flow electrophoresis can also be achieved in a nonuniform electric field between two stationary cylinders (*3*). Yet another approach to

continuous flow electrophoresis is to fill the separation chamber with nonporous monodisperse spherical particles as an anticonvective media (*4*). All these methods are limited either by the larger dispersion or small flow rates.

Giddings et al. (*5, 6*) proposed a method for field-flow fractionation of different particles and macromolecules across a narrow slit, in which the species were separated into thin layers. Lightfoot and coworkers (*7*) fractionated a mixture of solutes inside hollow fibers. A small volume of the mixture was introduced into the fiber. An electric field was then applied across the fiber for a limited period of time, which pushed the solutes toward the fiber wall. Finally, after the electric field was terminated, different solutes came out from the hollow fiber at separate time intervals.

Giddings (*8*) proposed a novel method of field-flow fractionation in which the field strength and/or direction of the sedimentation, thermal diffusion, electrical, crossflow, or magnetic field was cycled up and down many times during a run. Cyclical-field flow fractionation was implemented experimentally (*9*) with a gravitational field force for a system in which the orientation of the channel was cycled relative to the gravitational field. The separation was generated along the single principal flow axis.

In this paper we propose a new concept for continuous free flow electrophoretic separation which employs a combination of an alternating electric field and a pulsating secondary buffer flow. In this method the charged solutes move in three different directions; continuously flowing downward with the main buffer flow, migrating electrophoretically perpendicular to the main buffer flow, and flowing in a secondary periodical buffer flow perpendicular both to the main buffer flow and the electric field directions (see Fig. 1). The three-dimensional movement is more complex than the two-directional fractionations of the previously known methods (*1–7*). However, it is capable of achieving precise separation with an increased throughput.

Electrophoretic fractionation is carried at a narrow slit between two flat, semipermeable, preferably uncharged, hydrophilic membranes. The ion-permeable membranes are permeable to the electric current but do not allow passage of any solute. Two electrodes are placed behind the membranes to provide an alternating electric field across the gap. A steady stream of a buffer electrolyte is pumped downward through the gap between the membranes. A mixture of charged species is introduced in a narrow streak in the upper corner between the membranes. The electric field across the gap is reversed after a certain period of time. As the species move downward with the buffer flow, they migrate back and forth between

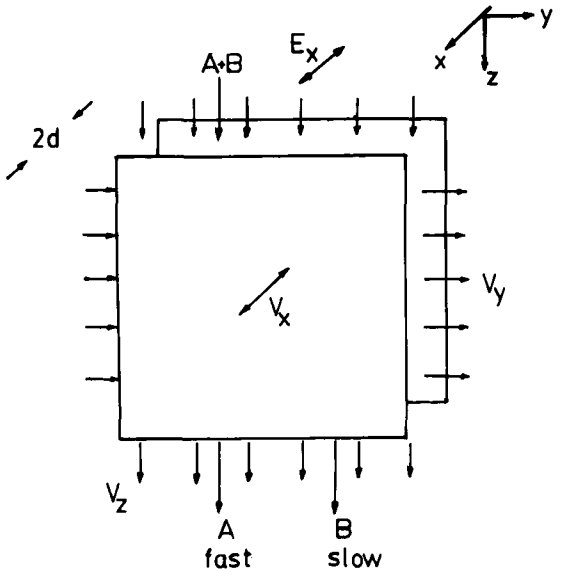

FIG. 1. Schematic drawing of the separation chamber.

the membrane walls as they are driven by the alternating electric field. Faster migrating species move from one membrane wall to the opposite in less time during each cycle, and as a result, move less downward than the slower migrating species. After the faster particles reach the membrane surface, they stay there until the electric field is reversed. Adsorption of the species at the membrane surface is prevented due to the short residence time and the nonadsorbing nature of the membrane.

At some time after the start of each time cycle, when at least some of the faster species have reached the membrane, an additional buffer flow is applied. This buffer flow is directed perpendicular both to the downward main buffer flow and to the electric field across the membrane. This additional buffer flow carries the slower migrating particles that have not reached the membrane wall and is discontinued when the electric field is reversed. Thus, different trajectories for the faster and slower migrating species are established, and this is responsible for their subsequent separation.

THEORETICAL ANALYSIS

A. Main Trajectories of the Charged Species

A mixture of two or more solutes with different electrophoretic mobilities is fractionated in a rectangular cell formed by two ion-permeable membranes which have membrane pores much smaller than the solute particles. A schematic drawing of the separation chamber is shown in Fig. 1. The two membranes are separated by a distance $2d$.

A continuous, steady flow of the buffer is pumped downward in the z direction through the gap between the membranes. An alternating electric field E_x is applied in the x direction perpendicular to the membrane surface by means of two electrodes placed behind the membranes. As the species flow downward with the buffer flow, they begin to migrate from one membrane surface to the other because of the electric field. The field also causes an electroosmotic flow in the x direction through the membrane (*11*). The electroosmotic flow reverses its direction when the electric field is alternated. As the faster migrating species move across the gap and finally reach the membrane surface, the slower migrating species are still at some distance away from the membrane surface. If one starts an additional buffer flow in the y direction at that moment (shown schematically in Fig. 2), the slower migrating particles will travel with that additional buffer flow in the y direction. Since the buffer flow velocity is very close to zero at the wall surface in the y and z directions, the faster migrating particles will not migrate in the y direction.

As the slower electrically migrating particles reach the membrane surface, the additional buffer flow in the y direction is stopped, the electric field E_x is reversed, and the separation cycle is repeated as shown in Fig. 2.

A continuous steady film of carrier electrolyte flows downward through the separation chamber. A symmetrical Poiseuille velocity profile is formed during the flow of the liquid through the rectangular cell (*10*),

$$V_z = V_{0z}\left[1 - \frac{x^2}{d^2}\right] \tag{1}$$

where V_z is the fluid velocity in the z direction at the distance x from the center of the gap, and V_{0z} is the fluid velocity in the center of the gap.

An alternating electric field E_x is applied with a time cycle T as shown in Fig. 2,

$$E_x = E_0 f_x(t) \tag{2}$$

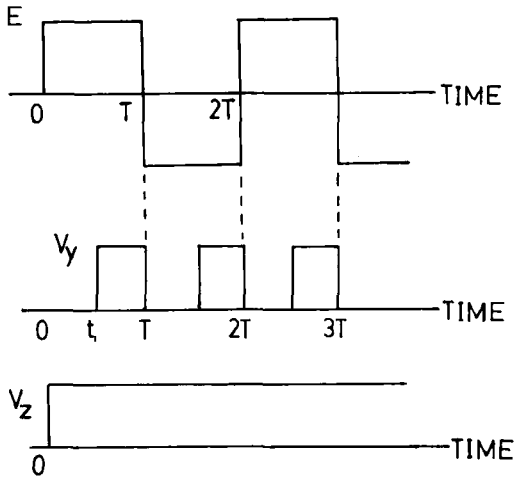

FIG. 2. Electric field and fluid velocity cycles with time.

where E_0 is the magnitude of the electric field. Function $f(t)$ is a periodical time function and can be expressed in terms of $\theta(t)$,

$$f_x(t) = \theta(t) + 2\sum_{1}^{\infty}(-1)^n\theta(t - nT) \tag{3}$$

where $\theta(t)$ is a Dirac function, equal to 1 when t is positive and 0 when t is negative. The derivative of θ is δ; it is equal to zero when $t \neq 0$, and δ is positive when $t = 0$.

The electroosmotic flow across the membrane is proportional to the electric field (*11*),

$$V_x = \alpha E_x \tag{4}$$

where α is the proportionality constant.

Periodic impulses of buffer flow in the horizontal direction are produced during the latter part of each time cycle as shown in Fig. 2. As a first approximation, a symmetrical Poiseuille velocity profile is assumed to represent the flow of liquid in the y direction (*10*),

$$V_y = V_{0y}\left[1 - \frac{x^2}{d^2}\right]f_y(t) \tag{5}$$

where V_y is the fluid velocity in the y direction at the distance x from the center of the gap, and V_{0y} is the fluid velocity in the center of the gap. Function $f_y(t)$ is a periodical time function equal to 1 during the latter part of each time cycle.

$$f_y(t) = \theta(t - t_1) + \sum_1^\infty [\theta(t - nT - t_1) - \theta(t - nT)] \tag{6}$$

For a thin-film, viscous buffer flow, fluid velocity in the x, y, and z directions is treated independently (*10*).

A heterogeneous sample is introduced into the separation chamber through the upper corner and it flows downward with a continuous steady film of buffer. Charged species are deflected from the direction of the carrier electrolyte in the x direction. The electrophoretic migration velocity in the x direction for Species i is V_x,

$$V_x = \mu_{0i} E_x \tag{7}$$

where μ_{0i} is the electrophoretic mobility of Species i.

In a general situation, the heterogeneous feed contains N species with electromobilities μ_{0i}:

$$\mu = \mu_{0i}, \quad i = 1, \ldots, N \text{ where } \mu_{0i+1} > \mu_{0i} \tag{8}$$

When the horizontal buffer flow impulse starts at t_1, the species have either already reached the opposite wall or not, thus all species can be classified as one of two different types. Therefore, only two general trajectories, for Species 2 that reach the wall before t_1, and for Species 1 that are still away from the membrane wall at time t_1, need to be solved.

The characteristic time t_1 is greater or equal to the time required for the faster Species 2 to migrate between the membranes and is less than the time required for the slower Species 1 to migrate from one wall to another.

$$\frac{2d}{V_x + \mu_{02} E_0} < t_1 < \frac{2d}{\mu_{01} E_0 + V_x} < T \tag{9}$$

where t_1 is the idle time and T is the cycle time as shown in Fig. 2.

During a single time cycle, Species 2 is carried with the buffer flow in the z direction, and Species 1 is carried in the y and z directions.

$$dz_2 = V_{0z}\left[1 - \frac{x^2}{d^2}\right]dt \tag{10}$$

$$dy_2/dt = 0 \tag{11}$$

$$dz_1 = V_{0z}\left[1 - \frac{x^2}{d^2}\right]dt \tag{12}$$

$$dy_1 = V_{0y}\left[1 - \frac{x^2}{d^2}\right]dt \tag{13}$$

Integration of Eqs. (10)–(13) during a single time cycle provides the change in the y and z coordinates for the 1st and 2nd species during one time cycle.

$$\Delta z_2 = \frac{4V_{0z}d}{3\mu_2 E_0} \tag{14}$$

$$\Delta y_2 = 0 \tag{15}$$

$$\Delta y_1 = V_{0y}\left[\frac{4d}{3\mu_1 E_0} - \frac{\mu_1 E_0 t_1^2}{d} + \frac{\mu_1^2 E_0^2 t_1^3}{3d^2}\right] \tag{16}$$

$$\Delta z_1 = \frac{4V_{0z}d}{3\mu_1 E_0} \tag{17}$$

where mobilities μ_1 and μ_2 also include the electroosmotic flow component α, $\mu_1 = \mu_{01} + \alpha$, $\mu_2 = \mu_{02} + \alpha$.

The number of cycles, m, required for the species to flow through a chamber of length L is approximately

$$m_1 = \frac{3L\mu_1 E_0}{4V_{0z}d} \tag{18}$$

$$m_2 = \frac{3L\mu_2 E_0}{4V_{0z}d} \tag{19}$$

The total distance traveled by Species 1 in the horizontal direction is equal to the number of steps multiplied by the horizontal distance traveled during each step.

$$Y_1 = m_1 \Delta y_1 \tag{20}$$

In order to separate two species with mobilities $\mu_1 < \mu_2$ in a separation chamber $2d$ wide having an electric field $\pm E_0$, the start of the periodic horizontal buffer flow should coincide with the moment that the faster Species 2 reaches the opposite wall,

$$t_1 = 2d/(\mu_2 E_0) \tag{21}$$

Since Species 2 does not deflect in the y direction, the distance Y_1 represents the separation distance between Species 1 and 2 as they flow out of the chamber. After substituting Eqs. (16), (18), and (21) into Eq. (20),

$$Y_1 = \frac{V_{0y}}{V_{0z}} \left[1 - 3\left(\frac{\mu_1}{\mu_2}\right)^2 + 2\left(\frac{\mu_1}{\mu_2}\right)^3\right] L \tag{22}$$

The dimensionless separation of Species 1 and 2, Y_1, is shown in Fig. 3, where μ_1/μ_2 is varied between zero and 1. The curve is sigmoidal in shape with an inflection point at $\mu_1/\mu_2 = 0.5$

If there are more than two different types of species to be separated, their relative separation can be readily estimated from Fig. 3, where μ_2 represents the species that migrates from one membrane to the opposite one in time $t = t_1$. It should be noted that the slower migrating species shows more deflection from the direction of the carrier electrolyte than the faster one.

B. Concentration Dispersion

Brownian diffusion is neglected in the analysis. The particles with different electrophoretic migration velocities flow out of the separation chamber at different Y coordinates where each species is supposed to have its own specific exit coordinate Y_i. The difference in the Y_i coordinates

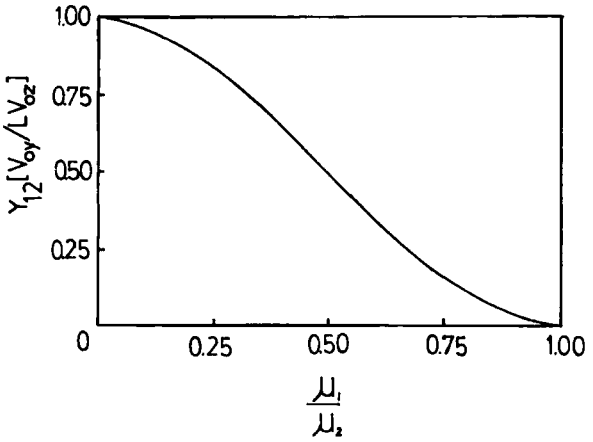

FIG. 3. Dimensionless separation of Species 1 and 2 versus the ratio of their electrophoretic mobilities.

determines the separation between the species. Unfortunately, the particles spread and flow out of the separation chamber within a certain concentration profile along the Y axis. The major reason of the dispersion is because the species are pumped into the separation chamber continuously and the electric field and the secondary buffer flow are applied periodically. This causes different entrance and exit effects which in turn lead to the varying exit X and Y coordinates.

The horizontal distance Y_1 (Eq. 22) is calculated by assuming that the species enter and exit the separation chamber along the membrane surface at exactly the start and the end of the separation cycle. Actually, the species enter and exit the separation chamber continuously and throughout the width of the gap between the membranes. If the particles enter the separation chamber at some moment within a certain time period, they may or may not end up at the membrane surface by the end of this period. Thus, the horizontal and longitudinal distances traveled by the species up to the end of one cycle and even, possibly, until the end of the next cycle will be less than that calculated in Eq. (17). The same phenomenon occurs near the exit of the chamber after the species reaches the membrane wall, where the remaining downward distance to the end of the separation chamber is less than Δz (Eqs. 14 and 17).

The concentration profile of the fractionated species can be determined from the conservation of mass in convective flow,

$$\frac{\partial c_i}{\partial t} + \bar{V}\nabla c_i + \mu_i \bar{E}\nabla c_i = 0 \tag{23}$$

Because the external fields (electric and buffer) are applied periodically, no attempt is made to solve Eq. (23) directly but, instead, the concentration profiles for the individual trajectories are calculated.

Suppose a mixture of species is fractionated in the separation chamber shown in Fig. 1, where $0 < z < L$, $0 < y < W$, and $-d < x < d$. The feed mixture containing Species A is introduced to the separation chamber in the form of an infinitely thin, uniform streak at $y = z = 0$.

If a Species A enters the separation chamber at $x_0 \in [-d,d]$ and at some time $t_0 \in [0,T]$, the x and y coordinates at $z = L$ where this species flows out of the separation chamber are calculated. In order to do this, we first calculate where the species will be at time T, at which time it should have either reached the membrane wall or not. If it did (Case a):

$$\mu E(T - t_0) \geq d - x_0$$

$$z_1 = \int_{x_0}^{d} \frac{V_{0z}}{\mu E}\left[1 - \frac{x^2}{d^2}\right]dx = \frac{V_{0z}}{\mu E}\left[\frac{2d}{3} - x_0 + \frac{x_0^3}{3d^2}\right] \tag{24}$$

If it did not (Case b):

$$\mu E(T - t_0) < d - x_0$$

$$z_1 = V_{0z}[T - t_0]\left[1 - \frac{3x_0^2 + 3x_0\mu E[T - t_0] + [\mu E[T - t_0]]^2}{3d^2}\right] \tag{25}$$

In Case b, the distance $x_0 + \mu E(T - t_0)$ is used as a new initial x coordinate for Species A for the next time period. By the end of the second time period, all species will have reached the membrane wall.

The total number of cycles required for Species A to flow through most of the length L until it reaches the membrane wall is then determined, where the remaining distance to the end of the separation chamber is less than Δz (Eq. 17). By knowing the remaining distance in the z direction, the exact time it will take Species A to flow out of the separation chamber during the last time cycle is calculated.

After determining all the steps in the z direction, the same calculations are then repeated in the x and y directions. Thus, the x and y coordinates at $z = L$, where Species A leaves the separation chamber, are obtained.

Next, the trajectories for all species that enter the separation chamber throughout the width of the separation chamber $[-d,d]$ and from the beginning to the end of the time period $[0,T]$ are determined. This is done by dividing the width of the chamber $[-d,d]$ into the N coordinates and the time period T into the M smaller periods. As a result, $M \cdot N$ different trajectories are determined. The relative density $\Phi(y)$ of the exit trajectories is then calculated, where the density of the exit points is averaged over time (0 to T) and the x coordinate ($-d$ to d), and is also multiplied by the relative flow factor.

$$\Phi(y) = \int_0^T \int_{-d}^{d} C(x,y,z=\mathrm{L}) \cdot V_z dx dt / T \int_{-d}^{d} V_z dx \tag{26}$$

A computer program was written to calculate concentration profiles for the species flowing out of the separation chamber.

RESULTS AND DISCUSSION

The results of calculations are shown in Figs. 4, 5, and 6. In all calculations the fractionation chamber was taken to be 40 cm long and 0.1 cm wide, the electric field was 100 V/cm, the buffer velocity in the z direction was $V_{0z} = 0.3$ cm/s, and the periodical buffer velocity in the y direction was $V_{0y} = 0.15$ cm/s.

Figure 4 shows the results of fractionating four different types of species with electrophoretic mobilities of 2.0, 1.5, 1.25, and 1.0 ($\mu m \cdot cm/V \cdot s$). These values of the electrophoretic mobility are common for real cells and proteins. The time T was taken to be 10 s for this fractionation and the idle time t_1 was 5 s. In this fractionation mode the fastest migrating species were able to cross the fractionation chamber during the idle time t_1, and the slowest migrating species took the full time T to travel across the chamber. Complete separation of all the species is predicted in this separation mode.

Figure 5 shows the results of fractionating another mixture of differently migrating species under the same operating conditions of $T = 10$ s and $t_1 = 5$ s. This time the species were not completely separated, with the most overlapping of the concentration profiles occurring between the species with 2.0 and 1.9 $\mu m \cdot cm/V \cdot s$ electrophoretic mobilities.

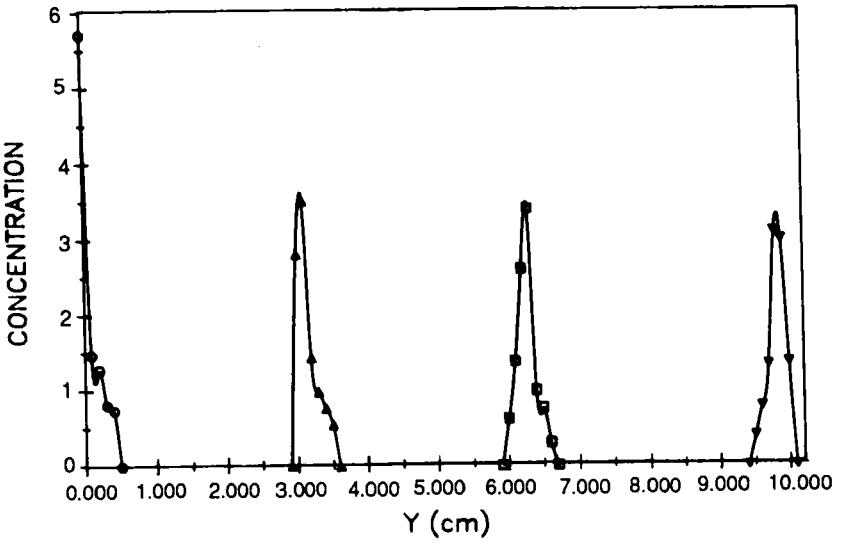

FIG. 4. Concentration profile of particles flowing from the separation chamber versus the distance migrated in the Y direction. [Mobilities in units of $\mu m \cdot cm/V \cdot s$: (○) 2.0, (▲) 1.5, (□) 1.25, and (▼) 1.0.] Cycle time T = 10 s, and idle time t_1 = 5 s.

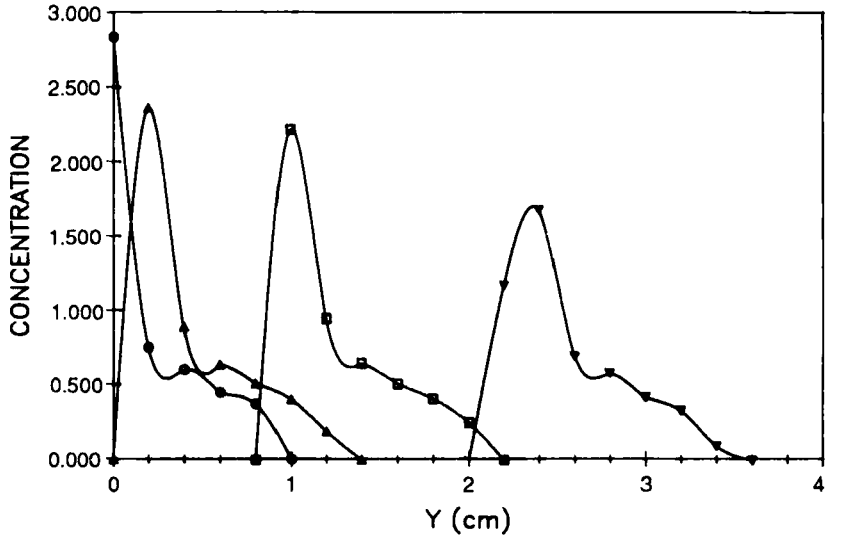

FIG. 5. Concentration profile of particles flowing from the separation chamber versus the distance migrated in the Y direction. [Mobilities in units of $\mu m \cdot cm/V \cdot s$: (○) 2.0, (▲) 1.9, (□) 1.8, and (▼) 1.7.] Cycle time T = 10 s, and idle time t_1 = 5 s.

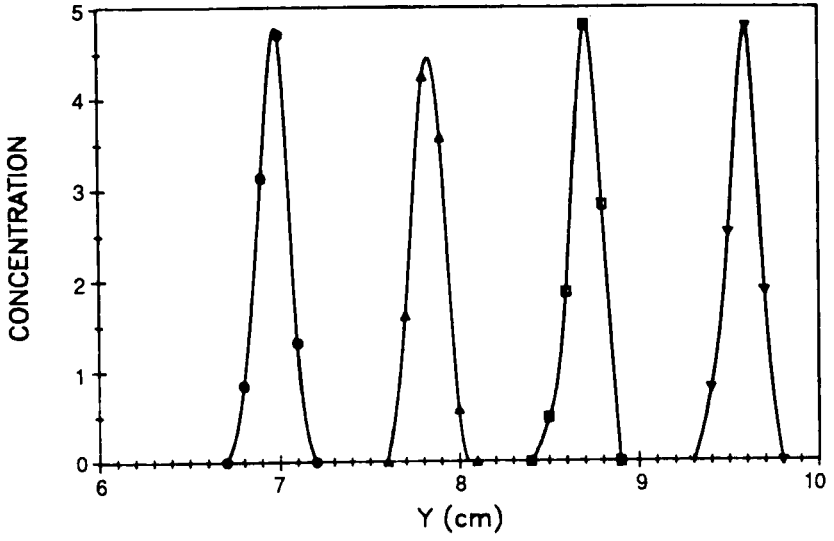

FIG. 6. Concentration profile of particles flowing from the separation chamber versus the distance migrated in the Y direction. [Mobilities in units of μm · cm/V · s: (○) 2.0, (▲) 1.9, (□) 1.8, and (▼) 1.7.] Cycle time T = 6 s, and idle time t_1 = 3 s.

In order to choose better operating parameters for fractionating this mixture, we need to look at Fig. 3. The best relative separation is predicted when the ratio of the electrophoretic mobilities between some particular species and a hypothetical fastest migrating species is approximately 0.5. By the hypothetically fastest migating species (μ_2) we mean the species that travels across the separation chamber during the idle time and that reaches the opposite membrane wall just before periodic buffer flow starts. Based on this approach, we choose new operating conditions where T = 6 s and t_1 = 3 s. The results of this fractionation are shown in Fig. 6, where complete separation of species is predicted.

CONCLUSIONS

Electrophoresis with a properly designed alternating electric field and variable buffer flow has been shown to be capable of fractionating charged species where the electrophoretic mobilities may differ by as little as 0.1 μm · cm/V · s with good resolution. Biological particles that typically

need to be separated (red blood cells, lymphocytes, organelles, proteins, etc.) have electrophoretic mobilities from 0.3 to 3.0 $\mu m \cdot cm/V \cdot s$, and their electrophoretic mobilities typically differ by at least 0.1 $\mu m \cdot cm/V \cdot s$.

The separation is not limited by the electroosmotic and crescent flow phenomena which are typical of existing thin film systems (*1*).

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