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Continuous Free Flow Electrophoresis in an Alternating Two-Dimensional Electric Field

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Abstract

A method of continuous free flow electrophoresis in an alternating two-dimensional electric field is proposed as a basis for continuous separation of biomolecules. Fractionation of species with different electrophoretic mobilities is investigated theoretically. Numerical calculations on particles' trajectories and dispersion are presented.

INTRODUCTION

Continuous free flow electrophoresis is used for fractionating cells, organelles, viruses, membrane proteins, and other solutes that are difficult or impossible to fractionate by other methods such as chromatography, centrifugation, solvent extraction, and filtration.

The most often used continuous free flow electrophoresis method is a "thin-film" approach, where a narrow streak of a feed mixture is fractionated by flowing a thin film of the carrier electrolyte between two parallel electrodes (*1*). This method is limited by a small throughput, typically 5–10 mL/h, and poor resolution.

Giddings et al. (*2*, *3*) proposed a method for field-flow fractionation of different particles and macromolecules in which the species were separated into thin layers across a narrow slit. Giddings (*4*) also described a cyclical-field flow fractionation (CFFF) in which the field strength and/or direction was cycled many times during a run. CFFF was implemented experimentally with a flow by gravity (*5*).

Lightfoot et al. (*6*) fractionated a mixture of solutes inside hollow fibers by applying an electric field across the fiber for a limited period of time. After the electrical field was terminated, different solutes came out of the hollow fiber at separate time intervals.

A new concept for continuous free flow electrophoretic separation which employs a combination of an alternating electric field and a pulsating secondary buffer flow was proposed by Shmidt and Cheh (*7*). In this method, charged solutes move in three different directions, with the main carrier electrolyte flow, in the electric field perpendicular to the main buffer flow, and in a secondary periodical buffer flow perpendicular both to the main buffer flow and the electric field. This movement is complex and requires a precise buffer flow. General principles of two-dimensional field flow fractionation method were discussed in a recent paper by Giddings (*8*).

In this paper a new concept is proposed for a continuous free flow electrophoresis which employs an alternating two-dimensional electric field. In this method the charged solutes move in three different directions, continuously flowing downward with the main buffer flow and migrating electrophoretically in two other perpendicular directions, as shown in Fig. 1a.

Electrophoretic fractionation is carried inside a narrow slit between two flat, semipermeable, preferrably uncharged, hydrophilic membranes as shown in Fig. 1a. The ion-permeable membranes let the electric current through but do not allow the passage of any solute. Two electrodes, A–A, are placed behind the membranes to provide an alternating electric field across the gap, as shown in Fig. 1b. A steady stream of carrier electrolyte is pumped downward through the gap between the membranes. Another pair of electrodes, B–B, is placed at the opposite sides of the gap, as shown in Fig. 1b. A mixture of charged species is introduced in a narrow streak in the upper corner between the membranes. An electric field is applied for a certain period of time between the first pair of electrodes across the gap perpendicular to the membrane surface. Then an electric field parallel to the membrane surface between a second pair of electrodes is applied for a specified time period. Immediately thereafter, the electric field perpendicular to the membrane surface between the first pair of electrodes is applied again, only in the opposite direction. This cycling of the electric field is continued throughout the separation.

As the species move downward with the buffer flow, they migrate from one membrane wall to the other, driven by the alternating electric field. When the species reach the membrane wall, they stay motionless until the electric field parallel to the membrane surface is applied. As the species migrate along the membrane surface, diffusion away from the membrane is minimized by the carrier electrolyte flowing through the membrane. When the direction of the electric field is changed by 90°, the species migrate across the gap to the opposite membrane.

Faster migrating species take less time to move across the gap, and as

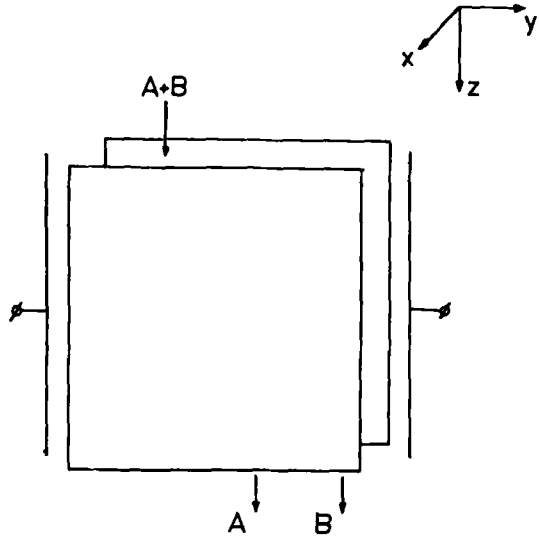

FIG. 1a. Schematic drawing of the separation chamber.

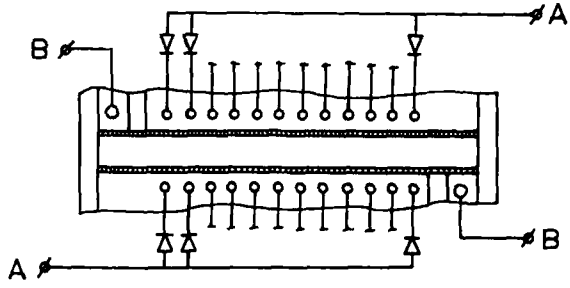

FIG. 1b. Cross-sectional view of the separation chamber.

a result, move less downward than the slower migrating species. At the same time, faster species migrate further along the membrane surface during each cycle. Thus, a dual fractionating effect is achieved, where faster species require more cycles to move through the length of the separation chamber and also migrate further along the surface during each cycle.

Therefore, different trajectories for the faster and slower migrating species are established, and these are responsible for the 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 with pores much smaller than the size of the solute particles. A schematic drawing of the separation chamber is shown in Fig. 1a. The two membranes are separated by a distance $2d$.

A continuous, steady flow of 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 A–A placed behind the membranes, as shown in Fig. 1b. 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 (*9*). The electroosmotic flow reverses its direction when the electric field is alternated. After the species have reached the membrane, the electric field is changed 90° and the species migrate along the membrane surface. To limit current leakage and to establish a uniform electric field along the membrane surface, electrodes A–A can be separated into multiple strands connected to a power supply through unidirectional diodes. The diffusion of particles away from the membrane surface is prevented by the carrier electrolyte flowing through the membrane. The transmembrane flow is achieved by a pressure differential across the membrane. The field E_y causes electroosmotic flow in the y direction along the membrane surface. After a certain period of time the electric field E_y is terminated and the electric field E_x is applied again. The electric field E_y cannot be applied continuously because it would result in a nonuniform electric field.

The two-dimensional electric field can be described by

$$\mathbf{E} = E_x f_x(t)\mathbf{i} + E_y f_y(t)\mathbf{j} \tag{1}$$

where E_x and E_y are the magnitudes of the electric field in the x and y directions as shown in Fig. 2, and $\mathbf{i}$ and $\mathbf{j}$ are unit vectors in the x and y directions. $f_x(t)$ and $f_y(t)$ are periodic time functions, as shown in Fig. 2, and can be expressed by

$$f_x(t) = \sum_{1}^{\infty} (-1)^n[\theta(t - nT) - \theta(t - nT - T_x)] \tag{2}$$

$$f_y(t) = \theta(t - T_x) + \sum_{1}^{\infty} [\theta(t - nT - T_x) - \theta(t - nT)] \tag{3}$$

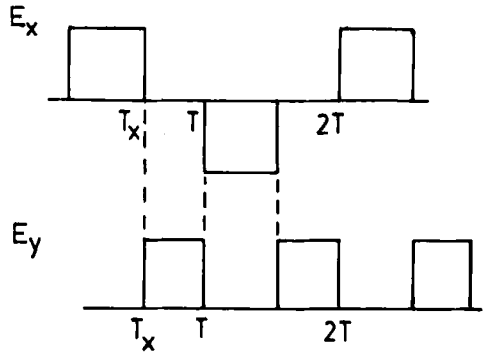

FIG. 2. Electric field and fluid velocity with time.

where $\theta(t)$ is a Dirac function which is equal to 1 when t is positive and 0 when t is negative. Time T is the half-period of a complete cycle, and time T_x is the period when the electric field is in the x direction.

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

$$V_z = V_{0z}\left[1 - \frac{x^2}{d^2}\right] \tag{4}$$

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

The electroosmotic flow in the separation chamber is assumed to be zero for the uncharged membranes (*9*).

A heterogeneous sample is introduced into the separation chamber through the upper corner, and it flows downward with a continuous steady film of the carrier electrolyte. Charged species are deflected from the direction of the carrier electrolyte flow in the x and y directions. The velocity for species i, $\mathbf{V}_i$, is comprised of the electrophoretic migration velocity in the x and y directions and the carrier electrolyte velocity in the z direction.

$$\mathbf{V}_i = \mu_{xi}E_x f_x(t)\mathbf{i} + \mu_{yi}E_y f_y(t)\mathbf{j} + V_z f_x(t)\mathbf{k} \tag{5}$$

where μ_{xi} is the electrophoretic mobility of species i in the x direction, and μ_{yi} is the electrophoretic mobility of species i along the membrane surface in the y direction. $\mathbf{k}$ is a unit vector in the z direction.

In a general situation, the heterogeneous feed contains N species with electrophoretic mobilities μ_{xi} and μ_{yi}. Let

$$\mu_{xi+1} > \mu_{xi} \text{ and } \mu_{yi+1} > \mu_{yi}, \qquad i = 1, \ldots, N \tag{6}$$

As a first approximation we only calculate trajectories of species that are able to migrate from one membrane wall to the opposite during the time interval T_x.

$$\mu_{xi} E_x T_x \geq 2d, \qquad i = 1, \ldots, N \tag{7}$$

During a single time cycle, species i is carried with the buffer flow in the z direction while it crosses the gap. It then migrates in the y direction along the membrane surface for a time period $(T - T_x)$.

$$dz_i = V_{0z}\left[1 - \frac{x^2}{d^2}\right] dt \tag{8}$$

$$dy_i = \mu_{yi} E_y dt \tag{9}$$

An integration of Eqs. (8) and (9) during a single time cycle provides the change in the y and z coordinates for species i during that cycle.

$$\Delta z_i = \frac{4V_{0z}d}{3\mu_{xi}E_x} \tag{10}$$

$$\Delta y_i = \mu_{yi} E_y (T - T_x) \tag{11}$$

where Δz_i and Δy_i are the changes in the z and y coordinates for species i after each cycle.

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

$$m_i = \frac{3L\mu_{xi}E_x}{4V_{0z}d} \tag{12}$$

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.

$$Y_i = \frac{3\mu_{xi}\mu_{yi}E_xE_y(T - T_y)}{4V_{0z}d} \tag{13}$$

where Y_i determines the exit point for species i. If the electrophoretic mobility is the same along the membrane surface as in the bulk, i.e., $\mu_{xi} = \mu_{yi} = \mu_i$, and the electric field is the same in the x and y directions, i.e., $E = E_x = E_y$, then the distance Y_i is

$$Y_i = \frac{3\mu_i^2 E^2 (T - T_x)}{4V_{0z}d} \tag{14}$$

In contrast to conventional thin-film electrophoretic separation methods in which migration distances are proportional to the migration velocities, the distance Y_i is proportional to the square of the migration velocity $\mu_i E$.

The relative separation of two or more species is equal to the difference of their respective distances Y_i

B. Concentration Dispersion

In this analysis, Brownian diffusion is neglected. Particles with different electrophoretic velocities flow out of the separation chamber at different Y coordinates, and each species is supposed to have its own exit coordinate, Y_i. The difference in the Y_i coordinates determines the separation of species. Unfortunately, particles spread and flow out of the separation chamber within a certain dispersion zone along the Y axis.

There are two major reasons for the dispersion. One reason is that dispersion occurs when particles migrate along the membrane surface. The same type of particles exhibits different migration velocities due to irregularities in the membrane surface, nonuniform transmembrane flow, and ionic and hydrophobic particle–membrane interactions. Another reason for dispersion is that the species are injected into the separation chamber continuously and the electric field is applied periodically. This causes different entrance and exit effects which in turn lead to varying exit X and Y coordinates.

The horizontal distance Y_i is calculated from Eq. (13) by assuming that species enter and exit the separation chamber along the membrane surface exactly at the start and the end of a separation cycle. Actually, the species enter and exit the separation chamber continously 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 reach the membrane surface by the end of this period. Thus, the horizontal and longitudinal distances traveled by the species for a complete cycle will be less than that calculated from Eqs. (10) and (11). The same phenomenon occurs near the exit of the chamber after the species reach the membrane wall, where the remaining downward distance to the end of the separation chamber is less than Δz as calculated from Eq. (10).

The concentration profile of the fractionated species can be determined from individual trajectories. Suppose a mixture of species is fractionated in a separation chamber as shown in Fig. 1a, where $0 \leq z \leq L, 0 \leq y \leq W$, and $-d \leq x \leq d$. 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 species A enters the separation chamber at x_0, where x_0 is between $[-d,d]$, and at some time t_0 between $[0,T]$, the x and y coordinates are calculated at the exit, $z = L$, where the species flows out of the separation chamber. It is first calculated where the species will be at the end of the first time cycle T, regardless of whether it has reached the membrane wall or not. After determining the exact distance where the particle first reached the membrane surface in either the first or the second time cycle, the total number of complete cycles required for species A to flow through length L is then determined. Knowing the remaining distance to the end of the separation chamber (less than Δz), the exact time it will take species A to flow out of the chamber during the remaining time cycle is calculated. After determining all steps in the z direction, the same calculations are repeated for the y direction. Finally, the X and Y coordinates at $z = L$, where species A leaves the chamber, are obtained.

The algorithm and the results of calculations are shown as follows.

A. The membrane wall is reached during the 1st time cycle.

$$\frac{d - x_0}{\mu_{xi} E_x} \leq T_x - t_0 \tag{15}$$

where $(d - x_0)$ is the distance to the wall.

1. The distance traveled in the z direction during the 1st step is Δz.

$$\Delta z = \int_{-d}^{d} V_{0z} \left(1 - \frac{x^2}{d^2}\right) dt = \frac{V_{0z}}{\mu_{xi} E_x} \left(\frac{2}{3} d - x_0 + \frac{x_0^3}{3d^2}\right) \tag{16}$$

2. Complete number of steps N.

$$N = \frac{3\mu_{xi} E_x}{4 V_{0z} d} (L - \delta z) \tag{17}$$

3. Distance traveled in the Y direction.

$$Y = (N + 1)\mu_{yi} E_y (T - T_x) \tag{18}$$

4. Distance traveled during the last step, Δz_f.

$$\Delta z_f = \frac{V_{0z}}{\mu_{xi}E_x}\left(x - \frac{x^3}{3d^2}\right), \qquad \text{STOP} \tag{19}$$

B. The membrane is reached during the 2nd time cycle, $t_0 > T_x$.

$$(d - x_0)/\mu_{xi}E_x > T_x - t_0 \tag{20}$$

1. The distance traveled in the Y direction during the 1st time cycle, Δy_0.

$$\Delta y_0 = \mu_{yi}E_y(T - t_0) \tag{21}$$

2. Distance traveled during the 1st time cycle, Δz_0.

$$\Delta z_0 = \frac{V_{0z}}{\mu_{xi}E_x}\left(\frac{2d}{3} + x_0 - \frac{x_0^3}{3d^2}\right) \tag{22}$$

3. Go to Step A-2.

C. The membrane is reached during the 2nd time cycle, $t_0 < T_x$.

1. Distance traveled in the z direction during the 1st time cycle, Δz_0.

$$\Delta z_0 = \frac{V_{0z}}{\mu_{xi}E_x}\left((T_x - t_0)\mu_{xi}E_x - \frac{[x_0 + (T_x - t_0)\mu_{xi}E_x)^3}{3d^2} + \frac{x_0^3}{3d^2}\right) \tag{23}$$

2. Go to Step A-1.

Next we determine 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 $[0,T]$. This is done by dividing the width of the chamber $[-d,d]$ into N coordinates and the time period T into M smaller periods. As a result, we calculate $M{\cdot}N$ different trajectories. Then we calculate the relative density $\Phi(y)$ of the exit trajectories, 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.

$$\Phi(y) = \int_0^T \int_{-d}^{d} C(x,y,z=L){\cdot}V_z dx dt / T{\cdot}\int_{-d}^{d} V_z dx \tag{24}$$

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

C. Temperature Field

The electric field dissipated in the gap is removed by continuously recirculating cooling electrolyte streams between the backside of the membranes and the electrodes. A schematic view of the separation chamber is shown in Fig. 3, where $2x_1$ is the width of the fractionation gap and $(x_2 - x_1)$ is the width of the membrane. The temperature is calculated from the conservation of thermal energy in the steady state.

$$C_v V \nabla T + \nabla k \nabla T + \sigma E_0^2 = 0 \tag{25}$$

where C_v is the volumetric heat capacity, k is the thermal conductivity, σ is the electric conductivity, V is the fluid velocity, and E_0 is the electric field strength.

For the negligibly small flow along and normal to the membrane surface (*10*), Eq. (25) is reduced to

$$k_i \frac{d^2T}{dx^2} + \sigma_i E_i^2 = 0, \qquad i = 1, 2 \tag{26}$$

where indexes 1 and 2 refer to the separation chamber and the membrane cross section, respectively. The solution to Eq. (26) subject to boundary conditions is, at $x = 0$:

$$dT/dx = 0 \tag{27}$$

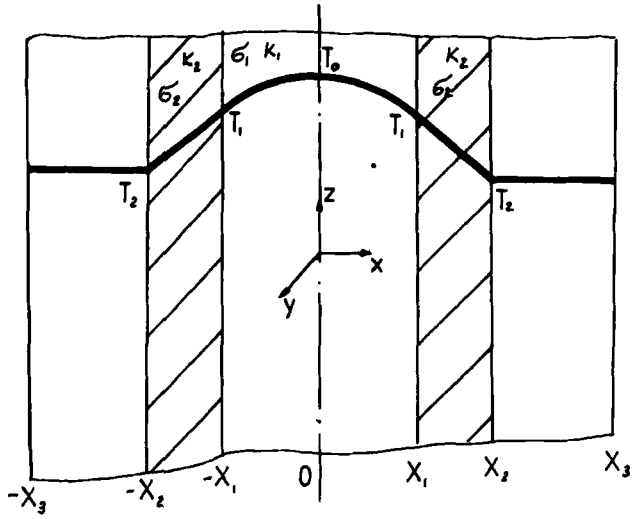

FIG. 3. Calculated temperature profile in the separation chamber.

at $x = x_1$:

$$k_1 \left.\frac{dT}{dx}\right|_{x \to x_1 + 0} = k_2 \left.\frac{dT}{dx}\right|_{x \to x_1 - 0} \tag{28}$$

at $x = x_2$:

$$T = T_2 \tag{29}$$

is

$$T_0 = \frac{\sigma_1 E_1^2}{2k_1} x_1^2 + \frac{\sigma_2 E_2^2}{2k_2} \left[(x_2 - x_1)^2 + \frac{2E_1 x_1}{E_2} (x_2 - x_1) \right] + T_2 \tag{30}$$

and

$$T_1 = \frac{\sigma_2 E_2^2}{2k_2} \left[(x_2 - x_1)^2 + \frac{2E_1 x_1}{E_2} (x_2 - x_1) \right] + T_2 \tag{31}$$

RESULTS AND DISCUSSION

The fractionation of a mixture of four different species with electrophoretic mobilities of 1.0, 1.1, 1.2, and 1.5 μm·cm/V·s was modeled, and the results of the calculations are shown in Figs. 4 and 5.

Figure 4 shows the results of fractionating in a separation chamber 20 cm long and 0.1 cm wide, with the electric field $E_x = E_y = 100$ V/cm, buffer flow velocity in the z direction $V_{0z} = 0.3$ cm/s, time T of 20 s, and time T_x of 10 s. In this fractionation mode the slowest migrating species took the full time T_x to migrate across the chamber from one membrane wall to the other. The species are shown by circles (1.0 μm·cm/V·s), triangles (1.1), inverted triangles (1.2), and squares (1.5), respectively. Complete separation of the 1.5 μm·cm/V·s mobility species and partial separation of the other three species are expected. The relative separation between bands of different particles increases proportionally to the square of the electrophoretic mobility.

To achieve complete separation of all four species, the length of the separation chamber was increased to 40 cm; all other operating parameters remain unchanged. The results of the calculations are shown in Fig. 5. All species were completely separated into four distinct bands.

When there is an electric field between electrodes B–B, some of the current goes through and behind the membranes. To limit the current leakage, electrodes A–A can be separated into individual strands connected to the power supply through unidirectional diodes, as shown in Fig. 1b.

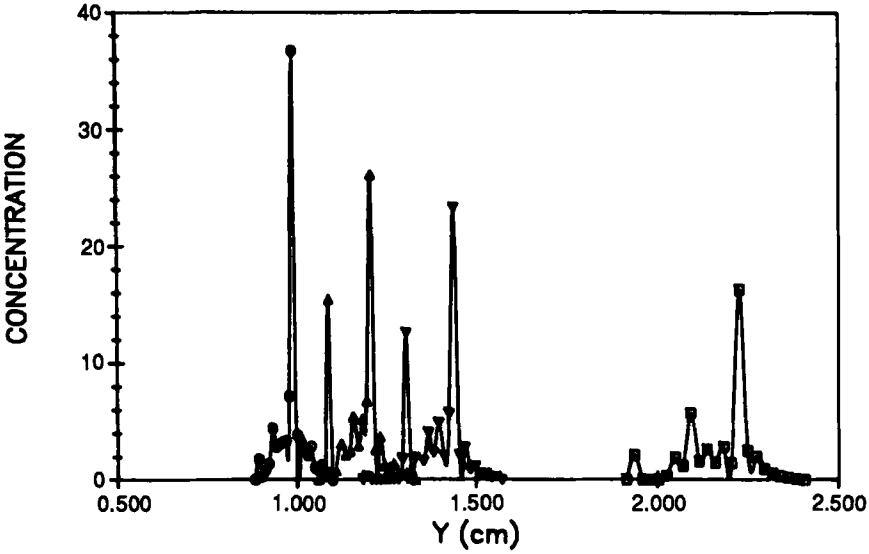

FIG. 4. Calculated concentration profile of particles flowing from the separation chamber versus the distance migrated in the Y direction. Chamber length $L = 20$ cm.

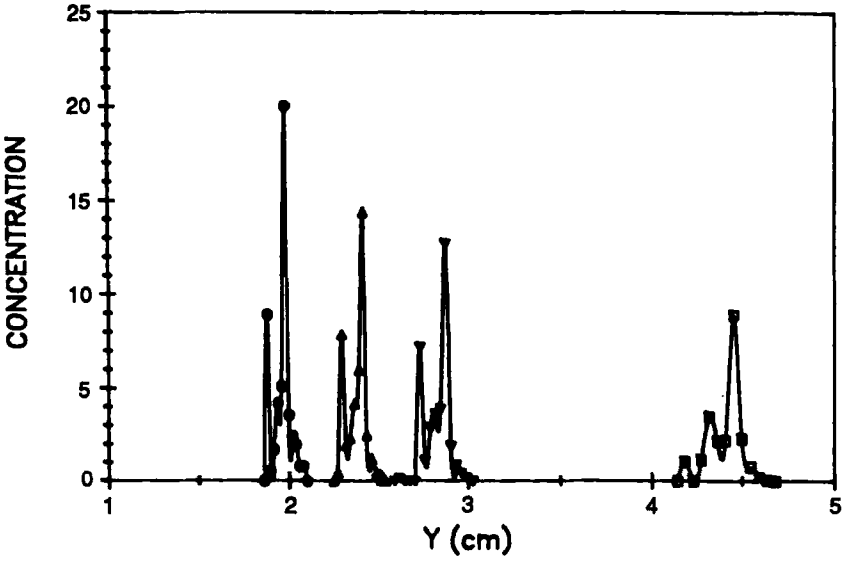

FIG. 5. Calculated concentration profile of particles flowing from the separation chamber versus the distance migrated in the Y direction. Chamber length $L = 40$ cm.

The temperature rise in the separation chamber was calculated from Eq. (30) to be 12.2°C, with $k_1 = 0.006$ W/cm·°C, $k_2 = 0.003$ W/cm·°C, $\sigma = 0.002$ (ohm·cm)$^{-1}$, $x_1 = 0.05$ cm, $x_2 = 0.07$ cm, and $E_x = E_y = 100$ V/cm.

CONCLUSIONS

Continuous free flow electrophoresis with an alternating two-dimensional electric field allows the fractionation of particles in proportion to the square of their respective electrophoretic mobilities. This method is expected to have good resolution and to be useful for separating a wide range of biomaterials including peptides, proteins, organelles, and whole cells.

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