Oscillatory Cross-Flow Electrophoresis

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Electrophoresis is a useful analytic technique for the separation of proteins and other large molecules, but has proven resistant to scale-up to production levels. In this article we describe a new technique that permits the use of much greater feed rates than possible in a conventional electrophoresis unit. The technique, in its simplest form similar to the cyclical field-flow fractionation technique developed by Giddings (1986) and extended by Shmidt and Cheh (1990), relies on the application of an oscillatory electric field across the narrow gap of the electrophoretic cell. The motion of the solute species induced by this field interacts with an oscillatory cross-flow to cause a separation based on the electrophoretic mobility of the species. This horizontal separation is combined further with a gradient in the strength of the oscillating electric field and oscillations in the vertical downflow to lead to different steady streamlines for species with different mobilities.

Introduction

Electrophoresis, originally developed over 50 years ago by Tiselius (1937), has proven to be an excellent analytical technique for the separation and purification of proteins and other biological macromolecules. Unfortunately, efforts to use the technique to produce large quantities of pure proteins have met with limited success. The primary impediments to scale-up of electrophoresis are many and complex, depending on the exact form of the electrophoresis technique employed. An excellent review of recent advances in electrophoresis techniques aimed at producing production level quantities of proteins is provided by Ivory (1988).

One attractive form of electrophoresis is known as free-flow electrophoresis. In the continuous, thin-film form of this technique, a uniform flow of a buffer is induced in the gap between two thinly-spaced plates (typical gap widths are on the order of 0.5 mm to 1 mm). A protein solution is injected at a point at one end of the cell and a transverse electric field is used to separate species with different electrophoretic mobilities into bands as they move with the buffer flow down the device. This approach to electrophoresis is attractive, since it is simple and allows proteins to be separated in their natural buffer—no additional chemicals need to be added to achieve separation which may later have to be removed.

Unfortunately, free-flow electrophoresis suffers from several drawbacks that ultimately limit the rate at which proteins can be separated. The primary impediments are Joule heating due to the large electric fields required to achieve separation, and loss in resolution due to crescent formation and electro-osmosis. The heating effect limits scale-up by placing an upper limit on the allowable gap width of the channel, in that wider gaps are more difficult to cool and are more sensitive to thermally-induced instabilities which would limit resolution. Crescent formation and electro-osmosis limit throughput by causing a nonuniform trajectory for species with the same electrophoretic mobility across the width of the thin gap, and hence increase the length/velocity ratio required for adequate resolution. As a consequence of these difficulties, the maximum feed rate of a conventional thin-film electrophoresis cell is about 4 mL/h. Efforts have been made to improve this feed rate through the use of different geometries [for example, stress stabilized free electrophoresis embodied in the commercially available Biostream separator (Philpot, 1973)] and through the use of recycle streams (Gobie et al., 1985); however, these approaches will not be discussed further here.

In this article we describe a novel approach, termed oscillatory cross-flow electrophoresis, which overcomes many of the difficulties involved in scaling up thin-film electrophoresis. The technique will be shown to increase throughput by more than three orders of magnitude over a conventional thin-film unit, while simultaneously concentrating the solute by approximately a factor of 20. The technique, in its simplest form similar to the cyclical field-flow fractionation technique developed by Giddings (1986) and extended by Shmidt and Cheh (1990), relies on the interaction of solute motion produced by an oscillatory electric field applied across the thin gap of an electrophoresis channel (the y direction in Figure 1) and an

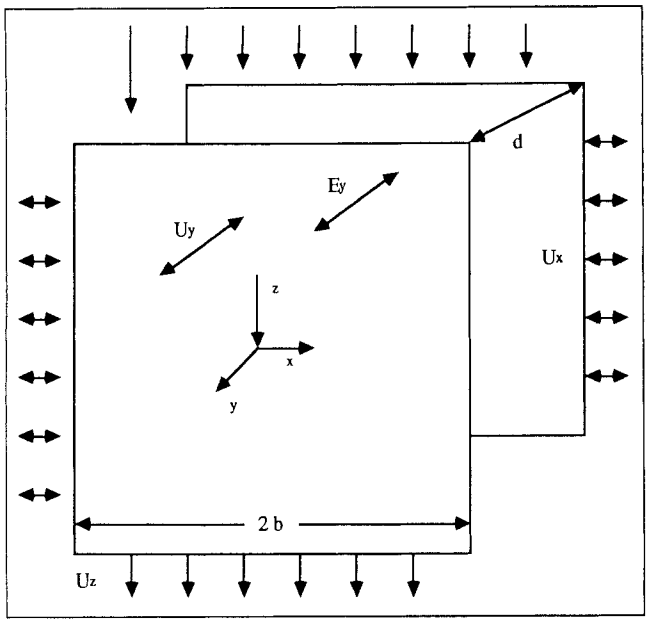


Figure 1. Electrophoresis cell.

oscillatory cross-flow in the x direction. This motion, combined with flow in the z direction will result in the continuous separation of species with different electrophoretic mobilities in much the same manner as conventional electrophoresis.

It has long been recognized that the interaction of an oscillatory field force with an oscillatory convective flow will separate species. Oscillatory gravity (produced by periodically inverting a device) has been employed by Giddings (1986) in the field-flow fractionation of particles. Giddings also suggested that an oscillatory electric field can lead to separation based on electrophoretic mobility. A general theory for the interaction of an oscillatory field force with oscillatory convection has recently been developed by Brenner and Shapiro (1990a), who applied it to particles sedimenting in a channel (Brenner and Shapiro, 1990b). In the next section we provide a simple analytic analysis for the interaction of motion induced by an oscillatory electric field with an oscillatory cross-flow in the limit of large Peclet numbers (the appropriate limit for the large molecules conventionally used in electrophoretic separations). While less general than the analysis of Brenner and Shapiro, which explicitly included the effects of diffusion, by restricting ourselves to this limit we obtain a very simple, closed-form solution for the time-averaged solute velocities. In particular, we demonstrate that if the position of the particle across the narrow width of the channel is sinusoidal in time, then the time average translational velocity in the x direction of these molecules is solely a function of three Fourier modes of an arbitrary time-dependent cross-flow. By combining this motion with a uniform downflow, a simple relation is derived for the degree of separation which may be achieved for species with differing electrophoretic mobilities. It is shown that the scheme suggested by Shmidt and Cheh (1990) is a special case of the general oscillatory cross-flow investigated here.

In the third section we explore the effect of adding an oscillatory component to the downflow (z direction). We show that appropriately chosen Fourier modes can retard the velocity of the species moving down the channel relative to that of the aqueous medium. This retardation can dramatically increase the degree of separation and shorten the required length of the electrophoretic cell. In the fourth section we present the primary result of our work, in which we combine these ideas with a gradient in the amplitude of the oscillating electric field in the x direction. The combination of such a gradient with the oscillatory cross-flow leads to a different steady-state position in the x direction for species with different electrophoretic mobilities. It is thus possible to feed the solution to be separated uniformly across the top of the electrophoretic cell and have separated streams emerge at the bottom, by analogy with the conventional thin-film technique of iso-electric focussing. With this scheme, however, no recycles or pH gradients are necessary. The final section summarizes our results and offers suggestions on how a practical device based on these principles can be constructed.

Solute Motion with Oscillatory Cross-Flow

Consider the electrophoresis cell depicted in Figure 1. The combination of a vertical down-flow, an oscillatory cross-flow, and an oscillating electric field will give rise to a time-averaged motion which depends on the electrophoretic mobility of each species. To see how this works, consider the trajectories of two molecules with different electrophoretic mobilities initially located at a wall shown in Figure 2. If we subject these molecules to a sinusoidally oscillating electric field, then they will move away from the wall in the y direction in an oscillatory fashion (Figure 2a). The amplitude of this motion and the position of the particle in the gap as a function of time, however, will differ for the two species. As a consequence, combining this oscillation with an oscillatory cross-flow (here we have taken the oscillatory cross-flow to be the first overtone of the oscillatory y-direction motion) and a steady down-flow will lead to the time-dependent x- and z-direction displacements shown in Figures 2b and 2c, respectively. Combining these x- and z-direction motions we obtain the two-dimensional trajectories given in Figure 2d, which shows that the species separate as they move down the channel, in much the same manner as in conventional electrophoresis. In this section we wish to quantify the relationship between the time-averaged trajectory and the fluid and field oscillations.

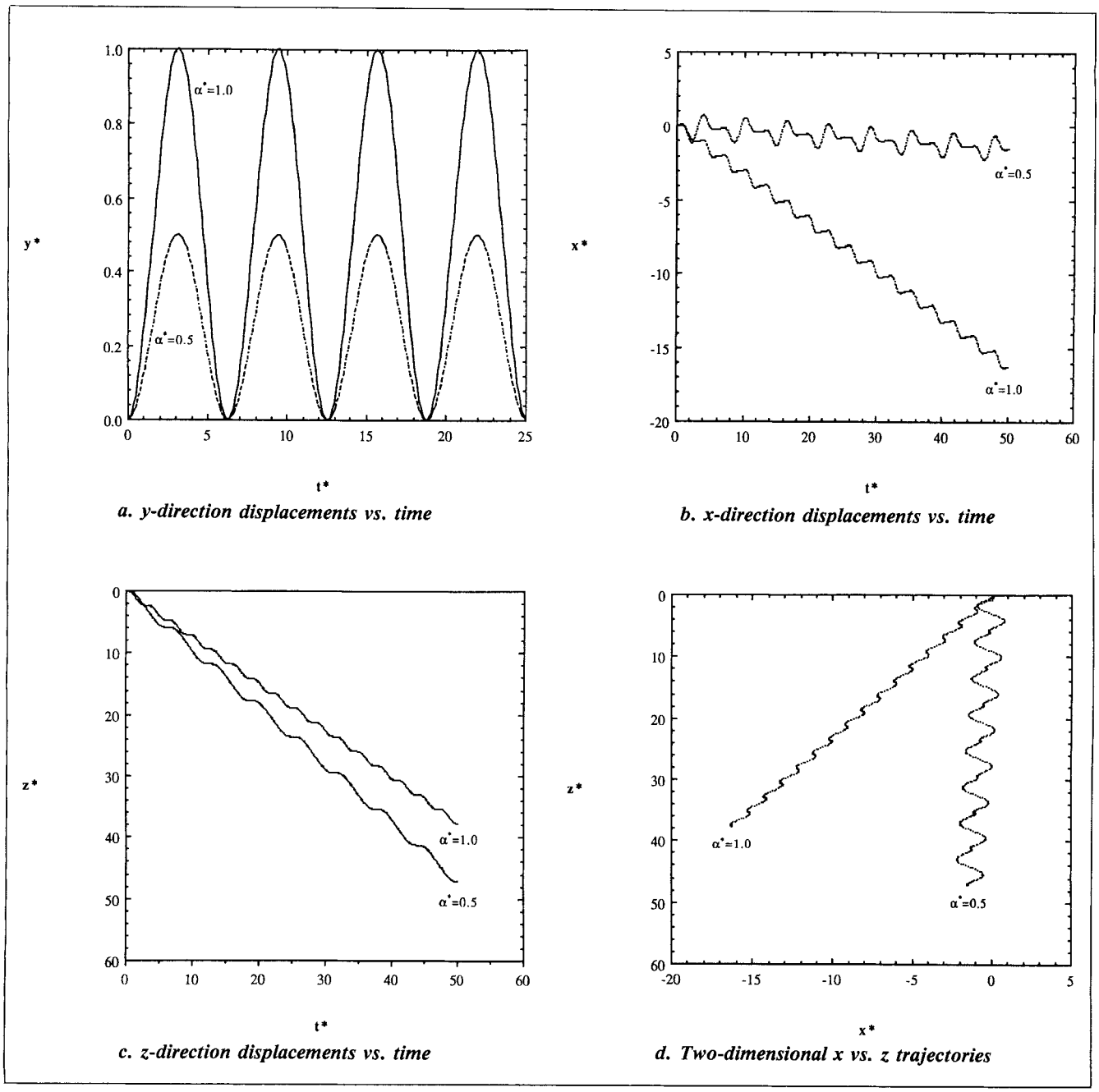
We shall take the vertical convective motion to be given by:

$$u_z = 6u_{z0}\frac{y}{d}\left(1-\frac{y}{d}\right) \tag{1}$$

where u_{z0} is the spatially-averaged magnitude of the down-flow and d is the gap width. The position of the molecules in the gap will be oscillatory in time, driven by the oscillatory electric field. We shall drive the molecules with the general oscillating field given by:

$$E=\frac{E_0}{\omega}\frac{d}{dt}[g(\omega t)] \tag{2}$$

where g is a positive periodic function, ω is the angular frequency of oscillation, and E_0 is the characteristic amplitude of the electric field. To simplify the problem and obtain an analytic solution for the solute motion, we shall restrict ourselves to the case where all solute molecules with the same sign



a. y-direction displacements vs. time

b. x-direction displacements vs. time

c. z-direction displacements vs. time

d. Two-dimensional x vs. z trajectories

Figure 2. Time-dependent displacements in oscillatory cross-flow and steady down-flow.

Cross-flow Fourier components were chosen as $B_0 = 2/29$, $B_1 = 0$, and $B_2 = 1.0$.

charge are in phase, and are at a position $y = 0$ at times $t = 0$, $2\pi/\omega$, and so on. This is achieved easily by adding a slight bias to the oscillating electric field (much smaller than the amplitude E_0) or by adding a dwell time during which the fluid velocity is zero and the electric field is set to a constant maximum value. Species of opposite charge will, of course, migrate to the opposite surface; however, they will otherwise behave the same. We shall also choose the parameters E_0, ω, and d such that the molecule of interest with the largest electrophoretic mobility μ_H just reaches the opposite side of the gap $d = h$ during each period. For these conditions, the motion of the solute molecules in the y direction is given by:

$$\frac{dy}{dt} = \frac{\mu E_0}{\omega} \frac{d}{dt} [g(\omega t)] \tag{3}$$

or by integrating,

$$\frac{y}{d} = \alpha g(\omega t) \tag{4}$$

where we have introduced the dimensionless group $\alpha = \mu E_0 / \omega d$ which is a measure of the amplitude of the oscillatory motion of the solute.

If we introduce the x-direction cross-flow:

$$u_x = 6u_{x0}\frac{y}{d}\left(1-\frac{y}{d}\right)f(\omega t) \tag{5}$$

where u_{x0} is the characteristic amplitude of the cross-flow and $f(\omega t)$ is an $O(1)$ function of time which may contain both oscillatory and steady components, the resulting z- and x-direction velocities of the solute are given by:

$$\frac{dz}{dt} = 6u_{z0}\alpha g(\omega t)[1-\alpha g(\omega t)] \tag{6}$$

$$\frac{dx}{dt} = 6u_{x0}\alpha g(\omega t)[1-\alpha g(\omega t)]f(\omega t) \tag{7}$$

We shall render these equations dimensionless using the variables:

$$z^* = \frac{\omega z}{u_{z0}}, \quad x^* = \frac{\omega x}{u_{x0}}, \quad t^* = \omega t \tag{8}$$

and taking the time average of Eqs. 6 and 7, we obtain:

$$\left\langle\frac{dz^*}{dt^*}\right\rangle = 6\alpha[\langle g(t^*)\rangle - \alpha\langle g^2(t^*)\rangle] \tag{9}$$

and

$$\left\langle\frac{dx^*}{dt^*}\right\rangle = 6\alpha[\langle g(t^*)f(t^*)\rangle - \alpha\langle g^2(t^*)f(t^*)\rangle] \tag{10}$$

where the brackets $\langle\,\rangle$ denote the time average over one period of oscillation.

In writing the equations above we have restricted ourselves to field strengths such that the solute molecule of interest with the largest mobility just comes into contact with the opposite side of the gap during each oscillation. If the dimensionless mobility of this species is represented by $\alpha_H = \mu_H E_0/\omega d$, then we may scale $g(\omega t)$ by this value; for example, let

$$g^*(t^*) = \alpha_H g(t^*) \tag{11}$$

where $g^*(t^*) > 0$ and $\max[g^*(t^*)] = 1$. The dimensionless time-averaged solute trajectories are thus governed by:

$$\left\langle\frac{dz^*}{dt^*}\right\rangle = 6\alpha^*[\langle g^*(t^*)\rangle - \alpha^*\langle g^{*2}(t^*)\rangle] \tag{12}$$

$$\left\langle\frac{dx^*}{dt^*}\right\rangle = 6\alpha^*[\langle g^*(t^*)f(t^*)\rangle - \alpha^*\langle g^{*2}(t^*)f(t^*)\rangle] \tag{13}$$

where $\alpha^* = \alpha/\alpha_H$.

It is interesting to note that the time-averaged velocities in the x and z directions are quadratic functions of the electrophoretic mobility for arbitrary choices of $f(t^*)$ and $g^*(t^*)$. This is a consequence of the parabolic velocity profile in these two directions and the requirement that $\alpha^* \le 1$. This will not be true for values of $\alpha^* > 1$, for which the migration of species in the y direction is limited by the finite width of the gap. We also note that with respect to calculating the time-averaged velocity in the x direction, the form of the periodic function $g^*(t^*)$ is arbitrary. This arises because the species with $\alpha^* = 1$ sample all positions across the gap, and thus any given time averaged x velocity can be achieved through an appropriate choice of $f(t^*)$. The precise choice of $g^*(t^*)$ does affect the time-averaged velocity in the z direction; however, for a steady down-flow the time-averaged velocity arising from different choices for the form of $g^*(t^*)$ will not differ greatly. In general we wish the time-averaged down-flow velocity of the solute species to be as small as possible to minimize the required length to achieve a given separation. If we represent the position of the particle in the gap by the Fourier cosine series:

$$g^*(t^*) = \sum_{n=0}^{n=\infty} C_n \cos(nt^*) \tag{14}$$

then the time-averaged z-direction velocity will be given by:

$$\left\langle\frac{dz^*}{dt^*}\right\rangle = 6\alpha^*\left[C_0 - \alpha^*\left(C_0^2 + \frac{1}{2}\sum_{n=1}^{n=\infty} C_n^2\right)\right] \tag{15}$$

Thus, to arrive at the smallest average velocity, we wish to maximize the sum of the squares of the coefficients C_n. We also have the conditions that $g^*(0) = 0$, $g^*(t^*) \ge 0$ and $\max[g^*(t^*)] = 1$. The first condition yields:

$$\sum_{n=0}^{n=\infty} C_n = 0 \tag{16}$$

However, the relation between the second and third requirement and the coefficients C_n is more difficult to determine. In general, the minimum velocity is obtained with the square wave:

$$C_n = \left\{\begin{array}{l} \frac{1}{2}, \text{ for } n = 0 \\ 0, \text{ for } n \neq 0 \;\&\; n \text{ even} \\ \frac{2}{n\pi}(-1)^{\frac{n-1}{2}}, \text{ for } n \text{ odd} \end{array}\right\} \tag{17}$$

however, this requires a very great electric field strength to achieve, as the field is proportional to the derivative of $g^*(t^*)$. For simplicity, we shall take $g^*(t^*)$ to be the simple sinusoidal function:

$$g^*(t^*) = \frac{1}{2}[1-\cos(t^*)] \tag{18}$$

which satisfies the conditions on $g^*(t^*)$ that $g^*(0) = 0$ and $\max[g^*(t^*)] = 1$, and which for $\alpha^* = 0.5$ yields a time-averaged, dimensionless, z-direction velocity of 15/16. This is quite close to the dimensionless velocity $\langle dz^*/dt^*\rangle = 3/4$ obtained for the

square wave given by Eq. 17 for a species with the same mobility.

We shall take $f(t^*)$ to be represented by the Fourier series:

$$f(t^*) = \sum_{n=0}^{n=\infty} A_n \sin(nt^*) + \sum_{n=0}^{n=\infty} B_n \cos(nt^*) \tag{19}$$

Taking the time average of $\langle g^* f \rangle$ and $\langle g^{*2} f \rangle$ we obtain:

$$\langle g^*(t^*) f(t^*) \rangle = \frac{1}{2}\left(B_0 - \frac{1}{2} B_1\right) \tag{20}$$

$$\langle g^{*2}(t^*) f(t^*) \rangle = \frac{1}{4}\left(\frac{3}{2} B_0 - B_1 + \frac{1}{4} B_2\right) \tag{21}$$

Hence, for this choice of $g^*(t^*)$ the time-averaged x-direction velocity of the solute depends only on three Fourier modes of an arbitrary oscillatory cross-flow: a steady cross-flow, an oscillating flow at the same frequency as the oscillating field, and the first overtone.

For the assumed form of $g^*(t^*)$ we have $\langle g^*(t^*) \rangle = 1/2$ and $\langle g^{*2}(t^*) \rangle = 3/8$; thus, the time-averaged trajectory followed by a solute species with dimensionless mobility α^* is given by:

$$\frac{\langle dx^* \rangle}{\langle dz^* \rangle} = \frac{[\langle g^*(t^*) f(t^*) \rangle - \alpha^* \langle g^{*2}(t^*) f(t^*) \rangle]}{\left(\frac{1}{2} - \frac{3}{8}\alpha^*\right)} \tag{22}$$

We wish to use Eq. 22 to determine the resolution provided by an electrophoresis cell with some dimensionless length $L^* = L\omega/u_{z0}$. The dimensionless, horizontal displacement of a species with mobility characterized by α^* after this distance is given by:

$$\Delta x^* = L^* \frac{\langle dx^* \rangle}{\langle dz^* \rangle} \tag{23}$$

where $\Delta x^* = \Delta x \omega / u_{x0}$.

We wish to choose the parameters B_0, B_1, and B_2 such that the resolution between species with different values of α^* is a maximum, e.g., maximize:

$$\frac{\partial \langle \Delta x^* \rangle}{\partial \alpha^*} = \frac{3}{8} \frac{L^*}{\left(\frac{1}{2} - \frac{3}{8}\alpha^*\right)^2} \times \left[\langle g^*(t^*) f(t^*) \rangle - \frac{4}{3} \langle g^{*2}(t^*) f(t^*) \rangle \right] \tag{24}$$

which, for the particular choice of $f(t^*)$ and $g^*(t^*)$ has the simple representation:

$$\frac{\partial \Delta x^*}{\partial \alpha^*} = \frac{1}{8} \frac{L^*}{\left(1 - \frac{3}{4}\alpha^*\right)^2} (B_1 - B_2) \tag{25}$$

which is proportional to $B_1 - B_2$. Simply increasing the amplitude of the oscillatory cross-flow will not act to increase the resolution, however, because the x-direction oscillations will tend to smear out the solutes at the collection end of the electrophoresis cell. The total spatially averaged amplitude of the oscillatory component of the crossflow is always less than $u_{x0}(|B_1| + 1/2|B_2|)/\omega$. To achieve complete separation between solutes separated by an electrophoretic mobility $\Delta\alpha^*$ we require the separation at $z = L$ to be twice the amplitude of the oscillatory component of the crossflow. Thus,

$$\frac{\partial \Delta x^*}{\partial \alpha^*} \Delta\alpha^* > 2\left(|B_1| + \frac{1}{2}|B_2|\right) \frac{u_{x0}}{\omega} \tag{26}$$

or

$$\frac{1}{8} \frac{L^* \Delta\alpha^*}{\left(1 - \frac{3}{4}\alpha^*\right)^2} (B_1 - B_2) > 2\left(|B_1| - \frac{1}{2}|B_2|\right) \tag{27}$$

Thus, the optimal resolution is provided when $B_1 = 0$ and $B_2 > 0$, for example, when only the first harmonic is employed for the cross-flow. For this cross-flow we obtain:

$$\frac{L^* \Delta\alpha^*}{\left(1 - \frac{3}{4}\alpha^*\right)^2} > 8 \tag{28}$$

or in terms of dimensional variables,

$$L > 8 \frac{\left(1 - \frac{3}{4}\alpha^*\right)^2}{\Delta\alpha^*} \frac{u_{z0}}{\omega} \tag{29}$$

which places a condition on the minimum length of the electrophoretic cell required to achieve complete separation for a given flow rate.

It is interesting to note that the minimum required length is independent of both the amplitude of the oscillatory cross-flow and of the amplitude of any steady component to the cross-flow. The distance required for separation is quite short for moderate down-flow rates. This can be best demonstrated by calculation of the parameters for a typical system. For example, if we are interested in separating species with electrophoretic mobilities that lie in the range 1 μm/s/(V/cm) $\leq \mu \leq$ 2 μm/s/(V/cm), and we wish to resolve species separated in mobilities by 0.1 μm/s/(V/cm) (for example, about 5% of the maximum electrophoretic mobility), then the minimum length will be determined by separating the species at $\mu = 1$ μm/s/(V/cm) from its nearest neighbor, for example, $\alpha^* = 0.5$ and $\Delta\alpha^* = 0.05$. The minimum length is thus given by:

$$L > 62.5 \frac{u_{z0}}{\omega} \tag{30}$$

which is proportional to u_{z0}/ω. The frequency of oscillation is not a free parameter, but rather is determined by the requirement that the species with the highest electrophoretic mobility ($\alpha^* = 1$) reach the opposite side of the gap ($y = d$) during each

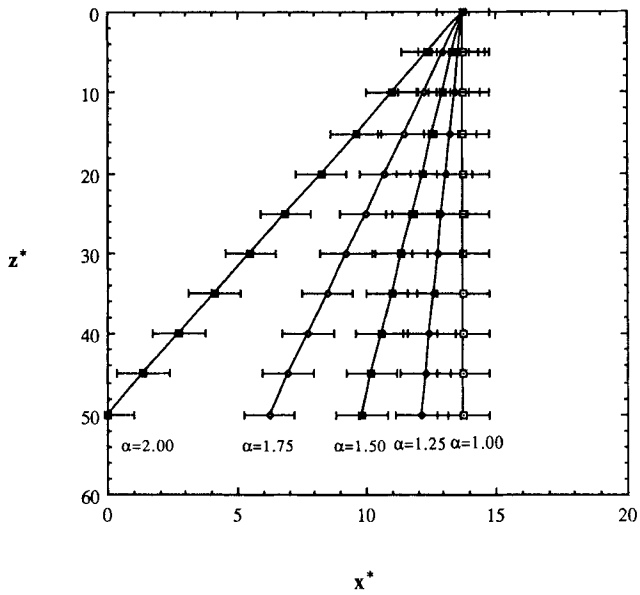


Figure 3. Simulated time-averaged trajectories in oscillatory cross-flow and steady down-flow.

Cross-flow Fourier components were chosen as $B_0 = 2/29$, $B_1 = 0$, and $B_2 = 1.0$.

oscillation. This frequency is thus given in terms of the characteristic electric field strength E_0, gap width d and mobility μ_H by:

$$\omega = \frac{\mu_H E_0}{d} \tag{31}$$

Assuming a maximum field strength amplitude of 100 V/cm (E_0, the characteristic magnitude, is twice this value), gap width $d = 1.0$ mm, and the mobility given above, we obtain an angular frequency of $\omega = 0.4$ rad/s or 0.064 Hz. If we take the down-flow to be 0.3 cm/s, the required length is approximately 47 cm. Note that with this down-flow and field strength, the mean position of two solutes with an electrophoretic mobility difference of 0.1 μm/s/(V/cm) would be separated by only 1.5 mm (1.5 gap widths) in a conventional electrophoretic cell of this length, which is why conventional thin-film electrophoresis must operate at lower flow rates and correspondingly lower throughputs. The simulated trajectories provided by the electrophoretic technique described here are presented in Figure 2 and the time-averaged trajectories are given in Figure 3. The amplitudes of the horizontal fluid oscillations are also shown.

Even in its simplest form, the oscillatory cross-flow electrophoresis technique described above offers several advantages over conventional electrophoresis approaches. First, the technique is unaffected by crescent formation because each molecule with a particular electrophoretic mobility follows the same time-dependent path across the gap—all of the molecules are in phase. In addition, there is no limitation in resolution due to electro-osmotic effects because the electro-osmotic velocity is parallel to that induced by the electric field, and hence simply acts to modify the effective electrophoretic mobility. Second, the cross-flow technique is much more amenable to scale-up. If we wish to have a greater total solute flow rate, it is simple to increase the horizontal (x-direction dimension) of the electrophoretic cell and simultaneously increase the amplitude of the cross-flow. The resolution of the device is unaffected by such a scale-up. In contrast, increasing the x-direction width of a conventional electrophoretic cell requires increasing both the local field strength and the total voltage drop to achieve the same resolution. Third, for a given local amplitude of the electric field strength, the total voltage required is very small—on the order of 10 V for the gap width and field specified above—rather than the much larger voltages required to maintain the same field strength in the x-direction across the much wider horizontal width of a conventional electrophoretic cell. Note that the total power dissipated in the oscillatory cross-flow electrophoresis cell will be approximately half that in a conventional cell of the same size, whose steady field strength is the same as the amplitude of the oscillating field. Finally, difficulties due to Joule heating and thermal instabilities may be easily circumvented since the gap width can be made quite thin without loss of capacity. This last advantage arises because the fluid velocity (both horizontal and vertical) is proportional to the frequency which, in turn, is inversely proportional to the gap width. The throughput, proportional to the product of the gap width and the velocity, is thus independent of the gap width. Decreasing the gap width can further decrease the total applied voltage which is required by the process.

It is useful at this point to contrast the technique presented above with the one-dimensional scheme suggested by Giddings (1986) and extended to two dimensions by Shmidt and Cheh (1990). In the work by Giddings, a square wave oscillatory field force (either gravity in the case of sedimentation or an electric field as given here) was combined with a steady, unidirectional flow to give different species different time-averaged convective velocities. In this proposed scheme, species with different mobilities would elute out of the one-dimensional gap at different rates. This is equivalent to the example described in this section with no horizontal cross-flow.

In the work of Shmidt and Cheh, the authors proposed a pulsed cross-flow electrophoresis device in which again a square wave electric field caused molecules to migrate across the gap of an electrophoretic cell and a *pulsed* unidirectional (non-oscillatory) cross-flow gave rise to the separation of species with different electrophoretic mobilities. This, combined with a uniform down-flow, yields a separation process very much like conventional thin-film continuous electrophoresis. Note that a steady cross-flow combined with a steady down-flow would result in no separation in a continuous system—all of the species would follow the same time-averaged trajectory. The oscillating electric field and pulsed cross-flow were sequential, such that there was no cross-flow during the time the electric field was applied. As may be easily seen, the proposed geometry is identical to that described in this section, and the pulsing cross-flow is a special case of the general oscillatory cross-flow described here. The resolution provided by this technique is similar to that calculated for the method described here, the difference arising from the different choice in the function $g^*(t^*)$ and its effect on the magnitude of the time-averaged down-flow velocity of the solute.

Effect of Oscillatory Down-Flow

There is no real difference between motion in the z direction and the x direction, thus it is reasonable to suppose that we

can retard the motion of the solute species relative to the solvent flow by superimposing an oscillatory component on the steady down-flow. We thus shall take the time-dependent down-flow velocity to be given by:

$$u_z = 6u_{z0}\frac{y}{d}\left(1-\frac{y}{d}\right)q(\omega t) \tag{32}$$

where $q(\omega t)$ is represented by the Fourier series:

$$q(\omega t) = \sum_{n=0}^{n=\infty} D_n \cos(n\omega t) + \sum_{n=0}^{n=\infty} F_n \sin(n\omega t) \tag{33}$$

As in the case of the oscillatory cross-flow, the dimensionless time-averaged z-direction velocity is given by:

$$\left\langle \frac{dz^*}{dt^*} \right\rangle = 6\alpha^*[\langle g^*(t^*)q(t^*)\rangle - \alpha^*\langle g^{*2}(t^*)q(t^*)\rangle] \tag{34}$$

Only three Fourier modes of the oscillatory down-flow contribute to the time-averaged motion, thus:

$$\left\langle \frac{dz^*}{dt^*} \right\rangle = 6\alpha^*\left[\frac{1}{2}\left(D_0-\frac{1}{2}D_1\right) - \frac{3}{8}\alpha^*\left(D_0-\frac{2}{3}D_1+\frac{1}{6}D_2\right)\right] \tag{35}$$

Because we wish to have the steady component of the down-flow to have magnitude u_{z0}, we shall take $D_0 = 1$ without loss of generality.

To provide the maximum degree of separation we wish to minimize this velocity. We may achieve $\langle dz^*/dt^*\rangle = 0$ for all α^* by requiring that $\langle g^* q\rangle$ and $\langle g^{*2} q\rangle$ vanish. While this is readily achievable even for nonzero, steady components of the down-flow, this would simply lead to the solutes piling up in the electrophoretic cell—nothing would ever emerge. Instead, it is better to require that the second term in Eq. 35 ($\langle g^{*2} q\rangle$) vanish and that the first be of dimensionless magnitude $\epsilon \ll 1$. Thus:

$$\left(1-\frac{2}{3}\frac{D_1}{D_0}+\frac{1}{6}\frac{D_2}{D_0}\right) = 0 \tag{36}$$

$$\left(1-\frac{1}{2}\frac{D_1}{D_0}\right) = \epsilon \ll 1 \tag{37}$$

or

$$\left(\frac{D_1}{D_0}\right) = 2(1-\epsilon) \tag{38}$$

$$\left(\frac{D_2}{D_0}\right) = 2(1-4\epsilon) \tag{39}$$

which yields the velocity:

$$\left\langle \frac{dz^*}{dt^*} \right\rangle = 3\alpha^*\epsilon \tag{40}$$

which is of order ϵ for all α^*. We can combine this result with the relation for $\langle dx^*/dt^*\rangle$ derived in the last section to obtain the time-averaged trajectories:

$$\frac{\langle dx^*\rangle}{\langle dz^*\rangle} = \frac{2}{\epsilon}[\langle g^*(t^*)f(t^*)\rangle - \alpha^*\langle g^{*2}(t^*)f(t^*)\rangle] \tag{41}$$

Note that with this formulation, the slope of the trajectories for different species is linear in α^*. Following the analysis in section for solute motion with oscillatory cross-flow, the dimensionless displacement of a solute species in a device with dimensionless length L^* is given by:

$$\Delta x^* = L^*\frac{\langle dx^*\rangle}{\langle dz^*\rangle} = 2\frac{L^*}{\epsilon}[\langle g^*(t^*)f(t^*)\rangle - \alpha^*\langle g^{*2}(t^*)f(t^*)\rangle] \tag{42}$$

We seek to maximize the relative displacement of two species separated by a dimensionless electrophoretic mobility difference $\Delta\alpha^*$. This relative displacement is given by:

$$\frac{\partial \Delta x^*}{\partial \alpha^*}\Delta\alpha^* = -\frac{2L^*}{\epsilon}\langle g^*(t^*)f(t^*)\rangle\Delta\alpha^* = -\frac{1}{2}\frac{L^*}{\epsilon}\left(\frac{3}{2}B_0 - B_1 + \frac{1}{4}B_2\right)\Delta\alpha^* \tag{43}$$

which, intriguingly, is nonzero even for a *steady* cross-flow—an oscillatory cross-flow is not required.

The overall resolution of an electrophoretic cell with both oscillatory cross-flow and oscillatory down-flow will be the result of the amplitude of horizontal and vertical oscillations. In the last section we showed that x-direction oscillations resulted in the solute stream being spread over some distance in the x direction at the bottom of the electrophoretic cell. The vertical oscillations will similarly degrade resolution by spreading the solute stream over some distance in the x direction proportional to the amplitude of the vertical oscillations and the slope of the solute trajectory. Combining these two sources of loss in resolution, we require:

$$\frac{\partial \Delta x^*}{\partial \alpha^*}\Delta\alpha^* > 2\left[\left(|B_1| + \frac{1}{2}|B_2|\right) + \frac{\langle dx^*\rangle}{\langle dz^*\rangle}\left(|D_1| + \frac{1}{2}|D_2|\right)\right] \tag{44}$$

for adequate resolution of two solutes with dimensionless electrophoretic mobilities which differ by $\Delta\alpha^*$. The optimum choice of ϵ, B_1, B_2, and B_0 depend on the value of α^* we are considering. For simplicity, we shall consider first cross-flows that are steady, for example, that B_1 and B_2 are zero. For this assumption, the optimum choice for all α^* is $\epsilon = 1/4$, corresponding to $D_1 = 3/2\ D_0$ and $D_2 = 0$. The minimum length is given by:

$$L^* > \frac{2}{\Delta\alpha^*}\left(1-\frac{3}{4}\alpha^*\right) \tag{45}$$

which, for characteristic values $\alpha^* = 0.5$ and $\Delta\alpha^* = 0.05$ used

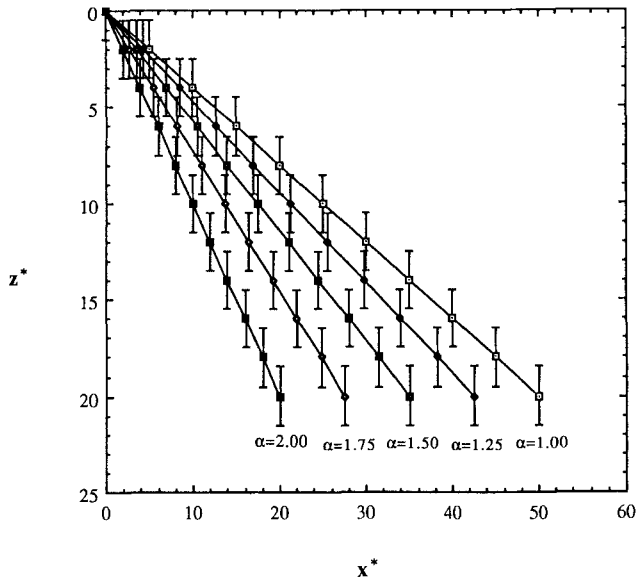


Figure 4. Simulated time-averaged trajectories in oscillatory down-flow and steady cross-flow.

Cross-flow Fourier components were chosen as $B_0 = 1.0$, $B_1 = 0$, and $B_2 = 0$; down-flow Fourier components were chosen as $D_0 = 1.0$, $D_1 = 1.5$, and $D_2 = 0$.

in the section for solute motion with oscillatory cross-flow, yields the length:

$$L^* > 25 \tag{46}$$

which is a factor of 2.5 better than was achievable with steady down-flow and x-direction oscillations. Essentially, by turning the electrophoresis device described in the section for solute motion with oscillatory cross-flow on its side, we can improve separation by a factor of 2.5. A plot of the trajectories for this choice of operating parameters is given in Figure 4.

If we are interested only in separating species about a single electrophoretic mobility α^*, it is possible to do even better, at the cost of loss of resolution away from this mobility. This can be done by choosing B_0, B_1, and B_2 such that the time-averaged x-direction velocity is zero for this species, but nonzero for species with slightly different electrophoretic mobilities. The trajectory for this particular species is thus vertical, and vertical oscillations no longer reduce the degree of resolution. Under these conditions the required length is proportional to ϵ, which can be made quite small. To achieve this condition we require:

$$\left(B_0 - \frac{1}{2} B_1\right) = \frac{1}{2} \alpha^* \left(\frac{3}{2} B_0 - B_1 + \frac{1}{4} B_2\right) \tag{47}$$

or

$$B_0 = \frac{\left[\frac{1}{2} B_1 (1-\alpha^*) + \frac{1}{8} \alpha^* B_2\right]}{\left(1 - \frac{3}{4} \alpha^*\right)} \tag{48}$$

which determines the magnitude of the steady cross-flow in terms of the two oscillatory modes. The minimum length for separation of this species is given by:

$$L^* = \frac{1}{\Delta\alpha^*} \frac{\left[\frac{4}{\epsilon}\left(|B_1| + \frac{1}{2}|B_2|\right)\right]}{\left(\frac{2}{\alpha^*}\left|B_0 - \frac{1}{2} B_1\right|\right)} \tag{49}$$

or substituting in Eq. 45,

$$L^* > 4\epsilon \frac{\alpha^*}{\Delta\alpha^*} \frac{\left(1 - \frac{3}{4}\alpha^*\right)}{(1-\alpha^*)} \frac{\left(|B_1| + \frac{1}{2}|B_2|\right)}{\left[\left|B_1 + \frac{1}{4}\left(\frac{\alpha^*}{1-\alpha^*}\right) B_2\right|\right]} \tag{50}$$

The righthand side of Eq. 50 is a minimum for values of $\alpha^* > 2/3$ when $B_1 = 0$ and for $\alpha^* < 2/3$ when $B_2 = 0$: that is, only one oscillatory Fourier mode should be used depending on the value of α^* of interest. The required lengths for these two cases are given by:

$$L^* = \left\{ \begin{array}{l} 4\epsilon\left(\frac{\alpha^*}{\Delta\alpha^*}\right)\left(\frac{1 - \frac{3}{4}\alpha^*}{1-\alpha^*}\right) \text{ for } \alpha^* < \frac{2}{3},\ B_2 = 0 \\ 8\epsilon\left(\frac{1 - \frac{3}{4}\alpha^*}{\Delta\alpha^*}\right) \text{ for } \frac{2}{3} < \alpha^* < 1,\ B_1 = 0 \end{array} \right\} \tag{51}$$

For the conditions used in the section for the solute motion with oscillatory cross-flow ($\alpha^* = 0.5$ and $\Delta\alpha^* = 0.05$) we have the length:

$$L^* > 50\epsilon \tag{52}$$

or, if we take $\epsilon = 0.10$, $L^* > 5$, which is a factor of 5 less than that calculated above for a steady cross-flow and more than an order of magnitude better than that which would be obtained with no vertical oscillations. A plot of the trajectories for the conditions where the time-averaged horizontal velocity of the species $\alpha^* = 0.75$ is zero is given in Figure 5.

Effect of Gradients in Field Strength Amplitude

In the last section we demonstrated that it was possible through suitable selection of oscillatory cross-flow frequencies to induce a species with a particular electrophoretic mobility α^* to have a zero time-averaged x-direction velocity, while species with different mobilities experience some horizontal displacement. This suggests the possibility of using a gradient in the amplitude of the oscillating electric field to cause species with different electrophoretic mobilities to acquire different steady-state positions in the x direction. The electrophoresis cell could then be used in a mode analogous to isoelectric focusing in conventional electrophoresis processes. In this section we explore this possibility.

Consider the electric field given by:

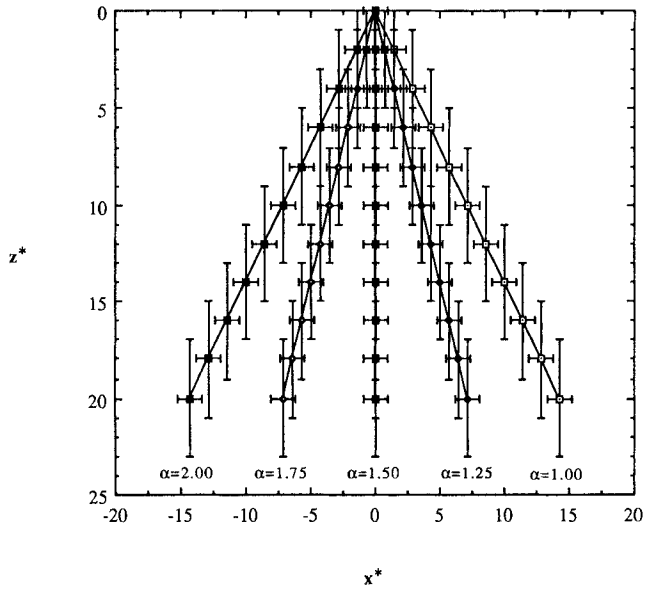


Figure 5. Simulated time-averaged trajectories in oscillatory cross- and down-flow.

Cross-flow Fourier components were chosen as $B_0 = 0.214$, $B_1 = 0$, and $B_2 = 1.0$; down-flow Fourier components were chosen as $D_0 = 1.0$, $D_1 = 1.8$, and $D_2 = 1.2$.

$$E = E_0\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right) g(\omega t) \tag{53}$$

where b is the x-direction half-width of the channel, and $\Delta E/E_0$ is the dimensionless variation in the amplitude of the electric field. The position of a species with electrophoretic mobility μ is given by:

$$\frac{y}{d} = \lambda\alpha\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right) g^*(t^*) \tag{54}$$

where

$$\alpha = \frac{\mu E_0}{\omega d} \tag{55}$$

and

$$g^*(t^*) = \frac{1}{2}\left[1 - \cos(t^*)\right] \tag{56}$$

as before.

The parameter λ is chosen such that the species with the largest electrophoretic mobility α_H just reaches the opposite side in the gap $y = d$ at the point where the electric field strength is the largest; for example,

$$\lambda = \frac{1}{\alpha_H\left(1 + \left|\frac{\Delta E}{E_0}\right|\right)} \tag{57}$$

Under these conditions, the x-direction velocity is given by:

$$\frac{dx}{dt} = 6u_{x0}\alpha\lambda\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right) g^*(t^*) \times \left[1 - \alpha\lambda\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right) g^*(t^*)\right] f(t^*) \tag{58}$$

We now wish to time-average Eq. 58 to obtain the time-averaged horizontal velocity. Because the righthand side of Eq. 58 depends on the position x, however, this cannot be done exactly. Instead, we shall assume that the tidal displacement due to the oscillatory cross-flow is much smaller than the length scale over which the electric field strength is varying; for example,

$$\frac{u_{x0}}{\omega b} \ll 1 \tag{59}$$

which would be required in any event for the horizontal oscillations to be contained within the width of the channel and to prevent the fluid oscillations from limiting the resolution. With this approximation, we may write:

$$\left\langle \frac{dx}{dt} \right\rangle = 6u_{x0}\alpha\lambda\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right)\left[\langle g^*(t^*) f(t^*)\rangle - \alpha\lambda\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right)\langle g^{*2}(t^*) f(t^*)\rangle\right] \tag{60}$$

The steady-state, time-averaged positions are determined by setting Eq. 60 equal to zero; for example,

$$\langle g^*(t^*) f(t^*)\rangle = \alpha\lambda\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right)\langle g^{*2}(t^*) f(t^*)\rangle \tag{61}$$

or

$$\frac{\langle g^*(t^*) f(t^*)\rangle}{\langle g^{*2}(t^*) f(t^*)\rangle} = \alpha\lambda\left(1 + \frac{\Delta E}{E_0}\frac{x}{b}\right) \tag{62}$$

We wish to adjust the parameters so that species with dimensionless mobilities between α_L and α_H are distributed between $x/b = -1$ and $x/b = 1$, respectively. We thus require:

$$\frac{\langle g^*(t^*) f(t^*)\rangle}{\langle g^{*2}(t^*) f(t^*)\rangle} = \alpha_H\lambda\left(1 + \frac{\Delta E}{E_0}\right) = \alpha_L\lambda\left(1 - \frac{\Delta E}{E_0}\right) \tag{63}$$

which has the simple solution:

$$\frac{\Delta E}{E_0} = -\frac{\alpha_H - \alpha_L}{\alpha_H + \alpha_L} \tag{64}$$

we can combine this result with Eq. 63 to obtain an explicit result for the parameter λ in terms of α_L and α_H:

$$\lambda = \frac{\alpha_H + \alpha_L}{2\alpha_H^2} \tag{65}$$

and for the ratio $\langle g^* f\rangle / \langle g^{*2} f\rangle$:

$$\frac{\langle g^*(t^*)f(t^*)\rangle}{\langle g^{*2}(t^*)f(t^*)\rangle}=\frac{\alpha_L}{\alpha_H} \tag{66}$$

As was the case for a constant amplitude oscillatory electric field strength, the time-averaged x-direction motion is the result of only three Fourier modes of the arbitrary oscillatory cross-flow:

$$\frac{(B_1-2B_0)}{\left(B_1-\frac{1}{4}B_2-\frac{3}{2}B_0\right)}=\frac{\alpha_L}{\alpha_H} \tag{67}$$

or

$$\frac{B_2}{B_0}=\left(8\frac{\alpha_H}{\alpha_L}-6\right)-4\left(\frac{\alpha_H}{\alpha_L}-1\right)\frac{B_1}{B_0} \tag{68}$$

where the ratio B_1/B_0 is undetermined. Substituting this expression into Eq. 60 yields:

$$\left\langle\frac{dx}{dt}\right\rangle\approx 6u_{x0}\frac{\alpha(\alpha_H+\alpha_L)}{2\alpha_H^2}\left(1-\frac{\alpha_H-\alpha_L}{\alpha_H+\alpha_L}\frac{x}{b}\right)\left[\frac{1}{2}B_0\left(1-\frac{1}{2}\frac{B_1}{B_0}\right)\right]\times\left[1-\frac{\alpha(\alpha_H+\alpha_L)}{2\alpha_H\alpha_L}\left(1-\frac{\alpha_H-\alpha_L}{\alpha_H+\alpha_L}\frac{x}{b}\right)\right] \tag{69}$$

The motion in the z-direction is similar to that for no gradient in the amplitude of the electric field:

$$\frac{dz}{dt}=6u_{z0}\alpha\lambda\left(1+\frac{\Delta E}{E_0}\frac{x}{b}\right)g^*(t^*)\times\left[1-\alpha\lambda\left(1+\frac{\Delta E}{E_0}\frac{x}{b}\right)g^*(t^*)\right]q(t^*) \tag{70}$$

where, for small amplitudes in the oscillatory cross-flow, we obtain the time-averaged down-flow:

$$\left\langle\frac{dz}{dt}\right\rangle\approx 6u_{z0}\alpha\lambda\left(1+\frac{\Delta E}{E_0}\frac{x}{b}\right)\langle g^*(t^*)q(t^*)\rangle\times\left[1-\alpha\lambda\left(1+\frac{\Delta E}{E_0}\frac{x}{b}\right)\left(\frac{\langle g^{*2}(t^*)q(t^*)\rangle}{\langle g^*(t^*)q(t^*)\rangle}\right)\right] \tag{71}$$

Again, taking $\langle g^{*2}q\rangle=0$ and $\langle g^*q\rangle=\epsilon/2$ we obtain:

$$\left\langle\frac{dz}{dt}\right\rangle=6u_{z0}\alpha\lambda\left(1+\frac{\Delta E}{E_0}\frac{x}{b}\right)\left(\frac{1}{2}\epsilon\right) \tag{72}$$

where we have again taken $D_0=1$ without loss of generality.

Combining this result with that for the x-direction velocity, we obtain the equation governing the trajectory:

$$\frac{\langle dx\rangle}{\langle dz\rangle}=\frac{u_{x0}}{u_{z0}}\frac{B_0}{\epsilon}\left(1-\frac{1}{2}\frac{B_1}{B_0}\right)\times\left[1-\frac{\alpha(\alpha_H+\alpha_L)}{2\alpha_H\alpha_L}\left(1-\frac{\alpha_H-\alpha_L}{\alpha_H+\alpha_L}\frac{x}{b}\right)\right] \tag{73}$$

It is convenient at this point to define new dimensionless variables $\hat{x}$ and $\hat{z}$ such that:

$$\hat{x}=\frac{x}{b}\quad\hat{z}=\frac{z}{b}\frac{u_{x0}}{u_{z0}}\frac{(-B_0)}{\epsilon}\left(1-\frac{1}{2}\frac{B_1}{B_0}\right) \tag{74}$$

and the scaled electrophoretic mobility $\hat{\alpha}$ and variation in mobility β:

$$\hat{\alpha}=\frac{\alpha(\alpha_H+\alpha_L)}{2\alpha_H\alpha_L}\quad\beta=\frac{\alpha_H-\alpha_L}{\alpha_H+\alpha_L} \tag{75}$$

which, for mobilities in the range $\alpha_L<\alpha<\alpha_H$, falls in the range:

$$\frac{1}{1+\beta}<\hat{\alpha}<\frac{1}{1-\beta} \tag{76}$$

In terms of these variables, the trajectory equation takes on the particularly simple relation:

$$\frac{\langle d\hat{x}\rangle}{\langle d\hat{z}\rangle}=-(1-\hat{\alpha})-\hat{\alpha}\beta\hat{x} \tag{77}$$

which has the solution

$$\langle\hat{x}\rangle=\frac{\hat{\alpha}-1}{\hat{\alpha}\beta}+\left(\hat{x}_0-\frac{\hat{\alpha}-1}{\hat{\alpha}\beta}\right)\exp(-\hat{\alpha}\beta\hat{z}) \tag{78}$$

where x_0 is the starting position of the solute at the top of the electrophoretic cell ($z=0$).

From Eq. 78 we find that the trajectories exponentially approach their steady value over a characteristic length scale z_c given by:

$$z_c=b\frac{u_{z0}}{u_{x0}}\frac{2\alpha_H\alpha_L}{\alpha(\alpha_H-\alpha_L)}\left(\frac{-\epsilon}{B_0-\frac{1}{2}B_1}\right) \tag{79}$$

Note that we require $(B_0-B_1/2)<0$ for the steady streamlines to be stable. If this quantity is positive, then the solute will exponentially diverge from this steady value.

To achieve the maximum resolution for a given flow rate, we wish to minimize the amplitude of the horizontal oscillations while minimizing the characteristic length given in Eq. 79. This corresponds to maximizing the ratio:

$$\frac{\left(\frac{1}{2}B_1-B_0\right)}{\left(|B_1|+\frac{1}{2}|B_2|\right)} \tag{80}$$

This ratio is a maximum for:

$$B_1=0,\ B_0<0,\ \frac{B_2}{B_0}=8\frac{\alpha_H}{\alpha_L}\left(1-\frac{3}{4}\frac{\alpha_L}{\alpha_H}\right) \tag{81}$$

yielding:

$$\max\left[\frac{\left(\frac{1}{2}B_1-B_0\right)}{\left(|B_1|+\frac{1}{2}|B_2|\right)}\right]=\frac{1}{4}\left[\frac{\left(\frac{\alpha_L}{\alpha_H}\right)}{\left(1-\frac{3}{4}\frac{\alpha_L}{\alpha_H}\right)}\right] \tag{82}$$

where for a ratio $\alpha_H/\alpha_L=2$, the amplitude of the first overtone is ten times that of the steady cross-flow. Adjusting B_1 and B_2 such that B_0 (the steady cross-flow) vanishes only decreases this resolution by a factor of two, however. This condition corresponds to $B_1<0$ and $B_2=-4B_1$.

In deriving the optimum form of the oscillatory cross-flow we have neglected the effect of vertical oscillations on the horizontal resolution. This is reasonable, since at steady state the time-averaged trajectories are vertical, and vertical oscillations will not degrade the resolution significantly.

For the choice of the oscillatory cross-flow given by Eq. 80 we obtain the characteristic length for the approach to steady state:

$$z_c=\left(\frac{-\epsilon}{B_2 b}\right)\frac{u_{z0}}{u_{x0}}\frac{2\alpha_H\alpha_L}{\alpha(\alpha_H-\alpha_L)}\left[8\frac{\alpha_H}{\alpha_L}\left(1-\frac{3}{4}\frac{\alpha_L}{\alpha_H}\right)\right] \tag{83}$$

The amplitude of the oscillatory component of the cross-flow is given by:

$$\Delta x_c=\frac{1}{2}\frac{u_{x0}}{\omega}|B_2| \tag{84}$$

In terms of this amplitude, the characteristic length is given by:

$$z_c=8\epsilon\frac{\left(\frac{u_{z0}}{\omega}\right)}{\left(\frac{\Delta x_c}{b}\right)}\frac{\alpha_H^2\left(1-\frac{3}{4}\frac{\alpha_L}{\alpha_H}\right)}{\alpha(\alpha_H-\alpha_L)} \tag{85}$$

If we choose $\alpha_H/\alpha_L=2$, $\Delta x_c/b=0.10$ for adequate resolution of species which differ in mobility by only 5% of the maximum mobility μ_H and $\epsilon=0.1$, then the characteristic length to approach steady state for the lowest mobility $\alpha=\alpha_L$ is given by:

$$z_c^*=\frac{z_c\omega}{u_{z0}}=20 \tag{86}$$

which, for $u_{z0}=0.3$ cm/s and $\omega=0.4$ rad/s, has a dimensional value of 15 cm. The amplitude of the vertical oscillation in the down-flow corresponding to these conditions is only 1.8 cm, which is much smaller than this characteristic length. A plot of the time-averaged trajectories of solute molecules fed across the top of the electrophoretic cell is given in Figure 6. A dimensionless distance $z^*=60$ (corresponding to a dimensional length of 45 cm) is sufficient to achieve complete resolution. The amplitude of the oscillatory cross-flow is also marked on the plot.

It is apparent from Figure 6 (and also from Eq. 83) that the rate of approach to steady state is different for species with different mobilities. Since the overall resolution of this device is no better than that for the species with the lowest electrophoretic mobility, we desire to alter the gradient in the electric field to induce all species to approach their steady streamlines at approximately the same rate. In addition, we find that the spacing between the species with different mobilities is nonuniform, and resolution can be improved if the spacing for the species is proportional to the difference in the mobility. A uniform spacing and a somewhat more uniform approach to steady state can be achieved if we adopt the electric field given by:

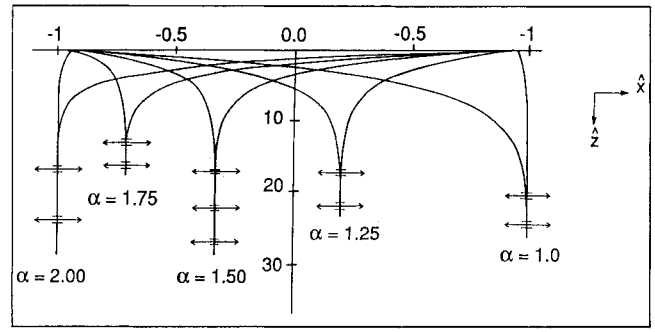


Figure 6. Simulated time-averaged trajectories in oscillatory cross- and down-flow with a linear gradient in the electric field strength.

Cross-flow Fourier components were chosen as $B_0=-1.0$, $B_1=0$, and $B_2=-10$; down-flow Fourier components were chosen as $D_0=1.0$, $D_1=1.9$, and $D_2=1.60$.

$$E=E_0\left[1+\frac{\Delta E}{E_0}s\left(\frac{x}{b}\right)\right]\cos(\omega t) \tag{87}$$

where

$$s\left(\frac{x}{b}\right)=\frac{1}{\beta}\left[1-\frac{(1-\beta^2)}{\left(1+\frac{x}{b}\beta\right)}\right] \tag{88}$$

for example, a hyperbolic function of x/b. In the limit that the ratio $\alpha_H/\alpha_L\to 1$ ($\beta\to 0$) this field reduces to the linear relationship given in Eq. 53. The time-averaged, steady-state streamlines for this field gradient are governed by:

$$\frac{\langle d\hat{x}\rangle}{\langle d\hat{z}\rangle}=-\frac{1}{\{1-\hat{\alpha}[1-\beta s(\hat{x})]\}} \tag{89}$$

where $\hat{x}$ and $\hat{z}$ are defined as before. This equation has the solution:

$$\hat{z}=-(\hat{x}-\hat{x}_0)-\frac{\hat{\alpha}}{\beta}(1-\beta^2)\log\left[\frac{\hat{x}-\left(\frac{1-\hat{\alpha}}{\beta}+\hat{\alpha}\beta\right)}{\hat{x}_0-\left(\frac{1-\hat{\alpha}}{\beta}+\hat{\alpha}\beta\right)}\right] \tag{90}$$

where we have given $\hat{z}$ in terms of $\hat{x}$. While the trajectories no longer have a simple exponential relationship for $\hat{x}$ in terms of $\hat{z}$, the approach to steady state occurs over a characteristic length approximately given by:

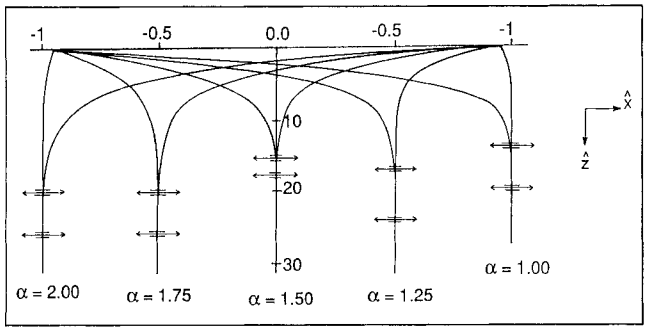


Figure 7. Simulated time-averaged trajectories in oscillatory cross- and down-flow with a hyperbolic gradient in the electric field strength.

Cross-flow Fourier components were chosen as $B_0 = -1.0$, $B_1 = 0$, and $B_2 = -10$; down-flow Fourier components were chosen as $D_0 = 1.0$, $D_1 = 1.9$, and $D_2 = 1.60$.

$$z_c \equiv 8\epsilon \frac{\left(\frac{u_{z0}}{\omega}\right)}{\left(\frac{\Delta x_c}{b}\right)} \frac{\alpha}{\alpha_L} \frac{\left(1 - \frac{3}{4}\frac{\alpha_L}{\alpha_H}\right)}{\alpha_H - \alpha_L} \tag{91}$$

by analogy with Eq. 85. For the parameters chosen above, this has a dimensionless value of $z_c^* = z_c\omega/u_{z0} = 15$ at $\alpha = (\alpha_H + \alpha_L)/2$; or for $u_{z0} = 0.3$ cm/s and $\omega = 0.4$ rad/s, a dimensional $z_c = 11$ cm. A plot of the solute trajectories for these trajectories is given in Figure 7. In this case, the approach to steady state is faster for species with low electrophoretic mobilities. By choosing a field gradient between those given here or by modifying the oscillatory component of the down-flow to selectively retard species with higher electrophoretic mobilities, the approach to steady state can be made approximately independent of α^*.

Summary

In the preceding sections we have demonstrated that the combination of an oscillatory electric field across the narrow gap of an electrophoresis channel and an oscillatory cross-flow will yield a separation based on the electrophoretic mobilities of the individual species. It is useful to compare the feed rate of solute possible with the designs suggested here with that achievable with conventional thin-film electrophoresis systems.

Consider first the oscillatory system with steady down-flow described in the section for solute motion with oscillatory cross-flow. We shall take the length of the device to be 100 cm, the width $2b = 50$ cm, and the gap width to be 1 mm. The width over which we feed the solute will not be on the order of the gap width as in a conventional device, but rather will scale with the amplitude of the horizontal cross-flow. Intriguingly, it may be seen from Eq. 29 that the down-flow velocity required to achieve a given separation is independent of the amplitude of the cross-flow, but rather depends only on the length of the electrophoresis device and the frequency of oscillation. As a consequence, it is possible to increase the total flow rate by simply increasing the width of the electrophoresis cell and correspondingly increasing the width over which the solute is injected and the amplitude of the cross-flow. For purposes of estimation, we shall assume that the fluid is injected over 1/20 of the width of the device, thus enabling the resolution of roughly ten species separated by an electrophoretic mobility of 0.1 μm/s/(V/cm). Under these conditions, the maximum down-flow velocity is given by:

$$u_{z0} = \frac{bL}{4} \frac{\Delta\alpha^*}{\left(1 - \frac{3}{4}\alpha^*\right)^2} \tag{92}$$

which has a numerical value of 0.64 cm/s if we take the frequency of oscillation to be that obtained for a system with maximum mobility of 2 μm/s/(V/cm) in the section for solute motion with oscillatory cross-flow. For a 50-cm total width, the feed rate is thus 0.16 mL/s or about 150 times that of an ordinary electrophoresis device (typically about 4 mL/h). Note that the feed rate is independent of the gap width since halving the gap allows the doubling of the frequency and velocities, leaving the feed rate unchanged.

The addition of an oscillatory component to the down-flow further increases the possible feed rate. If we are interested in separating a range of components, the increase in throughput made possible by vertical oscillations is about a factor of 2.5, or about 1.6 cm/s and 0.4 mL/s. If we are interested in only a single species and adjust the Fourier components of the cross-flow such that its trajectory is purely vertical, then the feed rate increases by a further factor of 5 to 8 cm/s or 2 mL/s.

The most startling increase in the amplitude of the feed rate is achievable if we operate the device with the gradient in electric field strength described in the last section. Under these conditions adequate resolution between the species is achieved after an effective length $L/z_c = 3.0$. For a 100-cm-long electrophoretic cell this results in a velocity of 0.9 cm/s for complete resolution of all of the species in the range 1 μm/s/(V/cm) $< \mu < 2$ μm/s/(V/cm). The corresponding feed rate is 4.2 mL/s or 15 L/h, more than a factor of 1,000 greater than what would be found for a conventional electrophoresis system with the same field strength. As was the case for no vertical oscillations, the total feed rate can be further scaled up by either increasing the length of the electrophoretic cell or by increasing its width. Because it operates in a manner analogous to isoelectric focusing, this technique also concentrates the species by about a factor of 20.

It is useful to study the ways in which the electrophoresis scheme described above could be implemented. Because of the narrow gap over which the electric field is applied, the total voltage required by this system is quite low, only on the order of ± 10 V rather than voltages in the kV range used in a conventional device. As a consequence, it is simple to apply an oscillatory voltage of this magnitude. Similarly, the total power dissipation in the electrophoretic cell is about the same as in a conventional cell with the same amplitude electric field, thus Joule heating should not create significant difficulties. The gradient in the electric field may be induced by simply designing the device with a gradient in the separation distance between the electrodes while maintaining a constant width of the gap through which the fluid flows. The greatest difficulty will be in imposing the combination of oscillatory down-flow and oscillatory cross-flow. Because the flow in the cell (as in the case of a Hele-Shaw cell) satisfies the Laplace equation

and the streamlines are time-varying straight lines, it is possible to create the desired flows by controlling the velocities at the edges.

The limitation in throughput with this device will likely come from the assumption that the solute molecules follow a precise time-dependent path across the narrow gap of the device. Effectively, this requires that at all times the solute must be concentrated in a very narrow fraction of the gap width. At some point, there will be a concentration polarization layer that will prevent the further concentration of the species, and hence will limit the resolution achievable between different species. The spreading of the solute bands will be largely mitigated in the nonuniform field case by the self-focussing nature of the electric field gradient. While the exact evaluation of this limitation will require further investigation, we expect that the maximum capacity of the device described in the last section will be on the order of 100 g/h of protein (10 g/h of each protein which is to be separated). This is far greater than the capacity of the best conventional thin-film electrophoresis devices (about 0.1 g/h total protein content) and appears to exceed the capacity of related free electrophoresis geometries such as the Biostream separator (Philpot, 1973) and recycle continuous-flow electrophoresis (Gobie et al., 1985).

Notation

A_n = Fourier mode constants
b = x-direction half width of the electrophoresis cell
B_n = Fourier mode constants
C_n = Fourier mode constants
d = y-direction gap width of the electrophoresis cell
D_n = Fourier mode constants
E = electric field strength
E_0 = characteristic amplitude of the electric field
$f(\omega t)$ = function of time
$g(\omega t)$ = oscillating forcing function
$g^*(t^*)$ = dimensionless oscillating forcing function
L = length of the electrophoresis cell
L^* = dimensionless length of the electrophoresis cell
$q(\omega t)$ = oscillating component for the down flow
s = electric field gradient
t = time
t^* = dimensionless time coordinate
u_x = x-direction convective velocity
u_{x0} = spatially averaged x-direction convective velocity
u_z = z-direction convective velocity
u_{z0} = spatially-averaged z-direction convective velocity
x = cross-flow direction
x^* = dimensionless x coordinate
$\hat{x}$ = dimensionless x coordinate
x_c = characteristic amplitude of the oscillation
y = direction of the electric field
z = direction of buffer flow/feed
z^* = dimensionless z coordinate
$\hat{z}$ = dimensionless z coordinate
z_c = characteristic length
z_c^* = dimensionless characteristic length

Greek letters

α = dimensionless group, measure of the amplitude of the oscillating motion of the solute
α_H = highest dimensionless mobility of the species
α_L = lowest dimensionless mobility of the species
α^* = ratio of α and α_H
$\Delta\alpha^*$ = difference in electrophoretic mobilities
$\hat{\alpha}$ = scaled electrophoretic mobilities
β = variation in electrophoretic mobilities
ω = angular frequency
μ = electrophoretic mobility of the species
μ_H = highest electrophoretic mobility
μ_L = lowest electrophoretic mobility

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