ELECTROPHORETIC SEPARATIONS USING SWEEPING FIELDS

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SUMMARY

Sweeping-field electrophoresis is investigated as a method for increasing the resolution of low-voltage slab gel separations. In this technique a low DC voltage is time multiplexed to an array of periodically spaced electrodes placed along the length of the slab in a manner that follows the band migration. Because the electrode spacing is smaller than the slab length, a larger field is generated yielding an improved separation. The effect of the non-uniform electric field on band distortion is studied in some detail. Experimental band distortion results showed good agreement with theoretical predictions in a macroscopic sweeping-field electrophoresis system. Both analytical and numerical results show that band distortion can be effectively minimized when an appropriate sweep rate is selected for a narrow band range. Using this scheme we have achieved the same number of theoretical plates as a DC driven system with one third of the drive voltage.
1 Introduction

While the demands for faster and inexpensive sequencing are enormous as exemplified by the Human Genome Project, currently available automated slab gel sequencing machines are slow, expensive to operate, and require large amounts of labor. A rapid, efficient, and automated sequencing machine is both technically and commercially attractive.

Cost reductions and increases in speed can be accomplished through miniaturization of the separation system. Recently, capillary electrophoresis systems have demonstrated the ability to separate DNA fragments with high resolution at speeds up to 25 times larger than conventional slabs [1, 2]. Further cost reductions can be potentially achieved using microfabrication techniques that integrate channels and detectors [3, 4] promising low operating costs and high speed sequencing in a disposable package.

An important limitation of integrated separation systems is the restriction on the voltage driving the separation. High-resolution CE systems require several hundred volts across the channel which can cause breakdown of thin films in microsystems [5]. In a linear DC separation channel, the channel voltage in turn determines the separation resolution. This quantity is represented by the number of theoretical plates $N_p$, which is defined as the square of the number of distinguishable bands that fit within the capillary length [6]

$$N_p = \frac{L^2}{\sigma^2}$$

(1)

where $L$ is the channel length and $\sigma$ is a measure of band width.

If band broadening is mainly caused by diffusion, the number of theoretical plates is proportional to the channel voltage [6].

$$N_p \approx \left( \frac{\mu}{2D} \right) V \approx \frac{V}{2V_T}$$

(2)
where \( \mu \) is the species mobility, \( D \) its diffusion constant, and at room temperature \( V_T = \frac{kT}{q} = 26 \) mV. In Eq. (2) we have used Einstein’s relationship which is roughly valid for charged DNA fragments moving on gels [7, 8]. At higher fields, thermal effects increase band-broadening [9], and the resolution drops. Luckey and Smith [9] found that the optimized value of electric field for DNA fragments is about 200 V/cm. If these numbers are applied for example to a 4 cm long micro-separation channel, a voltage drop of 800 V is required. This high voltage produces a large electric field which is sufficient to cause dielectric breakdown in most thin films; therefore a lower potential difference must be used.

The direct relationship between channel voltage and separation efficiency of Eq. (2) is only valid if the electric field is constant throughout the channel. This relationship breaks down if the electric field is nonuniform. For this case

\[
N_p = \frac{\mu E L}{2D} \tag{3}
\]

where \( \overline{E} \) is the average electric field sensed by the moving bands over the separation time

\[
\overline{E} = \frac{1}{\tau} \int_0^\tau E(t) dt \tag{4}
\]

Equation (3) states that rather than a large \( V \), a large \( \overline{E} \) is required on the region occupied by the migrating bands. Therefore a high efficiency separation can be achieved using a low voltage if the distance between the electrodes \( L' \) is reduced since

\[
E = \frac{V}{L'} \tag{5}
\]

where \( L' < L \). In order for the separation to be complete the field must be applied throughout the entire length of the channel. But since \( L' < L \), the voltage must “follow” the bands. This is the basic principle of the separation mechanism described here. An obvious consequence of the moving potential difference is that some of the bands may reach either electrode. Fast bands can reach the upper
electrode and slow bands may be left behind. Therefore, only bands that move in synchronization with the field are separated. Some of these can also be subject to band bending distortion due to proximity to the electrodes.

In the sections below the two dimensional electric field between the electrodes and its effect on band distortion are calculated. From these calculations, a set of rules is determined that minimize the distortion. Finally, the validity of band bending calculations and the effectiveness of the sweep field method are demonstrated in a macro-scale slab separation experiment.

2 Theory

Figures 1(a-c) show the working principle of sweeping-field electrophoresis. In addition to the reservoir electrodes, the gel contains a series of \( n \) electrodes placed along the walls with spacing \( L'/2 \). At time 0, voltage is applied from electrode 1 to electrode 3 (Fig. 1(a)). As bands move to some point between electrodes 2 and 3, the voltage is switched to electrodes 2 and 4 (Fig.1(b)), then to electrodes 3 and 5 (Fig.1(c)) and so on until the bands sweep through the entire length of the channel. At any time voltage is applied across a distance of two electrodes apart. Bands that move within the moving field region always see the larger electric field, and an effective voltage \( n \times V/2 \).

In this configuration, two intrinsic problem arise. Because the field region is small only bands with a narrow range of mobilities

\[
\frac{(L - L') L'}{V \tau} = \mu_L \leq \mu \leq \mu_H = \frac{LL'}{V \tau} \tag{6}
\]

will be traveling with the field. Bands with mobility \( \mu > \mu_H \) are compacted at the front of the moving field while those with \( \mu < \mu_L \) are left behind. Therefore a wide range separation can only be achieved using several of these type of channels spanning the entire mobility range of interest.
The bands that fall within the mobility window may also be affected by the close proximity of the electrodes. A non-uniformity in the electric field can induce band distortion and degrade the resolution of separation. In the next paragraphs these are discussed in some detail.

### 2.1 Electric Field

In Fig.1(a-c), each electrode consists of a thin platinum wire which contacts the entire thickness of the slab. In this electrode arrangement, the electric field in the channel is two dimensional. Figure 2(a) shows the configuration of a pair of these electrodes. In order to find an approximate expression of the electric field in the slab, two assumptions are made. First, we assume that the electrodes are small hence can be treated as point electrodes. The effect of finite sized electrodes is discussed later based on numerical calculations. Secondly we also assume that the channel length is much larger than $L'$. This assumption is reasonable since beyond the electrodes the electric field is small, as we will show later.

The electric field in the channel can thus be found using the method of images [10]. As illustrated in Fig. 2(b), the two point charges are repeated infinitely at periodic spacing $w$ which by symmetry makes $E_y = 0$ along the boundary of the channel. The $E_x$ and $E_y$ fields are

$$E_x \approx \sum_{n=-\infty}^{\infty} \frac{x + L'/2}{(x + L'/2)^2 + (y + w/2 + nw)^2} - \frac{x - L'/2}{(x - L'/2)^2 + (w/2 + nw - y)^2}$$

$$E_x = \frac{Q\pi}{w} \left[ \frac{\sinh \frac{2\pi(x+L'/2)}{w}}{\cosh \frac{2\pi(x+L'/2)}{w} + \cos(2\pi \frac{y}{w})} - \frac{\sinh \frac{2\pi(x-L'/2)}{w}}{\cosh \frac{2\pi(x-L'/2)}{w} + \cos(2\pi \frac{y}{w})} \right]$$

$$E_y \approx \sum_{n=-\infty}^{\infty} \frac{y + w/2 + nw}{(x + L'/2)^2 + (y + w/2 + nw)^2} - \frac{w/2 + nw - y}{(x - L'/2)^2 + (w/2 + nw - y)^2}$$

$$E_y = \frac{Q\pi}{w} \left[ \frac{\sin \frac{2\pi y}{w}}{\cosh \frac{2\pi(x-L'/2)}{w} + \cos(2\pi \frac{y}{w})} - \frac{\sin \frac{2\pi y}{w}}{\cosh \frac{2\pi(x+L'/2)}{w} + \cos(2\pi \frac{y}{w})} \right]$$
where \( L' \) is the electrode spacing, \( w \) is the channel width, and \( Q \) is a constant determined by the applied voltage. Figure 3 shows a plot of \( E_x \) for \( L' = 2 \) cm and \( w = 1 \) cm using the expression above. Beyond the electrodes, electric field drop to zero quickly. So it is valid to assume that channel length \( L \) is much larger than \( L' \) for the calculation of field. Figure 4 shows a comparison of the fields with numerically calculated values (from Laplace equation) using a boundary element method code[11] along the center of the channel. In the numerical simulation, the channel length is 4 cm with the electrodes distance \( L' = 2 \) cm. The channel width \( w \) of 1 cm and 0.1 cm are both studied. The electrodes have finite width of 0.1 cm. The boundary conditions are as following: positive electrodes are fixed with voltage 1 V, negative electrodes with voltage -1 V, and all other boundaries have zero normal field. (see the inset of Fig. 4) Due to the lateral voltage drop near electrodes, for the case \( w = 1 \) cm, the maximum field is about 20% lower than uniform field situation (\( E = V/L' \)). However, as \( w \) decreases or \( L'/w \) increases, the maximum field is quickly getting closer to \( V/L' \). Also plotted in Fig. 4 is the calculated value of \( E_x \) from Eq. (7) (solid lines) with proper choice of \( Q \). The analytical expressions are good approximations except in the regions outside the electrodes or very near them where the effect of finite size electrodes become important. When the electrodes have a width of 0.02 cm (\( L' = 2 \) cm and \( w = 1 \) cm) there is very little difference in electric field over the region of interest.

### 2.2 Band Distortion

For the band distortion calculations we neglect any additional dispersion due to diffusion. In this case, the transport of a charged particle is simply governed by

\[
\frac{d\vec{r}}{dt} = \mu \vec{E}
\]

(9)

where \( \vec{r} \) is the position vector of the charged particle, \( \mu \) its mobility, \( \vec{E} \) the electric field, and \( t \) the time. Since we have expressions for the field, Eq. (9) can be
integrated giving the position \( r' \) of a band fragment as a function of time and the band distortion. Because the expression for \( E' \) is complex, direct integration of Eq. (9) is not simple. However, we can first find the equation regulating the track of the charged particle. From Eqs. (7)-(8),

\[
\frac{dy}{dx} = \frac{E_y}{E_x} = \frac{\sin \frac{2\pi y}{w} \sinh \frac{2\pi x}{w}}{\cosh \frac{\pi L'}{w} + \cos \frac{2\pi y}{w} \cosh \frac{2\pi x}{w}}
\]

which can be converted to a total differential using the integration factor

\[
0 = \frac{1}{\sin^2 \frac{2\pi y}{w}} \left[ \sin \frac{2\pi y}{w} \sinh \frac{2\pi x}{w} \, dx - \left( \cosh \frac{\pi L'}{w} + \cos \frac{2\pi y}{w} \cosh \frac{2\pi x}{w} \right) \, dy \right]
\]

\[
= d \left[ \cosh \frac{2\pi x}{w} + \cos \frac{\pi L'}{w} \cosh \frac{2\pi y}{w} \right] \sin \frac{2\pi y}{w}
\]

and applying the initial condition \( y_0 = y(x = 0) \) yields

\[
\cosh \frac{2\pi x}{w} = \frac{\sin \frac{2\pi y}{w} \sin \frac{2\pi x}{w} \sin \frac{2\pi y_0}{w} \sin \frac{2\pi x}{w}}{\sin \frac{2\pi x}{w}} + \left( \cosh \frac{\pi L'}{w} \right) \frac{\sin \frac{2\pi (y-y_0)}{w}}{\sin \frac{2\pi y_0}{w}}
\]

Equation (12) describes the trajectory of each charged particle. In order to determine the band shape, we must find the position for each particle starting at \((x, y_0)\) at any given time. Substituting Eq.(12) in Eq. (7), and assuming \( \cosh \frac{\pi L'}{w} \gg 1 \) which is a good approximation when \( L' \geq w \) yields

\[
\frac{2\pi \mu Q}{w} \, dt \approx \left( \coth \frac{\pi L'}{w} + \frac{\cos \frac{2\pi y_0}{w} \cosh \frac{2\pi x}{w}}{\sqrt{\cosh^2 \frac{\pi L'}{w} - \sinh^2 \frac{2\pi x}{w} \sin^2 \frac{2\pi y_0}{w}}} \right) \, dx
\]

Integrating the above equation and using the initial condition \( x(t = 0) = 0 \) yields

\[
\mu Q \frac{2\pi}{w} t = \left[ \left( \coth \frac{\pi L'}{w} \right) x + \frac{w}{2\pi} \cot \frac{2\pi y_0}{w} \arcsin \left( \frac{\sin \frac{2\pi y_0}{w} \sinh \frac{2\pi x}{w}}{\sin \frac{2\pi x}{w}} \right) \right]
\]

We can now calculate the band distortion from Eqs. (12) and (14). Figure 5 shows an example illustrating the progression in the distortion of the band. Most of the distortion occurs when the band is close to the electrode, and the largest distortion occurs near the channel walls. The distortion can be minimized if the band is confined to the central region of the slab away from the wall.
distortion is also reduced if the applied voltage is switched before the band gets too close to the forward electrode. The first method requires a complex injection method; hence, it is not considered here. For the second method, we define the band distortion as

$$B_d(t, y_0) = x(t, y_0) - x(t, 0)$$

(15)

where \(x(t, y_0)\) and \(x(t, 0)\) are shown in Fig. (5). The worst-case distortion happens at the channel walls \((y_0 = \pm w/2)\). Using \(y_0 = 0\) and \(y_0 = w/2\) in Eq. (14) we arrive to

$$\left[x_e(t) - x_0(t)\right] \cosh \frac{\pi L'}{w} = \frac{w}{2\pi} \left[\sinh \frac{2\pi x_e(t)}{w} + \sinh \frac{2\pi x_0(t)}{w}\right]$$

(16)

where \(x_e(t)\) is the \(x\) position of band end at time \(t\) and \(x_0(t)\) is the \(x\) position of the band center. Assuming \(L' \leq w\) and \(x_e \sim x_0 \sim L'/2\), each hyperbolic term can be replaced with its dominant exponential term. Further, assuming \(\frac{2\pi}{w}(x_e - x_0) \sim 0\) then \(e^{\frac{2\pi}{w}(x_e - x_0)} \approx 1 + \frac{2\pi}{w}(x_e - x_0)\). Hence, the worst-case distortion is

$$B_d(t, w/2) = x_e - x_0 \approx \frac{w}{\pi} e^{\frac{\pi (2x_e - L')}{w}} = \alpha w$$

(17)

The distortion increases exponentially from the origin hence most of it occurs near the electrodes. When the band center is 0.5\(w\) away from the forward electrode, the total band distortion is only about 0.01\(w\).

The distortion is accumulated throughout the entire sweep; hence the segment distortion is multiplied times the number of sections in the channel. This determines the maximum number of theoretical plates achievable

$$N_{p,\text{max}} = \frac{L^2}{\eta^2 B_d^2} = \frac{L^2}{\alpha^2 w^2 n^2}$$

(18)

with a typical \(n = 5\) and \(\alpha = 0.01\), \(N_{p,\text{max}} \approx 400 \cdot L^2/w^2\), and with \(L/w = 50\) then \(N_{p,\text{max}} = 10^6\). This number is well within the limits of conventional CE channels; therefore in most cases the distortion due to the nonuniform field can be neglected.
These results are valid for rectangular slab channels and capillaries. For many microfabricated capillary channels, their cross sections are rectangular. For other geometries, the results will be different.

3 Experiments

Two electrophoresis channels were built on 8 cm × 10 cm gel trays. Segmented separation channels were defined by filling the tray with two pieces of styrofoam or plastic (Delrin from E.I. DuPont). The first channel was 2 cm wide and 9 cm long with five pairs of electrodes spaced 2 cm along the channel. With this channel the band distortion was recorded for \( L'/w \) ratio of 1/1, 2/1, 3/1, and 4/1. A second channel 0.4 cm wide and 9 cm long with nine electrodes equally spaced by an amount of 1 cm along the channel was constructed to record separations. A buffer reservoir is built at the position of each electrode.

Agarose gel was used for all experiments in this study. The gel is prepared with 0.5×TBE (49.5 mM Tris, 49.5 mM boric acid, and 1 mM EDTA) buffer and the agarose concentration is 0.01 g/ml. Agarose and TBE are heated up on a hot plate until the agarose dissolves in the buffer solution. The solution is then poured into the channel and cooled down to get to gel. Gel is removed in the reservoirs, and the reservoirs are filled with 0.5×TBE buffer. The injection wells are formed using a 1 mm thick comb (from Fisher Scientific). The band migration was recorded using two different dyes (Bromophenal Blue, Orange G from Sigma-Aldrich) and a commercial CCD camera (TOPICA, TP-8002A). The resulting video images were digitalized and then analyzed on a computer.

Using the first channel with \( L'/w = 6/2 \), at \( t = 0 \) the Bromophenal Blue dye band was introduced in the gel at the midpoint between the two electrodes. Next a DC voltage of 80 V was applied, and the migration was recorded. Figure 6 shows a snapshot of the band distortion at \( t = 5 \) min, superimposed with that
predicted using Eqs. (12) and (14) in reasonably good agreement. We speculate that the small band tails shown at the both sides of the channel could be caused be electroosmotic flow [12]. The band shape at other times was examined and also agree with corresponding predictions. As anticipated, when the band is more than \( w/2 \) away from the forward electrode little distortion is observed, severe band distortion occurring only near the forward electrodes. The experiment was repeated using \( L'/w \) ratios of 2/2, 4/2, and 8/2. For all cases the band distortion is very close to the theoretical prediction (Fig. 5). The maximum observed distortion for these cases was similar to that for \( L'/w = 6/2 \) as predicted by Eq. (17).

Another experiment was next performed with the second channel. Figure 7 shows the separation of two different dye bands, Bromophenal Blue and Orange G, using fixed-field electrophoresis and that using sweeping-field electrophoresis. Figure 7(a) is the band right after injection. Figure 7(b) shows the result of regular fixed-field electrophoresis. A 140 V voltage is applied from one channel end to the other \((L = 8 \text{ cm})\). After 30 minutes, the two bands are separated by an amount of 1.2 cm. Orange G moves faster than Bromophenal blue. The band widths of Orange G and Bromophenal blue are 2.8 mm and 2.6 mm respectively. Figure 7(c) shows the result of sweeping-field electrophoresis. A 60 V voltage is first applied from electrode 1 to 4 and then from 2 to 5 and so on. Compared with fixed-field electrophoresis, the applied voltage is lower and the effective electric field remains unchanged. After approximately the same amount of time, the same two bands are separated with lower voltage. The band widths are 2.4 mm and 3 mm respectively. In this case, using sweeping-field, a voltage about 2.3 times lower than that of fixed-field electrophoresis gives the same resolution as the latter. Also we noticed that band distortion due to non-uniformity of electric field is negligible.

The sweeping mode used in Fig. 7(c) is slightly different from Fig. 1. In Fig.
1 field is first applied from electrode 1 to 3, then 2 to 4, and so on. With that sweeping mode, the maximum separation distance is $L'/2$ or 1 cm in this case. As we can see from Fig. 7(b), the separation of the two dye bands exceeds 1 cm; hence it is not possible to separate them with a 2 cm electrode separation. Using the new sweeping mode, the maximum separation distance is increased to $2L'/3$ or 2 cm, and the separation of dye bands is possible. The cost of this improvement is a higher required voltage. Since $L'$ is increased from 2 cm to 3 cm, the required voltage is increased by 1.5 times to attain the same field. This is an intrinsic trade-off of sweeping field electrophoresis. Another method for increasing the separation range involves the use of multiple columns covering different mobility ranges, as pointed out in the theory section.

In Fig. 7(b), the distance between two electrodes $L$ is 8 cm, while $L'$ in Fig. 7(c) is 3 cm. The expected voltage improvement should be 2.67 times. However, the actual voltage improvement is 2.3 times. This is due to the lateral voltage drop near electrodes as partly explained by Fig. 4.

With these simple experiments, we have demonstrated the feasibility of sweeping-field electrophoresis. In this scheme bands move faster for a given voltage thus achieving a high resolution separation with lower voltage. If the distance between electrodes decreases by a factor of 10, the required voltage also decreases by 10 retaining the same resolution with a narrower mobility range.

4 Conclusion

The method of sweeping field electrophoresis has been investigated as a low-voltage, high-resolution alternative to conventional high-voltage DC separations. In this method, the driving voltage is applied to a small segment of the channel that moves with the bands. This scheme requires a lower potential to attain a high electric field at the expense of a narrow separation mobility range. The
effects of the non uniform electric field on band distortion have been calculated and experimentally confirmed. For capillary channels with high aspect ratios, the band distortion is negligible.

References


FIGURE CAPTIONS

Fig.1 Principle of sweeping-field electrophoresis. The electric field is first applied from electrode 1 to 3 (a), then 2 to 4 (b), and so on. The voltage is always applied on a shorter distance; thus the effective electric field is larger.

Fig.2 Calculation of electric field using the image method. The field within the channel may be calculated by using an infinite array of point electrodes in the y direction.

Fig.3 Electric field in x direction calculated using the image method (Eq. 7). The channel width is 1 cm and the distance between two electrodes is 2 cm.

Fig.4 Electric field along the center of the separation channel calculated using a boundary Element solver with finite size electrodes. The solid line is the result of Eq.(7).

Fig.5 Band distortion due to non-uniform field. Band start from the middle of two electrodes and is distorted when it comes near electrodes.

Fig.6 Comparison of experimented and predicted band distortion. The band curvature is in good agreement with Eq. (14).

Fig.7 Separation of two dye bands showing: (a) the initial band; (b) separation with regular electrophoresis; and (c) separation using sweeping field with a voltage 2.3 times lower than that in (b). The results of (b) and (c) are almost identical demonstrating a voltage improvement with sweeping field.