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Binary Oscillatory Crossflow Electrophoresis

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Abstract.

We present preliminary results of our implementation of a novel electrophoresis separation technique: binary oscillatory cross flow electrophoresis (BOCE). The technique utilizes the interaction of two driving forces, an oscillatory electric field and an oscillatory shear flow, to create an active binary filter for the separation of charged species. Analytical and numerical studies have indicated that this technique is capable of separating proteins with electrophoretic mobilities differing by less than 10%. With an experimental device containing a separation chamber 20cm long, 5cm wide, and 1mm thick, an order of magnitude increase in throughput over commercially available electrophoresis devices is theoretically possible.

Introduction.

Electrophoresis has long been shown as an effective process for the separation of particles and biological macromolecules based on small differences in electrophoretic mobility. Since Tiselius [1] discovered electrophoresis in 1937, the technique has become a cornerstone for biochemical analysis and has branched into several different subfields. Despite its attention, several key problems have prevented electrophoresis techniques from scaling up to commercial processes. Among these are Joule heating and crescent dispersion caused by electroosmosis, parabolic velocity profiles of the carrier electrolyte, and electrohydrodynamic effects [2], [3].

The present research project, binary oscillatory crossflow electrophoresis, has the potential for significant improvements in throughput and resolution over conventional CFFE techniques. This technique relies on the interaction of an oscillatory electric field and an oscillatory shear flow, shown in Figure 1.

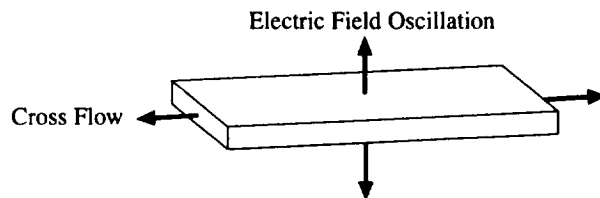


Figure 1: Direction of motions in a binary oscillatory crossflow electrophoresis cell.

By appropriate selection of frequency and phase of oscillation of the two interacting driving forces, an effective filter can, in principle, be created that will allow proteins either higher or lower than a chosen electrophoretic mobility to pass through the device. The technique could be used for both analytical and preparative work by programming the filter setpoint with time and collecting proteins that pass through the device at each setpoint. For continuous isolation of a particular fraction from a complex mixture feed, a network of four of these devices could be used.

Analytical Model

The driving forces in the binary separator are an oscillatory convective flow and an oscillatory electric field. The solute molecules will have some net motion that is a complex function of the two driving forces, the electrophoretic mobility, and the diffusion of the species. The cross flow may be written as

$$u_x = 4u_{max} \frac{y}{b} \left(1 - \frac{y}{b}\right) f(\omega t) \tag{1}$$

where u_x is the fluid velocity in the x direction, u_{max} is the velocity of the center line, y is position in the gap, b is the gap thickness, ω is the frequency of oscillation, t is time, and f is some periodic function of ωt . The motion of a particular solute molecule across the channel will depend on $f(\omega t)$ and the position of the molecule in the gap. If we neglect diffusion, we may write the motion of the solute across the gap due to an oscillatory electric field as

$$\frac{dy}{dt} = \frac{\mu E_o}{\omega} \frac{d}{dt} g(\omega t) = \mu E_o h(\omega t) \quad (2)$$

where μ is the electrophoretic mobility, E_o is the characteristic amplitude of the electric field, $g(\omega t)$ is the oscillatory motion of the solute, and $h(\omega t)$ is the oscillation of the electric field. Integrating this equation, we can solve for the position across the gap of the solute molecule.

$$\frac{y}{b} = \alpha g(\omega t) \quad (3)$$

where $\alpha = \frac{\mu E_o}{\omega b}$ is the dimensionless electrophoretic mobility and $g(\omega t)$ is bounded between 0 and 2. Substituting the position of the solute molecule into equation 1 and taking the time average, we will have an equation that describes the motion of a solute molecule in the absence of molecular diffusion:

$$\left\langle \frac{dx}{dt} \right\rangle = 4u_{max}\alpha \left(\langle g(\omega t)f(\omega t) \rangle - \alpha \langle g(\omega t)^2 f(\omega t) \rangle \right) \quad (4)$$

where $\langle \cdot \rangle$ is the time average over one period. Equation (4) is limited to dimensionless mobilities $\alpha \leq 0.5$. The maximum value of α corresponds to a solute molecule that just reaches the opposite wall during one period of oscillation. With equation (4) we may evaluate the solute velocity across the channel for combinations of arbitrary choices for $f(\omega t)$ and $g(\omega t)$. Chandhok and Leighton [4] chose $g(\omega t) = 1 - \cos(\omega t)$ and showed that the only Fourier modes of a general periodic function $f(\omega t)$ that contributed to a migration velocity were a steady flow, $\cos(\omega t)$, and $\cos(2\omega t)$. While this analytic solution is very useful for qualitatively determining the performance of such a separations device, the solution is seriously limited by neglecting the effects of diffusion. This is particularly true since the interaction of diffusion with an oscillatory shear flow has been shown to increase the dispersion in the direction of motion by several orders of magnitude [5], [6]. Furthermore, bounding the maximum value of α significantly limits the possible operating parameters of the system.

In order to better characterize the motion of the solute, we numerically simulated the solute motion in the channel including the effects of diffusion. To perform the numerical simulation, we used a molecular dynamics approach. At each time step, we added a random walk of length $\sqrt{2\lambda\Delta t}$ to the electrophoretic motion of a solute molecule and used the resulting position to calculate the displacement in the direction of the fluid motion. The simulation was carried out for 1000 molecules and the average position and variance of the solute as a function of time were recorded. From the position data we determined the time averaged solute velocity and the effective diffusion coefficient in the direction of motion of the solute. We ran the simulation until the velocity reached a steady value, usually after only a few periods of oscillation.

For our simulation we have chosen the gap width $b = 0.1$ cm, and by arbitrarily choosing the dimensional electrophoretic mobility of a particular species and the oscillation frequency ω , we fix the electric field amplitude for a desired value of α . Consequently, our choice of the above parameters fixes the dimensionless diffusivity. We simulated the solute motion for an electric field of amplitude $h(t) = \sin(\omega t) - 0.05$ coupled with both $f(\omega t) = \cos(\omega t)$ and $f(\omega t) = \cos(2\omega t)$. The small steady component to the electric field strength of 0.05 is important to refocus the solute at the lower wall during each period. The velocities for the solute as a function of α for $f(t)$ equal to $\cos(\omega t)$, $\cos(2\omega t)$, and $\cos(\omega t) - \cos(2\omega t)$ coupled with $h(t) = \sin(\omega t) - 0.05$ were simulated and are shown in Figure 2.

Note that $f(t)$ equal to $\cos(\omega t)$ and $\cos(2\omega t)$ both lead to negative velocities, with the maximum amplitude occurring at different mobilities. For a binary separation to be possible, the time averaged velocity for the solute molecules must change sign at some critical value of the electrophoretic mobility. Because the fluid motion does not directly influence the migration across the gap, the effect of each mode of the imposed solvent velocity on the time averaged solute velocity is linearly additive. As a result, we chose to subtract the two modes. The velocity resulting from $f(\omega t) = \cos(\omega t) - \cos(2\omega t)$ coupled with $h(t) = \sin(\omega t) - 0.05$ meets the constraints for a binary separation. In addition, $\frac{d(v_A/u_{max})}{d\alpha}$ is large near the mobility with zero net velocity, a condition for sharp separation.

To determine the characteristic throughput and selectivity of the device, we consider a cell connecting two reservoirs of fluid. In one reservoir, we impose some concentration c_{A1} , in the other reservoir we impose a concentration of zero by flushing it with buffer. The cell is of length L , width W , and thickness b with operating parameters as listed above. The cross flow will oscillate with $f(\omega t) = \cos(\omega t) - \cos(2\omega t)$ and $u_{max} = \Delta x \omega$ where Δx is the characteristic amplitude of fluid oscillation.

To find the flux though a cell we start with the governing equation

$$k_{eff}^* \frac{\partial^2 c_A^*}{\partial x^{*2}} = v_A^* \frac{\partial c_A^*}{\partial x^*} \tag{5}$$

where k_{eff} is the dispersion coefficient determined numerically, x is the direction of flow, c_A is the concentration of species A, and v_A is the velocity of species A depicted in Figure 2. Equation (5) is the result of substituting the flux into the mass balance. By integrating twice and imposing the boundary conditions of $c_A^*(x^* = 0) = 1$ and $c_A^*(x^* = L/\Delta x) = 0$, we can solve for the dimensionless concentration profile. Substituting the dimensionless concentration profile into the flux equation, we find the the dimensionless flux is

$$N_A^* = v_A^* \frac{1}{1 - \exp\left(\frac{-v_A^* L}{k_{eff}^* \Delta x}\right)} \tag{6}$$

Figure 3 shows a plot of N_{Ax}^* as a function of α for a value of $\frac{\Delta x}{L} = \frac{1}{5}$ where v_A^* is as shown in Figure 2 and k_{eff}^* is determined numerically. The curve labeled high pass represents the flux

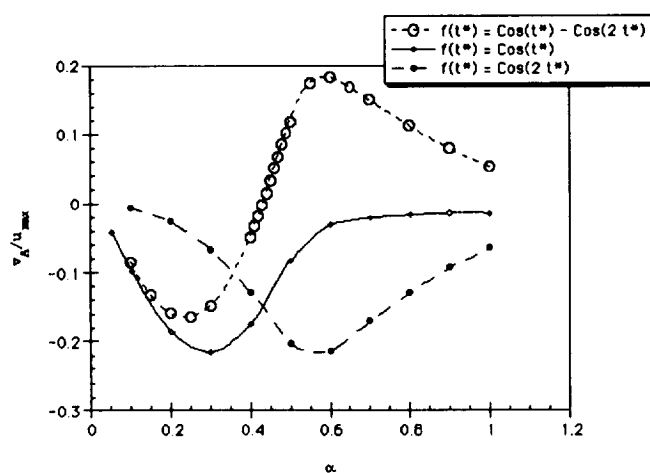


Figure 2: Plot of dimensionless velocity versus dimensionless electrophoretic mobility including the effects of cross-streamline diffusion.

when $f(\omega t) = \cos(\omega t) - \cos(2\omega t)$, and the curve labeled low pass represents the flux when $f(\omega t) = \cos(2\omega t) - \cos(\omega t)$.

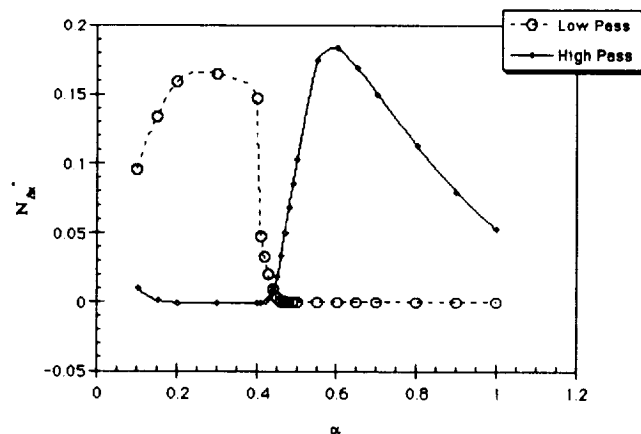


Figure 3: Plot of Dimensionless Flux versus dimensionless electrophoretic mobility.

Clearly the flux changes very dramatically near $\alpha = 0.41$ for both high and low pass operation. For a 10% change in α about this point, the flux increases two orders of magnitude.

Experimental.

To verify the analytical and numerical results of the BOCE technique, an experimental system including a single binary separation cell, two reservoirs and an electrolyte bath was constructed. The cell is similar to that of Giddings [7], and allows continuous flow of solute in a buffer solution through a separation chamber 20.0 cm long, 5.0 cm wide, and 0.1 cm thick. Care was taken to address problems inherent in electrical flow cells of this type; mainly gas generation from electrode reaction, Joule Heat and membrane flexure.

The BOCE cell is approximately 24.0 cm long, 7.0 cm wide, and 2.0 cm thick. The central separation chamber of the cell is machined from a plastic sheet that is divided into two halves to accommodate feed and exit channels. Fluid is introduced at either end of the chamber by means of a stainless steel syringe connected to polypropylene fittings extending through the electrolyte bath and connecting to the reservoirs. The separation chamber is sandwiched between two cellulose membranes, supported by the electrode screens. The layers are clamped between two plexiglass blocks. In the center of each block a rectangular opening approximately the size of the separation chamber was machined. This design allows the central chamber to be sealed with the membranes and screen support using bolts to provide uniform pressure around the perimeter.

Giddings [7] found in experiments using a similar device that cellulose membranes are prone to flexing even upon small pressure fluctuations. The requirement of fixed volume in the separation chamber thus necessitates the use of a support. Aside from chemical compatibility with the buffer solution and mechanical strength, the electrical resistance was an important consideration. It was therefore decided that the electrode screens could be used as supports, thus serving dual purposes. This reduced the electrode separation to under 1.1 mm.

Gas bubbles and Joule heat are removed by submerging the entire cell in an electrolyte bath open to atmosphere. The bath accommodates up to 6 liters of electrolyte fluid and copper cooling coils. The cell is positioned in the bath with the 7.0 cm side forming the vertical axis, and gas bubbles generated from electrode reaction are removed from the electrodes through the machined

openings in the clamping block halves.

The two oscillatory driving forces were provided by a KEPCO BOP100-4M bipolar power supply capable of constant current operation and a specially designed syringe pump. The electric field was controlled using LabVIEW software and a 100 MHz Pentium personal computer. Bulk fluid conductivity data was acquired into LabVIEW using a Cole-Parmer conductivity meter and the amplitude of the current waveform was simultaneously adjusted to maintain the desired electric field. The flow waveforms were delivered to the syringe pump using a Galil DMC-1500 motion controller and software. Position data from the syringe pump was fed back into LabVIEW allowing phase locking of the two driving forces to within a few milliseconds. Temperature was monitored using the Cole-Parmer conductivity meter and protein concentration was analyzed using a Varian UV-VIS spectrometer.

Results

The focus of preliminary experiments was to verify qualitatively that the interaction of a purely oscillatory shear flow with an oscillatory electric field could indeed lead to the net convection of a single charged protein species under conditions of uniform concentration throughout the system. Furthermore, we sought to demonstrate that by changing the dimensionless mobility, α , by changing the amplitude of the electric field, we could change the sign and magnitude of the velocity of the protein. To this end preliminary experiments were conducted using Bovine Hemoglobin (BHb) dissolved in a sodium acetate/acetic acid buffer of pH 4.5 and conductivity of approximately $375.0 \frac{\mu S}{cm}$. Based on results from Douglas et. al. [8], the electrophoretic mobility of BHb was approximately $17.4e^{-5} \frac{cm^2}{V_{sec}}$. The choices of waveforms included $h(wt) = \sin(wt) - 0.05$ for the electric field and $f(wt) = 2 \cos(2wt)$ for the crossflow. The concentration throughout the system was initially uniform using a 0.021 wt% solution of BHb. The syringe, which served as the inlet reservoir, was filled with a volume of 8mL of protein solution, while the outlet reservoir contained approximately 40mL of the same solution.

For each of the experiments, the frequency of oscillation was chosen to be $0.068 \frac{1}{sec}$ and the stroke volume to be 1.5 mL. For $\alpha = 0.25$, an electric field amplitude of $9.795 \frac{V}{cm}$ was required, and for $\alpha = 0.5$, the amplitude was doubled. In both experiments, the concentration of the outlet reservoir was monitored with time. The results of the experiment using $\alpha = 0.5$ is shown in Figure 4. Note the concentration of the outlet reservoir increased by over 10% for 20 minutes of run time while the syringe concentration (not shown) was almost entirely depleted. The results of these experiments indicate that even with initially uniform concentrations, a net flux could be obtained for both choices of α , and furthermore, the magnitude of the velocity qualitatively agreed with theory.

Experiments will continue with single protein species using various oscillatory shear flows and dimensionless mobilities under both transient and steady state conditions. From these experiments we hope to achieve quantitative comparisons of the net velocity and effective diffusivity of various protein species. Additionally, we will conduct multi-protein experiments in an attempt to verify that binary oscillatory crossflow electrophoresis as an effective binary separation technique.

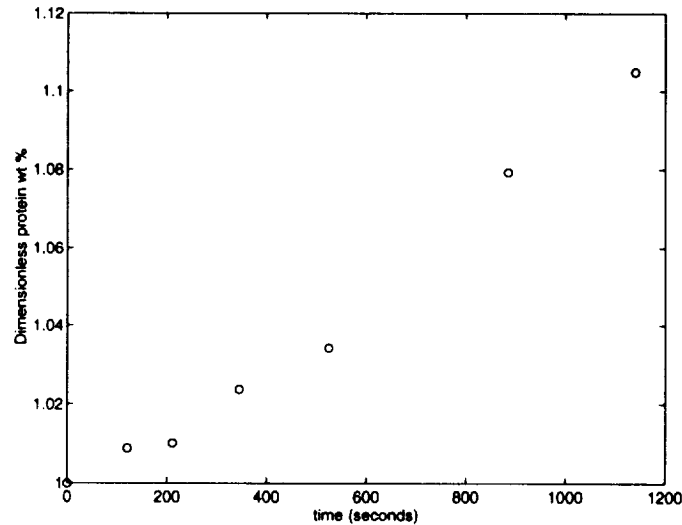


Figure 4: Plot of dimensionless weight percent of Bovine Hemoglobin in the outlet reservoir versus time for a dimensionless electrophoretic mobility of $\alpha = 0.5$.

References

- [1] Tisilius, A., "A New Apparatus for Electrophoretic Analysis of Colloidal Mixtures," *Trans. Farad. Soc.*, **33**, 524 (1937)
- [2] Rhodes, P.H., R.S. Snyder, and G.O. Roberts, "Electrohydrodynamic Distortion of Sample Streams in Continuous Flow Electrophoresis" *J. Colloid and Interface Sci.* **Vol 129 No 1**, 78 (1988)
- [3] Sayville, D.A., "Electrohydrodynamic Deformation of a Particulate Stream by a Transverse Electric Field" *The American Physical Soc.* **Vol 71 No 18**, Nov 1, 2907 (1993)
- [4] Chandhok, A.K., and D.T. Leighton, Jr., "Oscillatory Cross-Flow Electrophoresis," *AICHE Journal*, **Vol 37 No 10**, 1537 (1991)
- [5] Aris, R., "On the Dispersion of a Solute by Diffusion Convection and Exchange Between Phases," *Proc. Roy. Soc. A*, **252**, 539 (1959)
- [6] Watson, E.J., "Diffusion in Oscillatory Pipe Flow," *Journal of Fluid Mechanics*, **Vol 133**, 233 (1983)
- [7] Giddings, J.C., M.N. Myers, K.D. Caldwell, and S.R. Fisher, "Analysis of Biological Macromolecules and Particles by Field-Flow Fractionation" *Methods of Biochemical Analysis*, **Vol 26** 79 (1976)
- [8] Douglas, N.G., A.A. Humffray, H.R.C. Pratt, and G.W. Stevens, "Electrophoretic Mobilities of Proteins and Protein Mixtures," *Chem Eng Sci*, **Vol 50 No 5**, 743 (1995)