Localized Asymmetric Electric and Velocity Field Effects during Counterflow Gradient Focusing at a Converging Channel

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Abbreviations:
EP (Electrophoretic), CE (Capillary Electrophoresis), CZE (Capillary Zone Electrophoresis), FASS (Field-Amplified Sample Stacking)

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Abstract

Electrophoretic exclusion is a counter-flow gradient focusing method that simultaneously separates and concentrates electrokinetic material at a channel entrance utilizing electric and fluid velocity fields. However, its effectiveness is heavily dependent on the mostly undefined field gradients about the entrance. This work examines the lateral fluid velocity and electric fields within the channel entrance region. A model using finite element analysis was constructed to simulate experimental conditions. Simulation results indicated decreased gradient uniformity for both electric and fluid velocity fields closer to the channel wall. The magnitude of each of these fields varied dramatically with respect to the centerline values, resulting in a localized concentration enhancement at lower applied voltages than previously observed or predicted. Simulation results are compared to the concentration of charged fluorescent dye monitored at the entrance using fluorescent microscopy and midway down the channel with visible spectroscopy. Results were consistent between the electrophoretic exclusion experiment and simulation, providing new insights for the lateral fluid velocity and electric fields about the channel entrance region that will aid future device design and fabrication strategies.
1 Introduction

The appeal of CE and related microfluidic electrophoresis separations often stems from the high resolution, low volume reagent use, and highly adaptable simple designs common with most systems. However, limited sensitivity has been historically regarded as a major drawback to the technique [1]. Consequently, a variety of on-line concentration enhancement strategies have been developed to overcome this issue [2-7].

Sample stacking, broadly defined as analyte concentration enhancement on a boundary by electrophoretic (EP) velocity change, is one such strategy which encompasses a variety of configurations [4, 8-10]. Among the simplest and most common configuration is on-line field-amplified sample stacking (FASS). FASS results when two solutions of different conductivity induce an electric field gradient at the solution boundary to where electrophoretically mobile analytes migrate, stack, and are subsequently separated using traditional CZE [11]. While this configuration has been widely accepted for on-line pre-concentration of charged analytes within the sample, it is largely dependent on the sample amount initially injected and limited by the conductivity ratio between the two solutions [10].

Counter-flow gradient focusing offers another general sample stacking approach that relies on the equilibrium gradient principle, summarized by Giddings, to achieve the improved sensitivity [12]. While the specifics of several focusing configurations have been described elsewhere [13, 14], all employ a constant force opposed to some gradient (electric field, pH, conductivity, temperature, micelle, etc.) to cause a unique and specific equilibrium position to where analytes with similar properties, such as net charge, mass, size, etc., migrate from all parts of the separation domain. Separation and concentration occur simultaneously and diffusional
band-broadening is minimized as restoring forces on both sides of the equilibrium position act to keep the concentration plug focused.

Electrophoretic exclusion is a form of counterflow gradient focusing where charged analytes with constant hydrodynamic velocities ($U$) oppose an electrophoretic velocity ($U_{EP}$) induced by an electric field residing at a channel entrance [15-17]. Generally speaking, when an exclusion condition exists for a given analyte, the analyte velocity drops to zero at the equilibrium boundary where it concentrates and never enters the channel. Analytes with higher EP mobility are also excluded from entering the channel while analytes with lower EP mobility pass through the entrance boundary until they exit the opposite channel end. Unlike FASS or related stacking techniques that rely on a conductivity ratio between solution plugs to create an electric field gradient, and unlike most other counterflow gradient focusing techniques that create a continuous gradient to separate analytes serially along the gradient, electrophoretic exclusion relies on distinct electric field and flow field gradients induced at a converging channels entrance with deliberately positioned electrodes to create a single differentiation zone. Advantages offered by this design include simultaneous separation and concentration that is not limited to creating and maintaining conductivity ratios near the entrance. Additionally, this design can be easily expanded to serial or parallel (array) formats where the electric field and detection element of each array unit could be specifically tailored and independently operated to concentrate a chosen category of analytes in bulk solution. However, the effectiveness of this design is heavily dependent on the shape and steepness of the field gradients [18].

The significance of the entrance geometry and spatial arrangement of the electric field and hydrodynamic velocity gradients for electrophoretic exclusion and related techniques has been realized [16, 19]. The coupling of simulated electrophoretic and fluid velocity fields along a
longitudinal entrance centerline demonstrated an electric field gradient sharpening effect with an electrode placed about the entrance, although the entrance geometry as a whole was ignored [20]. An electrode with no radial symmetry and patterned a few micrometers from the entrance of a PDMS microdevice produced a gradient still capable of achieving electrophoretic exclusion, but the specific impact of symmetry and location on performance was not quantified [21]. In a recently published theoretical description of the electrophoretic exclusion construct, resolution was found to be directly dependent on the steepness of the electric field gradient at the entrance [18]. Despite the useful information obtained from the previous studies, evidence exists that flow and electric field gradients also vary laterally across the entrance, especially near the walls and corners, necessitating further examination of this region to better understand the field gradient components that directly affect the exclusion condition [16, 20].

This work examines the lateral fluid velocity and electric fields within the channel entrance region by simulation and experiment to aid future device design and fabrication strategies. A model using finite element analysis was constructed to perform simulations with experimentally-similar electrophoretic exclusion conditions in an effort to extrapolate information pertaining to lateral electric fields, fluid velocity fields, and any transport dynamics that may be associated with diffusion, convection, and electrokinetic dispersion. To experimentally assess the gradient field and transport effects for comparison to the simulation, the concentration of charged fluorescent dye was monitored at the entrance using fluorescent microscopy and midway down the channel with visible spectroscopy. Simulation and experimental results were consistent and indicated decreased gradient uniformity for both electric and fluid velocity fields closer to the channel wall that resulted in a localized concentration enhancement at lower applied voltages than previously observed or predicted.
2 Materials and methods

2.1 Experiment design

Figure 1. (A) Schematic of experimental design. A flat-glass plate reservoir (far left) above the microscope objective is filled by applying nitrogen gas to a sample feed reservoir. A capillary channel connects the plate reservoir, which serves as the inlet, and cuvette outlet reservoir (far right). Bulk fluid velocity ($U$) and direction were controlled by adjusting the gas and hydrostatic pressure (induced by the fluid level height difference ($\Delta h$) between the feed reservoir and outlet). EP velocity ($U_{EP}$) was controlled based upon electric field magnitude and analyte EP mobility. (B) Representative 2-D finite element analysis model with shaded lines representing boundary electrodes. Dashed boxes in A and B represent channel entrance regions of interest.

The general design and fabrication of the device (Figure 1) consisted of a 20 μL glass plate inlet reservoir (9 mm × 6 mm × 365 μm) and 2 mL outlet vial fluidically connected using a
10 cm long fused silica capillary (75 μm i.d. 365 μm o.d., Polymicro Technologies, Phoenix, USA). An electrode was constructed exactly at the capillary channel entrance of the inlet by sputter-coating 30 nm titanium, then 50 nm platinum on the cleaved tip where the polyamide coating had been removed [15, 17]. This electrode was connected to two parallel electrodes running along the edges of the inlet reservoir. Another platinum electrode was placed in the 2 mL outlet vial completing the circuit.

Fluid flow was controlled using both hydrostatics and house nitrogen. Setting the total pressure by countering one another allowed changes to flow magnitude and direction with simple adjustment of the nitrogen pressure, monitored with sensor (GPS-BTA, Vernier, Beaverton, USA), and/or vial height via bench-top scissor lift. Volume flow rate was calculated using density and weight of liquid collected from the channel during a set time and temperature, and then converted to average flow velocity. The velocities from each pressure source were tested independently by removing the other pressure source during measurement.

The bulk fluid utilized in the experiments was a 5 mM phosphate buffer (Sigma Aldrich, St. Louis, USA) with pH of 2.2. Rhodamine 123, a cationic fluorescent dye (Molecular Probes, Grand Island, USA), was prepared at 5 μM for use in the concentration monitoring experiments. Prior to conducting experiments, the reservoirs and channels were preconditioned with buffer for a minimum of 30 minutes and then the rhodamine 123 was added and allowed to reach a steady state concentration throughout the system.

Concentration monitoring was accomplished using fluorescent microscopy at the channel entrance and on-capillary visible spectroscopy midway down the channel. An Olympus IX70 inverted epifluorescent microscope (Tokyo, Japan) with a 4x, UPlanAPO, 0.16 NA objective, mercury short arc light source, and QICAM CCD camera (QImaging, Burnaby, Canada) with
StreamPix III image capturing software (Norpix, Montreal, Canada) were employed for fluorescence imaging. An Ocean Optics USB 4000 visible spectrometer (Dunedin, USA) with optical fibers positioned perpendicular to and 5.2 cm down the channel was employed for the spectroscopic measurements.

2.2 Model design

A 2-dimensional finite elemental analysis model developed with COMSOL Multiphysics 4.4 software (COMSOL, Inc., Los Angeles, USA) was used to calculate the transport dynamics of the electrophoretic exclusion construct. The model geometry closely mirrored that of the fabricated device with the exception of the reservoir sizes and length of the feed reservoir tubing. To minimize unnecessary meshing, the model reservoir dimensions were reduced approximately 10-fold without causing a significant change to the overall hydraulic and ohmic resistances. The ~1000-fold reduction in the length of the model feed reservoir tubing was accounted for by using the software’s built-in correction factor under the laminar flow interface where the length of the inlet channel outside the model domain can be defined (46 cm in this case). Liquid, silica glass, and copper materials were selected from the built-in library and assigned to the respective geometric entities. The liquid electrical conductivity was modified to reflect that of the phosphate buffer used in the experiments. The laminar flow, electric current, and transport of dilute species interfaces were coupled during the simulations. Electric potential (800 V) was assigned to an electrode boundary located 1 mm from the exit in the reservoir, while ground was assigned to both the electrode boundary on the capillary face and to the electrode boundaries along the inlet reservoir edges parallel to the length of the capillary (Figure 1). All other boundaries were defined as electrical insulation. Laminar, incompressible flow was assigned to all domains and a no slip condition to all boundary walls. The inlet boundary condition was set
to a laminar inflow entrance pressure of 2068 Pa and entrance length of 46 cm. The outlet boundary condition was set to a 1582 Pa no viscous stress pressure resulting in a net pressure of 486 Pa towards the outlet. Rhodamine 123 was assigned an initial concentration of 0.001 mol/m$^3$ for all domains and an EP mobility of $1.8 \times 10^{-8}$ m$^2$/Vs, determined experimentally using traditional CE with similar concentration and buffer composition. The quadratic shape function order within the Transport of Diluted Species interface was selected in place of the linear option to improve the accuracy of the results for low Reynolds number flow such as those in this model. A 5 µm wide and 75 µm high rectangular domain probe was inserted midway down the channel simulating a spectroscopic detection zone for monitoring local average concentration. All other model parameters were left at default and, where applicable, closely mirrored the fabricated device and experimental conditions.

3 Results and discussion

3.1 Simulation demonstrating localized exclusion and concentration enhancement

A simulation was performed to first assess the lateral fluid velocity field within the channel entrance region. Given the low Reynolds number for this system, the characteristic features of laminar flow were assumed within the channel. Here, fully developed flow, or the lack of a longitudinal gradient, results along all laminae a few microns inside the channel. The maximum velocity lamina ($U_{\text{max}}$) resides at the longitudinal centerline and the effective (sometimes “average”) velocity lamina ($U_{\text{eff}} = \frac{1}{2} U_{\text{max}}$), resides parallel to and between $U_{\text{max}}$ and the channel wall, where $U_{\text{wall}} = 0$ µm/s. The simulation was consistent with the laminar flow description evidenced by $U_{\text{max}}$ residing along the centerline and decreased velocities along laminae nearer the wall (Figure 2A).
Figure 2. Flow (A), electric field (B), and concentration profiles (C) near capillary entrance ($x = 0$) calculated from finite element simulation. Note patterned lines in right panels with corresponding univariate plots in the left panels. The combination of flow and electric field lateral asymmetries create a concentration bolus (C) along the wall near the entrance.

A simulation was also used to assess the lateral electric field within the channel entrance region. The electric field gradient remained largely unchanged from beyond 50 µm outside and 50 µm inside the entrance regardless of proximity to the channel wall (Figure 2B). However, a pronounced, non-uniform gradient resulted near the entrance along the cutline nearest the wall. The localized electric field spike as predicted by the simulation had twice the magnitude of the global electric field making it a potentially important feature within the electrophoretic exclusion construct.

The features of the independent flow and electric field calculations resulted in a prediction of a local increased concentration not noted in previous assessments (Figure 2C). Although the global pressure and electric field settings do not suggest the formation of a bolus under these conditions, the combination of localized velocity minimums and electric field
maximums near the entrance wall gave rise to a noticeable concentration enhancement near the corners.

Figure 3. (A) Surface concentration plot from finite element simulation with voltage on (upper left) and immediately after it was removed (subsequent panels starting from middle top moving to bottom right). (B) Concentration plot after potential was removed as detected midway down the channel (detection location at vertical line across channel in A).

The simulations can be presented such that experimental data can be directly compared to the results. Simulated time-dependent surface plot concentrations during applied potential and
upon potential removal provided a visual and spectroscopic-like means to monitor concentration changes (Figure 3). Transport dynamics from diffusion, convection, and electrokinetic dispersion were included in the simulation to more closely match the conditions with which experimental data were collected.

### 3.2 Flow velocity and model validation

![Graph showing flow velocity vs. pressure](image)

**Figure 4.** Comparison of calculated and measured fluid velocity. Average fluid velocity determined by fluid volume weight at various pressures induced by hydrostatic effects (circle markers) and nitrogen gas (square markers). Theoretical estimates of the system: Poiseuille’s law (solid line) and model simulation (dashed line) for comparison.

Since flow and electric fields must be carefully controlled and the electric field can be trivially controlled via external power supply, establishing known and controllable magnitude of fluid velocities was necessary to properly assess the experimental results. Gravimetrically calculated average fluid velocities from both hydrostatic forces and nitrogen gas pressures were compared to the 2-D finite element computer simulations and Poiseuille’s law. As expected, the average velocities increased linearly with increased pressure (Figure 4). The calculated and
simulated values fell within the standard error of the experimentally determined values indicating reliable use of the simulation, fluid level height difference, and nitrogen pressure sensor as accurate and precise predictors of fluid velocity.

Some assessment of the electric field can be made. The simulated electric field was compared to values according to a simple Ohmic model. The field well within the length of the capillary is estimated to be constant \( E_{\text{global}} \), according to \( E_{\text{global}} = \frac{V}{L} \), where \( V \) is applied voltage, and \( L \) is channel length. Within the simulation, inputs of 800 V across the 10 cm channel generated a global electric field solution of 8000 V/m (Figure 2), which was consistent with the Ohmic result. Although the global electric field was not measured experimentally due to the device design prohibiting sample plug injection, the agreement between the monitored current, Ohmic model, and the 2-D simulation supported the validity of the 2-D model.
3.3 Experiment demonstrating local exclusion and concentration enhancement

Figure 5. Images of dye dynamically exiting a capillary and plot of bolus passing a detector within the capillary. Rhodamine 123 exiting capillary entrance (A) after local exclusion and flow direction reversal. Capillary width = 360 µm, channel diameter = 75 µm, frame interval = 1.6 s. See Figure 1 Microscope for region of interest probed. Spectroscopic plot of Rhodamine 123 midway down channel (B) after a 60 s applied voltage was removed at 90 s. Plot shape is representative of other data sets collected under similar conditions. Dashed plot is a normalized overlay of model data from Figure 3B offset from this experimental data for comparison. See Figure 1 detector for region of interest probed.

A local concentrated bolus is predicted by the simulations at a location that is difficult to image. Two temporal methods were used to confirm (or refute) the presence of a bolus under conditions where previous assessments suggest none should form. The examination of the simulation cutlines approaching the channel wall (Figure 2) exhibited varying localized velocity and electric field gradients relative to flat (non-gradient) global longitudinal velocity and global
longitudinal and lateral electric fields found inside the channel. To determine whether these local gradients and their effects exist under experimental conditions, the concentration of rhodamine 123, a fluorescent and electrophoretically mobile dye, was monitored during conditions set similar to the simulation. While the lateral fields themselves cannot be directly visualized or assessed, monitoring the concentration at the entrance using fluorescent microscopy and midway down the channel with visible spectroscopy provided a means to test the model.

During the application of the same electric potential used in the simulation, no concentration increase was observed at the entrance area using the epifluorescent microscope. The simulation indicated that the concentrated zone resides just inside the channel as potential was applied and is consistent with no increase of fluorescence near the entrance (Figure 5A, first image on left). Figure 5A illustrates a 6.4 s montage of rhodamine 123 exiting the capillary after the local exclusion condition was established and subsequently pushed from the channel using reversed fluid flow so any local concentration enhancement could be visibly detected. The concentrated zone exiting the channel in both the simulation and experiment contained higher concentrations on the periphery compared to the center (Figures 3A and 5A). A similar experiment was performed without changing flow direction or magnitude where the applied potential was removed to allow any collected sample to flow down the channel towards the spectroscopic detection zone (Figure 3B and 5B). Based on the bulk fluid velocity and the time to reach the detector, the original location of the concentrated zone was back-calculated to the channel entrance, consistent with the simulation results (Figure 3A). Similarities between the spectroscopic and simulated probe peak shapes (Figures 5B and 3B, respectively) as well as the consistent shape and general appearance between the concentration zones in both the microscope
and simulation studies, further supported the notion that the localized lateral field effects observed during the simulation were also present in the experimental.

Under normal exclusion conditions as demonstrated in previous works, where $U_{EP}$ of the analyte meets or exceeds $U_{eff}$ at steady state, complete analyte exclusion would occur and any analyte initially inside the channel prior to applied voltage would be evacuated. However, under the conditions described in this work, where $U_{EP}$ (calculated using the globally applied voltage) was five times less than $U_{eff}$, no exclusion or discernable concentration enhancement would be expected, especially with convective and diffusional dispersive forces inherent with the system. Instead, the coupling of the sudden decrease in fluid velocity with the sharp local electric field at the corner created a localized concentration bolus confined to the entrance corner region when voltage was applied.

With the characterization of both the longitudinal and lateral velocity and electric fields of the currently adopted EP exclusion design, strategic manipulation of the device geometry and electrode configuration can be considered for improved resolution and overall performance. One such non-trivial approach would be the suppression of any lateral gradient so an analyte approaching the channel entrance from any direction would be subject to only a single, well-defined, sharp longitudinal gradient for either the velocity or electric field components. Another attempt may instead be the manipulation of the geometry and electrodes of the current system to further exploit the corner gradient spikes generated at relatively low applied voltages. With either approach, the continued modeling and experimental testing of the field effects within the EP exclusion construct will play an important role for efficient device design.
4 Concluding remarks

To design a high resolution separation interface utilizing a punctuated electrophoretic counterflow gradient approach like EP exclusion, the electric and velocity fields across the entire separation domain must be understood. This work demonstrated the relationship between localized asymmetric electric and velocity fields near a converging channel entrance and revealed their impact on the EP exclusion condition. With the goal of establishing a separation interface where analytes of a certain EP mobility do not enter the channel and simultaneously pre-concentrate relative to those of a slightly lower EP mobility that pass through the channel, the need to limit interfacial field gradient variations that directly influence analyte velocities, and consequently separation resolution, becomes apparent. The agreement between the experimental and simulation data from this study warrants future use of the simulation to explore how changes to device design parameters like entrance corners, channel diameter, and electrode configuration can impact resolution. Tailoring the device design to maximize resolution at a single interface will further support the feasibility of engineering high resolution parallel interfaces for separating and concentrating electrophoretic species from complex samples.
5 Bibliography


