Analysis of Sample Injection and Band-Broadening in Capillary Electrophoresis Microchips

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ABSTRACT

An implicit finite-volume numerical scheme has been developed to solve the three-dimensional transport equations that govern the electroosmotic and electrophoretic phenomena. The scheme consists of solving the ion transport equation and electro-neutrality condition coupled with Navier-Stokes equations for fluid flow in a sequential fashion until convergence is achieved. This capability is used for accurate modeling of electrokinetic focussing and of sample injection in cross-channels, and band-broadening in microchips. The simulations show that sample injection using electrokinetic switching technique produces sample plug with a large tail which has been observed in experiments. The simulations also show the sample plug broadening due to turns in the separation channel.

Keywords*: Electrophoresis, Electroosmosis, microfluidics, simulation, focussing*

1 INTRODUCTION

Capillary Electrophoretic (CE) devices have been successfully fabricated on microchips for biological/chemical sensing applications. Microchip implementation of these devices has the advantages of compact size, integrated functions of separation and detection, low cost due to batch production and potential parallel analysis. However, design, fabrication and commercialization of this technology requires understanding of fundamental physical mechanisms associated with transport of various species and fluid flow in the presence of electric fields. Numerical analysis can provide insight into the interactions between various physical processes and improve the design of such microdevices.

Electroosmosis and electrophoresis are two physical phenomena that occur in fluids containing ions under the influence of external electrical field. Electroosmosis refers to the bulk movement of an aqueous solution past a stationary solid surface due to an externally applied electric field. This requires the existence of a charged double-layer at solid-liquid interface. Electrophoresis, on the other hand, does not produce bulk motion, but moves charged specie in the buffer solution under the action of an applied electric field. Velocity of the specie depends on the electrophoretic mobility and local electric field. The electroosmotic flow produces a flat velocity profile across the channel. There are several ways of modeling this flow: a) if the elctroosmotic mobility is known, then the velocity at the wall can be calculated from the local potential gradient, and it can be imposed as the wall velocity, and b) the axial electrostatic force in the double layer can be added as a source term [1]. Since the double layer thickness is really small, the second approach requires very fine mesh near the wall, which is not desirable. Electrophoresis is readily modeled using the electromigration term in the species conservation equation. The only numerical difficulty this introduces is in correct upwinding of the convection term when the elctromigration velocity is in the opposite direction to the bulk flow.

In this paper, the electroosmotic flow is modeled using the wall velocity approach. Fluxes due to electromigration are included in the convective term of species transport. Numerical implementation of this approach and boundary conditions are discussed. The model is applied to study sample injection and band-broadening in typical CE microchip operating conditions.

2 MATHEMATICAL MODELING

To model electrophoretic phenomena of charged specie, the equations governing advection, diffusion and electromigration in an electric field must be solved [2,3]. The conservation equation for a given specie "i" is expressed as:

$$\frac{\partial c_i}{\partial t} = -\left(\quad ?J_i \right) + \mathbf{r} \tag{1}$$

where the flux vector J_i is given by

$$J_i = \mathbf{V}c_i - z_i\omega_i c_i \quad \Phi - D_i \quad c_i \tag{2}$$

In the above equation c_i is the concentration, D_i is the diffusion coefficient, z_i is the valence and ω_i is the electrophoretic mobility of i^{th} specie. **V** is the velocity vector and Φ is the electric potential. The production rate

term, **r**, is usually neglected when equilibrium assumption is made.

The next step is to calculate the electromigration term. For this, one needs to calculate the electric field

$$E = - \quad \Phi \tag{3}$$

This is achieved by solving Laplace equation for potential:

$$?(\sigma \quad \Phi) = 0 \tag{4}$$

where the electrical conductivity σ is defined as:

$$\sigma = F \quad z_i^2 \omega_i C_i \tag{5}$$

Here, F is the Faraday constant. Equation (5) assumes electroneutrality condition and neglects the diffusional effects of the ions on the electric field [4]. Electroosmosis is simulated via applying a slip velocity at the wall calculated using specified electroosmotic mobility. The bulk fluid velocity due to electroosmotic mobility is expressed as:

$$\mathbf{V}_o = w_o \quad \Phi \tag{6}$$

where w_o is the electroosmotic mobility. The velocity, $\mathbf{V}_o$, is imposed as slip velocity at the wall.

1.1 Boundary Conditions

All the walls are assumed to be insulated and no-flux boundary condition is applied to both specie and electric potential equations. Voltages are specified at the inlet and exit of the channel.

3 RESULTS AND DISCUSSIONS

3.1 Sample Injection and Pinching

Capillary-electrophoresis is the most popular technique in the field of macromolecule separation due to its ability to yield high resolution in a short period of time. The resolution depends on the electric field strength and not on the length of the column as in liquid/gas chromatography. A schematic of the channel geometry in the microchip is shown in Figure 1. Geometry used in the present study has a simple cross configuration, with a straight injection channel of 1.5 mm long and a separation channel of 1.5 mm long and 30 μm wide. The injection channel is connected to analyte reservoir on the left and analyte waste reservoir on the right (numbered 1 and 3 respectively). The separation channel is connected to the buffer reservoir at the top and a waste reservoir at the bottom (numbered 2 and 4 respectively). A "pinched" sample loading technique [5] is used, followed by sample separation. In the "pinched" sample loading mode, a potential of 150 V is applied at the analyte reservoir and the analyte waste reservoir is grounded. The buffer reservoir and waste reservoir voltages are fixed at 75 V. In the separation mode, a potential of 150 V is applied to the buffer reservoir with the waste reservoir grounded and analyte and analyte waste reservoir voltages at approximately half of the potential at the buffer reservoir. An electroosmotic mobility of 4.0e-08 m^2/Vs is used.

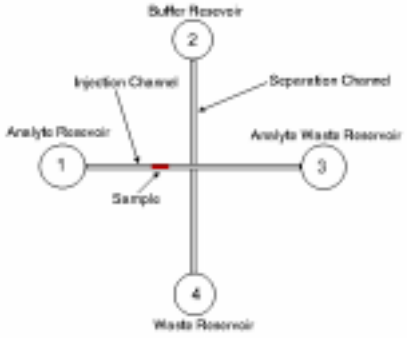
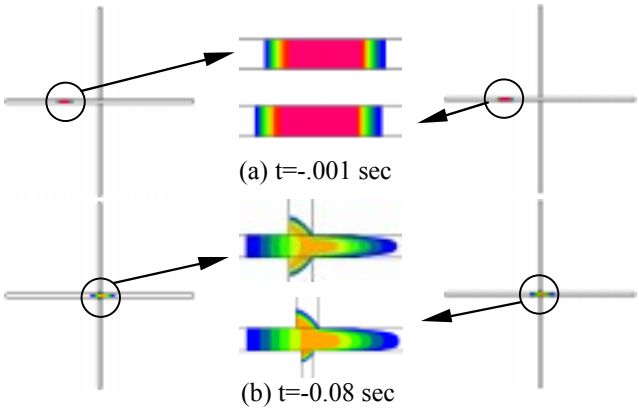


Figure 1. Schematic of the Cross-Channel Geometry Used in Sample Injection and Separation

Figure 2 shows a sequence of operation at different time levels for two different cross-channel configurations. In the first configuration (shown on the left) the width of the separation channel is same as that of the injection channel. In the second configuration (shown on the right), the width of the separation channel is half that of the injection channel. The operating conditions and boundary conditions are kept same in both the cases. During loading mode, the sample flows from Reservoir 1 to 3 due to electroosmotic forces. As the sample reaches the junction, it is squeezed due to the electrokinetic focussing. Consequently, the sample at the injection cross has a trapezoidal shape. After the sample reaches the intersection, the voltages are switched from the sample loading mode to sample separation mode, i.e., a potential of 150 V is applied to the buffer reservoir with waste reservoir grounded and analyte and analyte waste reservoir voltages at approximately half of the potential at the buffer reservoir. Consequently, buffer starts flowing from the reservoir 2 to 4, pinches the sample at the junction and carries with it. A rectangular shape of the injection plug from the analyte stream is mandatory for good performance.



(a) t=-.001 sec

(b) t=-0.08 sec

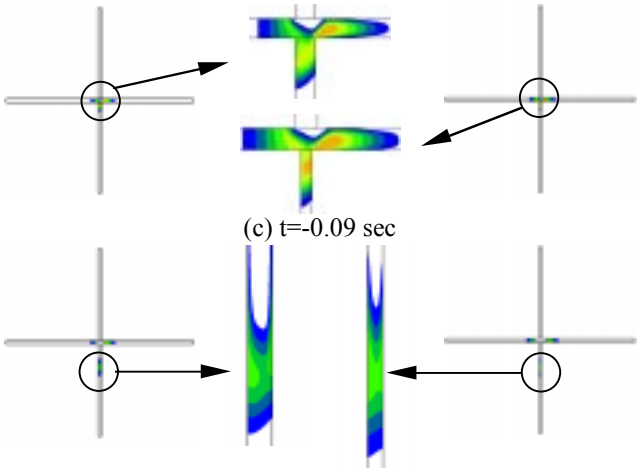


Figure 2. Sample Injection and Pinching in Cross-Channel: Plug Shape is Shown at Different Time Levels for a Regular Cross Channel at Right and Narrower Separation Channel on the Left. (Not to Scale)

In the present simulation, the plug is distorted in the separation channel and tailing effects can be noticed. Reducing the cross-section of the separation channel did not help to obtain a nice rectangular plug shape. Electric field around the corners is critical in determining sample plug shape. A MEMS designer can use the present simulation tool to optimize the potential at different reservoirs to obtain a rectangular plug.

1.2 Band-broadening in Microchannel

Band-broadening or band-dispersion/spreading refers to the process of dilution of signal (sample concentration) as the sample moves across the micro-channel. Dispersion/diffusion is the main cause of broadening. Besides nonuniform electric field may also cause the different regions of the sample to migrate at different velocity [5]. A nonuniform electric field occurs primarily due to the curvature in the channel geometry. In the present study, electroosmotic flow in channels of two-different configuration, S and rectangular shapes, are studied to understand the influence of curvature on band-broadening in the sample.

S-shaped channel is 50 µm wide, 2.25 mm outer diameter, and 1 mm in pitch. An electric potential of 150^{o}V is applied at the inlet and grounded at the exit. The electroosmotic force has been modeled as a slip velocity in the momentum equation through electroosmotic mobility. An electroosmotic mobility of 4.0e-08 m^2/Vs is used. The shape of the sample plug is shown in Figure 3 at different time levels. As the sample plug moves through the channel, higher current density along the inner wall of the bend causes the inner region of the band to move faster than the outer region. This results in an asymmetric profile and eventually causes band-broadening. As the sample continuous its journey, more band-broadening occurs. As it completes the journey around the first bend, sample attains a shape of a highly distorted parallelogram. CFD-ACE+ simulation correctly predicts the experimental observation [6] reported in the literature. As it continues its journey through the second bend, outer region of the sample in the first bend becomes inner region and is accelerated due to higher potential gradient. Thus, the distorted sample shape is corrected, to some extent, by letting the sample go through another bend of equal and opposite curvature.

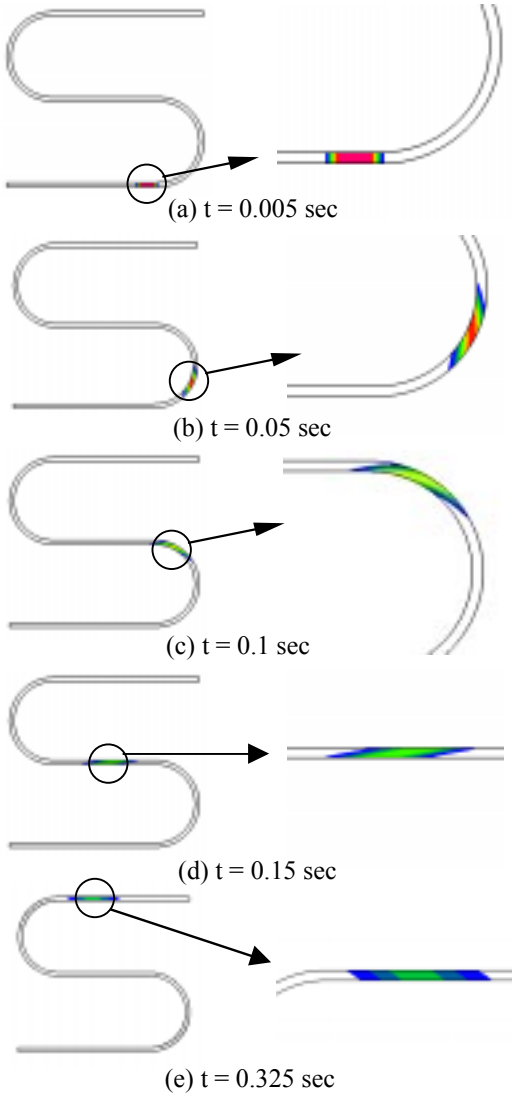


Figure 3. Flow of a Sample Plug in S-Shaped Microchannel: The Shape of the Plug is Shown at Different Time Levels. (Not to Scale)

In the next test case, the sample is moved though a "L" shaped bend twice in a rectangular channel. The channel is 50 µm wide and 4mm long. The inlet voltage is set to 150 V and the exit is grounded. An electroosmotic mobility of 4.0e-08 m^2/Vs is applied. The results from the simulation are shown in Figure 4 at various time levels. The geometry of the turn produces higher current density at the inner wall

than that at the outer wall. This causes a large distortion to the sample. As the plug negotiates the second turn, it gets distorted even further. The geometrical effects could be corrected partially at the end of the second 180° turn.

In the next case, band-broadening due to dispersion is illustrated. Here electrophoretic migration of three different species of same initial concentration and location is shown to cause band-broadening. In Figure 5, capillary electrophoresis in a straight microchannel is illustrated. The channel is 1.5 mm long and 30 µm wide. Three different species are introduced at time t = 0 secs. A potential of 150 V is applied at the inlet and exit is grounded. An electroosmotic mobility of 4.0e-08 m^2/Vs is applied. The first specie (shown at the top) is negatively charged and its electrophoretic mobility (2.53e-08 m^2/Vs) is opposing the main flow. The second specie (shown in the middle) is positively charged and its electrophoretic mobility (2.53e-08 m^2/Vs) is acting along with the main flow. The third specie (shown at the bottom) is neutrally charged and is passively carried by the main flow. In Figure 5, shape and the location of the samples are shown at different time levels. As expected, difference in the charge causes the species to separate. The positively charged specie exits the microchannel first, followed by the neutral specie.

2 CONCLUSIONS

The simulation tool reported in the present study will help the CE microchip designers to understand sample injection and pinching and sample distortion in the micro-channel configuration. The model predicts that the shape of the sample plug is a strong function of the electric field at the cross of the injection channel. This, in turn, depends on the geometry (length and cross-sectional shape) of the channel and the applied voltage. A uniform plug shape can be achieved by manipulating these parameters. Prediction of the band-broadening phenomenon using the developed tool will help to optimize the microchip design based on the trade-off between compactness and separation performance.

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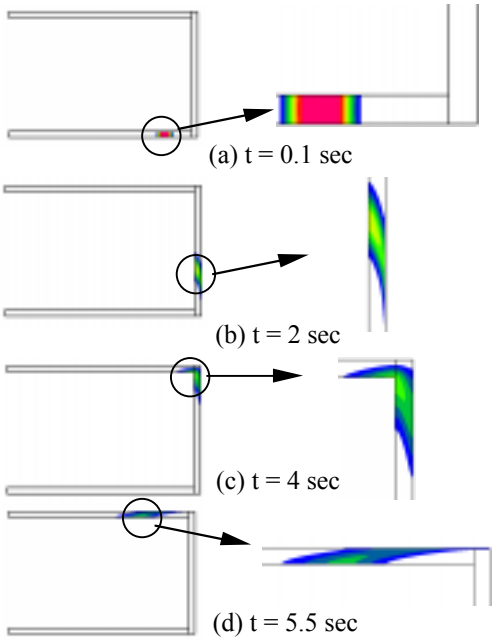

Figure 4. Flow of Sample Plug in a Rectangular Microchannel. The Shape of the Plug is Shown at Different Time Levels. (Not to Scale)

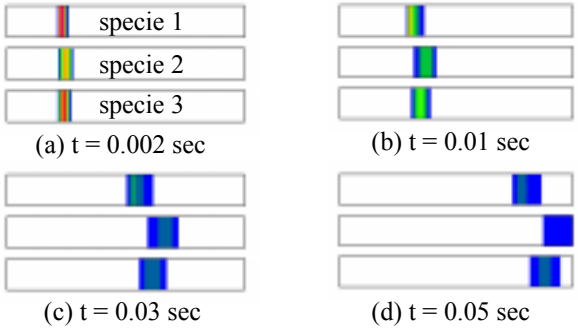

Figure 5. Capillary Electrophoresis in Separation Channel (Not to Scale). Specie 1 is Negatively Charged and Its Electrophoretic Migration Opposite to the Main Flow. Specie 2 is Positively Charged and Its Electrophoretic Migration is in the Direction of the Main Flow. Specie 3 is Neutrally Charged.