Simulation of Stacking for Concentration of Samples in a MicroFluidic Device

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Capillary electrophoresis (CE) is one of the methods used in analytical chemistry for separation and detection of charged species. One of the newer technologies is to implement electrophoresis on a single-use plastic device (so called lab-on-a-chip). The quality of the CE separation can be improved by preconcentrating the sample, which improves the analytical sensitivity and resolution. One method of preconcentration is known as stacking. In this technique, differences in the conductivity and mobility of the injected sample relative to the background electrolyte (BGE) cause a higher electric field strength in the injection zone, which subsequently leads to a sharpening of the concentration profile (stacking). We have investigated both sharpening from the trailing edge (back-stacking) and leading edge (front-stacking) of fluorescein using the microfluidic structure known as a double - T injector (see figures below).

The sample used in this investigation was fluorescein (1 μM) in buffer. Computer simulations (with software from CFDRC, Inc.) of the stacking phenomena were also performed. Images are shown below of experimental and simulation results of back stacking. There is excellent quantitative agreement between the experimental and simulation results. Validation of front-stacking showed equally good agreement.

The theoretical model for electrophoresis requires the simultaneous solution to the following three equations:

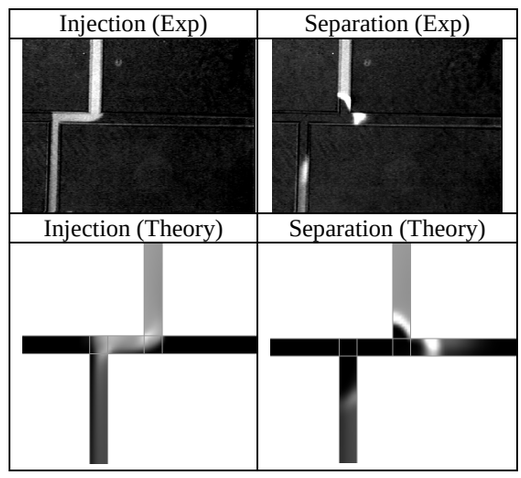
$$\frac{\partial c_i}{\partial t} = \nabla \cdot \left(D_i \nabla c_i + z_i \mu_i c_i \nabla \phi \right) \quad (1)$$

$$\nabla \cdot \left(\sigma \nabla \phi \right) = 0 \quad (2)$$

$$\sigma = F \sum_i \left| z_i \right| \mu_i c_i \quad (3)$$

Equation (1) expresses the electromigration of species (c_i) as a function of its diffusion coefficient (D_i), charge (z_i), mobility (μ_i), and the applied potential (ϕ_i). The potential field equation is shown in equation (2), with a dependence on conductivity (σ_i). Finally, equation (3) describes the dependence of conductivity on concentration, charge, mobility and the Faraday constant (F). We determined the value of the diffusivity from the mobility by the Nernst-Einstein relationship, equation (4) below:

$$D_i = RT \mu_i \quad (4)$$

In performing the simulations, numerical diffusion was minimized by using a very fine grid, third-order spatial differencing and Crank-Nicolson time differencing. Once the experimental model has been validated, it becomes easy to predict the effects of alteration in the experimental conditions of component concentrations in the buffer and sample, geometry of the injector, electric field strength, and duration of injection and separation. In addition, the theoretical model enables one to understand the migration of all the ionic species, conductivity gradients, and the non-linear potential gradients that occur.

The talk will present detailed results and comparisons of back- and front- stacking, the injection efficiency (fraction of injected material entering the separation channel), experimental and theoretical electropherograms, distributions of other co- and counter-ions, potential gradients during stacking, and animations of stacking. The concept of a virtual detector, in which the response-vs.-time profile from a hypothetical detector of optically fluorescent analytes is predicted for a given combination of device, sample, and measurement conditions, will be presented. Finally, the theoretical basis, capabilities, and limitations of the commercial software will be discussed.