MULTIMODE COMPUTATIONAL MODELS OF ISOTACHOPHORETIC SAMPLE CONCENTRATION COMBINED WITH BINDING REACTION KINETICS
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ABSTRACT
Simulation of isotachophoresis is a computationally difficult problem, because the physics involved are inherently stiff. We describe the use of a moving mesh model to speed up the solution time by 2 to 3 orders of magnitude.

KEYWORDS: Modeling, Isotachophoresis, Sample Concentration, Reaction Kinetics

INTRODUCTION
We have described a high sensitivity immunoassay system, based on isotachophoretic (ITP) sample concentration, followed by capillary electrophoresis. By making use of a very large sample volume (around 80 nL), high sensitivity can be achieved, in the order of 0.01 pM for alphafetoprotein [1]. The ITP concentration step also concentrates the DNA-antibody conjugate, due to its charge, and thereby greatly speeds up the binding kinetics of this reagent with the sample. In this paper, we describe a novel multiphysics modeling technique that allows all species concentrations to be calculated as a function of time much more quickly than before.

Finite element models of ITP have up to now been difficult and computationally costly to implement. The field across the moving boundary creates sharp transition zones for ionic species, whose width is in the order of $kT/qE$, or typically around 1 µm. Accurate modeling of this interface thus requires submicron mesh density, even when the ITP channel is 50 mm long, which implies an impractically large number of elements.

MODEL SETUP
Our model combines several physics modes of a finite element simulator (COMSOL). The first mode is the electrostatic problem. To correctly model ITP, we do not assume electroneutrality, but rather use the net charge of all ionic species as a source term in Poisson’s equation. This forces the system to generate the sharp boundaries characteristic of ITP. The second mode is the convection diffusion equation, which models ion transport (Fig. 1).

We also model the two buffer system equilibria with a derivative version of the equilibrium equation, and include the two binding reactions. The resulting model generates the concentrations of 13 different species: H+, Cl-, Na+, four buffer species, sample, two antibody conjugates, and three different immunocomplexes (Fig. 2).

The most important step in reducing the model complexity is to use a moving mesh that allows us to place a dense mesh only near the moving boundary, and move
Figure 1: Two dimensional model of a stacked sample peak as it travels across a field-free side channel. The three figures show the sample band at 0.85s, 0.9s, and 1 s after the beginning of the simulation. The first two figures show that a significant fraction (about 6%) of the sample does go to the side channel. However, almost all of it diffuses back to the high field region behind the moving boundary, where it quickly returns to the sample band (right-hand figure). After 1 second, the sample loss is below 1%.

Figure 2: Evolution of the pH in ITP when the pH of the sample and the antibody complexes are different than the Leading Buffer (Tri/NaCl-based, pH8) and the Trailing Buffer (Hepes, pH 7.5). Left: pH at t=0. Middle, pH at t=0.3, Right at 0.6 s. The pH increases in the high-field zone created behind the moving boundary.

it together with the moving boundary. We believe the application of moving meshes to ITP is a novel method to significantly reduce the computation time needed. A third physics mode (Arbitrary Lagrangian Eulerian displacement) is added to the two described above, and the velocity of the boundary movement is calculated from the species flux in the boundary region.

RESULTS

For a 50 mm long channel, the moving mesh has about 100 times fewer elements, but the computation time savings are even greater. With a moving mesh, the time steps increase up to the 1 s range because the problem becomes closer to time-invariant in the moving coordinates. For a 33 mm channel we observed a factor of 600 saving in computation time, and for the 50 mm long model it is even greater.

We have used this method for full-geometry simulations of the model with 13 concentrations, where we observe the generation of the detected immunocomplex (Fig. 3). The fraction of sample that becomes a detected complex depends on the DNA-antibody concentration, the electric field, and the binding kinetic constants. A modeled curve of the conversion fraction can be compared with experimental data, which allows the determination of these kinetic constants. Figure 4 shows an example, in which we have not yet matched the geometry and the electric field exactly.
Figure 3: Formation of the immunocomplex peak in a 34 mm geometry with 13 species modeled. At t = 30 s, a small peak begins to appear, as the stacking front reaches the dye-antibody reagent. Subsequent traces every 2 seconds show the growth of this peak. The moving mesh simulation required about 3 hours of computation time.

Figure 4: Modeled and experimental data of the sample conversion fraction as a function of the DNA-antibody concentration. Left side: modeled data at 600 V applied over 34 mm, and an 18 mm sample zone. Right side: experimental data for a 14 mm sample zone. This type of comparison, with matching parameters, can be used to determine experimental values of the kinetic constants of the binding reactions.

CONCLUSIONS

The many orders of magnitude of time savings resulting from the moving mesh allow the simulation of much more complex isotachophoretic problems, such as the formation of immunocomplexes with 13 different species concentrations reported here.

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REFERENCE