A GENERALIZED COMPUTATIONAL FORMULATION AND MODEL FOR TRANSPORT AND STOICHIOMETRY OF MULTIVALENT WEAK ANALYTES IN CAPILLARY ELECTROPHORESIS TECHNIQUES

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ABSTRACT

A generalized mathematical and computational model for various Capillary Electrophoresis (CE) techniques is presented. Accurate analysis of these electrokinetic phenomena can only be performed when the chemical interaction of the analytes with the system are taken into consideration. It should also be taken into consideration that majority of constituents in the electrolyte systems are weak, multivalent analytes, which have complex stoichiometric behavior intricately linked with the local transport phenomena. A generalized formulation to couple the association/dissociation stoichiometry of weak analyte with transport phenomena is presented along with a broad, fundamental treatment of macroscopic transport phenomena that will render common approximations like absence of bulk flow, a priori assumption of pH and conductivity, etc., redundant. Novel domain decomposition based Multi-Block Finite Volume scheme is developed and implemented. These formulations and strategy constitute the core of the microfluidics module of MEMS software *Intellisuite*® being developed by Corning Intellisense.

1. INTRODUCTION

Contemporary CE applications are typically characterized by plethora of physical phenomena. This, in addition to their ever-increasing scope and complexities, makes their realistic modeling a daunting task. In this work we report efforts towards developing a comprehensive mathematical and computational model for realistic, contemporary CE applications, and other microfluidics applications. The approach and philosophy adopted here are geared towards generalization without having to resort to restrictive assumptions like simplicity in geometry, absence of inertial effects, presence of only strong analytes or presence of only univalent weak analytes, etc. Accordingly, a generic approach is adopted, which is scalable with respect to physical phenomena and amenable to efficient algorithmic implementation.

Much of the work that has appeared in the literature in this context has focused on limited individual aspects of physical phenomena in microfluidics applications with numerous simplifying assumptions. Patankar and Hu [1] reported a finite-volume scheme for electroosmotic flow in cross-channel devices. Similarly, Dutta et. al. [2] reported a spectral element method for studying electroosmotic flow control in complex 2D geometries. Recently, Qiao and Aluru [3] presented an alternate way of modeling electroosmotic flows (with step changes in zeta potential) in fluidics network by modeling them as simplified electrical circuits. These works, although insightful, are pure fluid dynamics studies in the sense that they do not touch upon the presence of analytes in the system. They are also restricted to simple geometries.

The transport behavior of charged or neutral analytes (or solutes) in electric field has received considerable attention from numerical modeling and simulation point of view. This phenomenon, which is referred to as electrophoresis, is at the heart of majority of microfluidics applications concerned with identification, separation and patterning of various analytes. Central to modeling electrophoresis is the understanding of both transport and stoichiometric behavior of analytes in the electrolyte system in the presence of numerous driving forces [4-7]. Ermakov et. al. [8] reported a 2D finite-difference work that describes electrokinetically driven mass transfer phenomena in chip devices. His formulation however is restricted to strong analytes (pure ionic forms with fixed charge). Plethora of work on modeling the behavior of weak analytes has been reported in literature. Saville and Palusinski [9] and Palusinski et. al. [10] reported 1D model for electrophoresis of soluble materials. Their model is restricted to monovalent analytes and doesn't account for the presence of lateral boundaries, bulk flow and temperature gradients. Mosher et. al. [11] presented a mathematical model of the electrophoretic behavior of proteins in simple geometries with similar restrictions. Recently Gas et. al. [12] presented a computational model for optimization of background electrolyte in Capillary Zone Electrophoresis. The model however neglects the effect of diffusion and is restrictive with

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respect to number of analytes, type of analytes and processes being modeled. In general majority of the work in this regard have been along the lines mentioned above.

In this work it is sought to redress the restrictions mentioned above and to provide a simulation tool for real-life applications. A novel multi-block finite volume scheme is implemented to solve all transport equations in a generalized framework. The use of domain decomposition based multi-block technique provides the power to tackle complicated geometry and also provides the means to harness the suitability of finite volume scheme for boundary layer flows [13]. In addition this technique is amenable to scalable strategies like parallelization. A succinct formulation to model the stoichiometry of any multivalent weak analyte and its coupling with transport phenomena is presented and implemented. This can account for all the major physical phenomena individually or together. To our knowledge such computational model doesn't exist and it can provide the foundation for a powerful analysis and CAD tool.

2. A GENERALIZED FORMULATION FOR TRANSPORT PHENOMENA IN CONTINUA

In this section we outline a generalized formulation for transport phenomena, which encompasses all pertinent conservation laws like conservation of momentum (Navier-Stokes equation), energy, mass and current. The basic governing equation of transport can be formulated as conservation of total flux $\vec{J}$, of a dependent variable f in the following form [14,15],

$$\frac{\partial f}{\partial t} + \nabla \bullet \vec{J} = S_f , \quad (1)$$

where $\vec{J}$ is the vector depicting the total flux of dependent variable f. Two basic components of $\vec{J}$ are the diffusive flux and the convective flux, and there can be more. In its most general form $\vec{J}$ can be represented as,

$$\vec{J} = \vec{U} f - \Gamma_f \nabla f + \vec{J}_{EK} . \quad (2)$$

In the above equations S_f is the source term for the dependent variable f and it can also contain terms that could not be included in the transient and divergence terms of Eq. (1), $\vec{U}$ is the bulk flow velocity vector, Γ_f is the diffusivity and $\vec{J}_{EK}$ in general can denote other fluxes other than the convective and diffusive ones. In our case we will reserve it for electrophoretic flux that arises in solute transport in electrokinetic flows. It can be easily shown that for appropriate choice of f, the flux conservation statement (Eq. 1) in conjunction with the basic continuity equation, which ensures basic mass conservation of the bulk fluid, can represent the whole gamut of conservation laws encountered in continuum fluid dynamics and heat and mass transfer problems [14,15]. For the incompressible case continuity equation in generalized form can be written as

$$\frac{\partial r}{\partial t} + \nabla \bullet \vec{U} = b \quad (3)$$

where r is the density and b is the mass source or sink term.

This approach of casting transport phenomena as a statement of conservation of convection-diffusion flux provides a generic platform for algorithmic implementation. The basic nature of all the relevant governing equations is similar mathematically and also physically since in all of them flux of a dependent variable is conserved. Hence generic numerical schemes can be adopted to solve them in coupled fashion [14,16].

3. MODEL FOR ELECTROPHORETIC TRANSPORT OF WEAK ANALYTES

Weak analytes (or solutes) undergo rapid association/dissociation reactions in aqueous solutions and exist in both neutral and ionic states. The extent of its ionic and neutral contents depends on a variety of local factors the most important one being the local pH value. As a result transport behavior of weak analytes is intricately linked with its chemical behavior and a dissociation model to describe this chemistry is required. Strong analytes on the other hand undergo complete dissociation and exist in pure ionic states.

3.1 Dissociation Model: From chemical behavior point of view analytes can belong to any of the three categories of acid, base and ampholyte. One of the most commonly used definitions for these categories are - an acid can donate protons (hydrogen ion), base can accept protons and an ampholyte (e.g. proteins) can exhibit both of the above properties [18]. Valency of an analyte is defined as the maximum number of dissociable hydrogen ions in either neutral or ionic states.

A general analyte A with n dissociable protons (valency of n) is considered. For such an analyte there are n number of dissociation reactions [18]. $[A_j]$ (j=0 to n) is designated as the concentration of one of the ionic (or neutral) states of A with j protons. Thus the n+1 possible states of A will be A_n, A_{n-1}, …, A_0. The dissociation

reactions and the corresponding equilibrium rate constants (K_i) can be represented compactly as

$$A_j \rightleftharpoons A_{j-1} + H^+ \; ; \; K_{n-j+1} = \left[A_{j-1}\right]\left[H^+\right] \Big/ \left[A_j\right]; \; j = n, n-1, n-2, \ldots, 1 \quad (4)$$

The total concentration of analyte in the solution can be expressed in terms of dissociation constants using the expressions above as

$$[A] = [A_n] + [A_n] \sum_{i=1}^{n} \left[H^+\right]^{-i} \prod_{l=1}^{i} K_l \; . \quad (5)$$

Using the above general expressions some useful quantities can be deduced that play important role in this modeling. They are:

(1) Degree of dissociation (α): Degree of dissociation of an ionic state containing j dissociable protons, A_j, is defined as the ratio of $[A_j]$ to [A];

$$\alpha_j = \frac{\left[A_j\right]}{[A]} = \frac{\left[H^+\right]^{-j} \prod_{l=1}^{n-j} K_l}{1 + \sum_{i=1}^{n} \left[H^+\right]^{-i} \prod_{l=1}^{i} K_l} \; ; \; j \neq n. \quad (6)$$

When j=n the numerator in the above expression is 1 and the denominator remains the same as it is independent of j.

(2) Effective charge (z_{eff}): In the formulation adopted here instead of treating each state as a separate entity, which becomes a logistical nightmare for multivalent analytes, the ensemble of all the states is treated as a single entity with effective charge, effective mobility, etc [20]. This approach not only renders a compact formulation for any multivalent analyte but also makes perfect sense physically. The time scales of the reactions are so small compared to the time scales of typical diffusion or convection mechanisms that the reactions can be appropriately considered to be instantaneous and the analyte can be considered as a single entity with concentration defined by Eq. (5) and effective properties. The effective charge can be derived as [20]

$$z_{eff} = \sum_{i=0}^{n} (n-i)\alpha_{n-i} \quad (7)$$

where n is the net charge of analyte possessing all n dissociable protons

(3) Effective mobility (Ω_{eff}): Similar to effective charge, z_{eff} , effective mobility, Ω_{eff} , can be defined as

$$\Omega_{eff} = \sum_{i=0}^{n} (n-i)\Omega_i \alpha_i \Big/ z_{eff} \quad (8)$$

in which case the electrophoretic flux takes the form

$$\vec{J}_{EK} = z_{eff}\Omega_{eff}[A]\vec{E} \; . \quad (9)$$

The association/dissociation model presented here, it should be noted, is for any multivalent analyte (acid, base or ampholyte). One has to choose parameters like n, n and dissociation constants, K_i, appropriately.

3.2 General Transport Model: With the stoichiometric model in place a transport equation for conservation of mass of analyte A can now be formulated. The total mass flux, Eq. (2), can be written as (replacing ϕ with [A])

$$\vec{J} = \vec{U}[A] - D_A \nabla[A] - z_{eff}\Omega_{eff}[A]\nabla\varnothing \; , \quad (10)$$

where $\varnothing$ is the electric potential, which is related to $\vec{E}$ as $\vec{E} = -\nabla\varnothing$, D_A is the diffusivity of A, and other parameters are as defined above. The mass conservation equation for analyte A then looks like

$$\frac{\partial[A]}{\partial t} + \nabla \bullet \vec{J} = 0 \; , \quad (11)$$

where $\vec{J}$ is as defined in Eq. (10). Electric potential, $\varnothing$, is obtained by imposing the current continuity condition [20]. $[H^+]$ is obtained by solving for the electroneutrality condition, which stipulates that at every point in the bulk sum of all charges charges is zero [20].

4. NUMERICAL SCHEME

The generalized flux conservation statement, Eq. (1), which encompasses the governing equations for velocities, temperature, concentrations of analytes and electric potential, along with the incompressible continuity equation, Eq. (3), dissociation model (Eqs. 4-10), and the explicit electroneutrality condition constitute the basic computational model for electrophoretic applications with appropriate initial and boundary conditions. No approximations regarding dimensionality of the problem or type of physical phenomena that can be incorporated, etc. are entailed. In this work a multi-block finite volume scheme with structured grids is used for numerical solution of this formulation. Unfortunately a detailed discussion of this technique is beyond the scope of this paper and readers are referred to Chatterjee [20] and references therein for details.

5. APPLICATIONS: CAPILLARY ISOELECTRIC FOCUSING (IEF) WITH AND WITHOUT BULK FLOW

Capillary isoelectric focusing is a popular and effective technique used to identify and separate ampholytes [4]. There exists a pH value (pI value), called isoelectric point, at which the net charge of an ampholyte is zero and hence is immobile to the electric field at that point. Simulation of isoelectric focusing in a straight channel 1

cm long (other dimensions are 1000 μm each) is presented here and validated for the cases of no bulk flow (all velocities zero) and electroosmotic bulk flow. Histidine, an ampholyte, is the sample being focused in a channel, and an immobilized pH gradient was created by controlled distribution of the buffer constituents, cacodylic acid (CACO) and tris (hydroxymethyl)-aminomethane (TRIS). In order to maintain an immobilized pH gradient, mobilities of CACO and TRIS are taken to be zero. Linear distributions of TRIS and CACO are maintained in the channel, and initially 1mM of Histidine is uniformly distributed in the channel as shown in Fig. (1). Properties of the analytes used in the model are as given in Palusinski et. al [10].

Transient simulations are performed for this setup. A current density of 0.2 A/m^2 is used. Anode is to the left. Calculated transient Histidine concentration profiles are shown in Fig. (2). Profiles are plotted for 10, 20, 30 and 200 minutes of focusing. As can be see, Histidine tends to concentrate towards its isoelectric point. This result agrees extremely well with the reported results of Palusinski et. al [10]. As a result of focusing other parts of the channel are left virtually Histidine-free. In the Histidine-free zones conductivity is very low and current is mainly carried by hydrogen and hydroxyl ions. The calculated conductivity profiles are shown in Fig. (3). Again the agreement with Palusinski et. al. [10] is excellent.

An interesting extension to this problem would be to study the effect of electroosmosis (EOM) on the focusing phenomenon. A wall zeta-potential of –0.002V is used to impart electroosmotic driving force at the upper and lower walls. Zero velocity gradient condition is imposed at the right end of the channel. A transient analysis of this problem is carried out along the same lines as above. The pH gradient no longer remains constant (immobilized); instead shows notable non-linear transient behavior as shown in Fig. (4). Effect of these phenomena on focusing of Histidine sample is shown in Fig. (5). As can be seen the focusing point (point of maximum concentration) shifts to the right in the presence of bulk flow. While the shift is small at 10 minutes, it becomes substantial as time progresses, as seen for the case of 50 minutes. For times later than this it can be seen that the focusing process becomes unfeasible as the focusing point shifts too far to right. Thus the presence of bulk flow imposes restriction on duration and tightness of focusing that can be obtained otherwise.

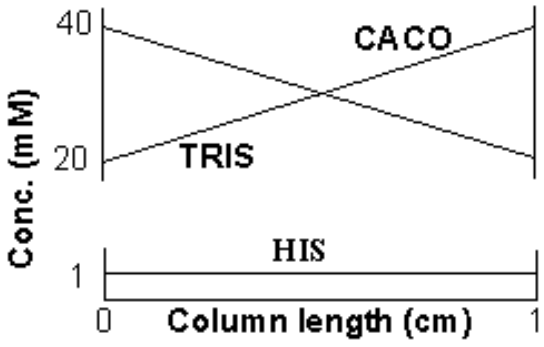

Figure 1: Initial concentration distribution in isoelectric focusing.

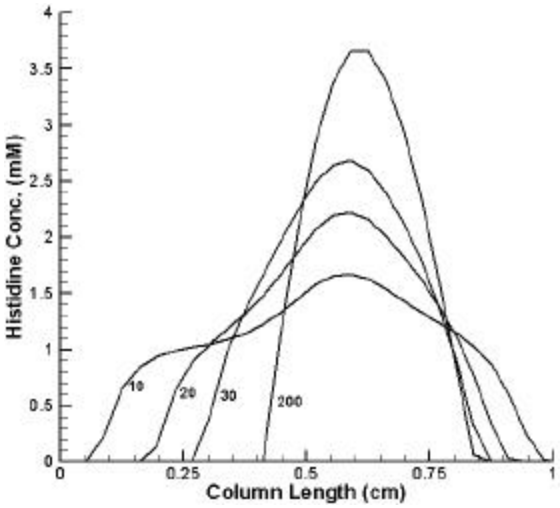

Figure 2: Transient Histidine concentration profiles in IEF (without bulk flow) after 10, 20, 30 and 200 minutes of focusing.

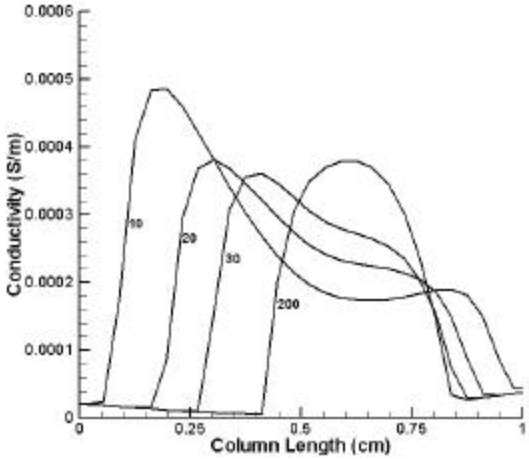

Figure 3: Transient conductivity profiles in IEF (without bulk flow) after 10, 20, 30 and 200 minutes of focusing.

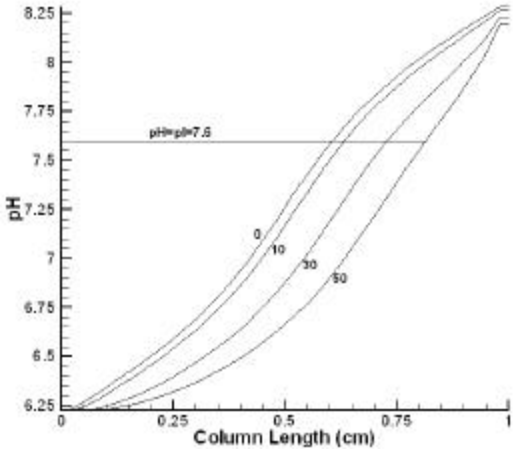

Figure 4: Transient pH profiles in IEF (with bulk flow) after 0, 10, 30 and 50 minutes of focusing.

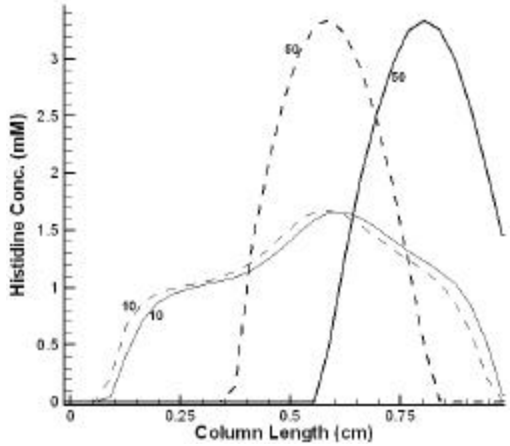

Figure 5: Transient Histidine concentration profiles in IEF with bulk flow (solid lines) and without bulk flow (dashed lines) after 10 and 50 minutes of focusing.

6. CONCLUSIONS

A generalized formulation for transport and stoichiometric phenomena in Capillary Electrophoresis type applications is presented. This formulation, apart from being genuinely general, is amenable to efficient algorithmic implementation and parallelization.

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