Theory of Electrophoretic Separations

Part I: Formulation of a Mathematical Model

A general model is developed for the electrophoresis of soluble materials. The model describes the evolution of concentration fields for a set of compounds which undergo transport by flow, diffusion, and migration in an electric field and simultaneously participate in rapid dissociation-association reactions. Modes of electrophoresis requiring special treatment can now be studied in a unified context. As an example of its utility, the model is used analytically to study a process known as isotachophoresis. In Part II two electrophoretic separation processes are simulated numerically, demonstrating the model's versatility.

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SCOPE

When a solution containing amphoteric compounds is exposed to an electric field, the migration of ions and uncharged species occurs in the presence of rapid dissociation-recombination reactions. If a zone containing several species is inserted in a column containing a homogeneous buffer, the various species will migrate at different rates according to their relative electrophoretic mobilities. Conversely, if the ends of the column are made impermeable to the amphoteric species, a stationary pH gradient will eventually be formed and sample constituents will migrate to their equilibrium isoelectric points under the influence of the electric field. These phenomena form the basis of several separation methodologies that have been difficult to model mathematically. The purpose of this paper is to present a generally applicable model of electrophoretic processes and apply it to a specific situation. The application is designed to illustrate the interplay between reaction, electromigration, and diffusion in simple configurations where complications due to lateral boundaries, bulk flow, and nonuniform temperature are supressed. The situation studied in detail is isotachophoresis in a one-dimensional column; more diverse applications that require numerical treatment of the equations are described in Part II.

CONCLUSIONS AND SIGNIFICANCE

Although electrophoretic processes can be described by familiar conservation relations, the structure of the mathematical model differs from that used to describe systems with strong electrolytes due to the presence of rapid dissociation-recombination reactions that tie the concentrations of ionic species to those of the undissociated solutes. It is shown that since the reactions are fast relative to transport by diffusion and electromigration, it is possible to treat the reactions as being in local equilibrium. Similarly, the ratio of an electrical length (the Debye scale) to the physical scale of the process is small, and this leads to the electroneutrality approximation. The model that is developed consists of a set of conservation equations for the total concentration of each amphoteric compound and the current. Appended to this set of partial differential equations is a set of algebraic relations describing chemical equilibria and electrical neutrality.

The model furnishes analytical results for isotachophoresis, one of the classical electrophoretic processes. Here more or less homogeneous zones are separated by transition bands that move with a uniform speed; analytical relations between the compositions of contiguous zones and the band speed for mixtures of amphoteric compounds were derived. However, the real utility of the model lies in its ability to treat complicated processes that must be studied by numerical methods.

As it now stands the model furnishes a means of establishing what can be termed the ultimate resolving power of a given technique. In this form the model can be used to explore the development of new techniques which may involve selective ion permeabilities at the boundaries or other novel features. The addition of a description of the behavior of noninteracting proteins, i.e., trace amounts, is straightforward. Much more work will be required to include proteins in amounts where interactions occur because their ionization processes are considerably more complex than those of simple ampholytes.

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INTRODUCTION

Although electrophoretic techniques are among the most widely used analytical tools for proteins and other biological materials, the pace of theoretical work aimed at modeling the complicated, interactive chemistry coupled with transport processes proceeds slowly. One reason for this slow progress is obvious; the processes themselves are complex and much of the work to date has relied on oversimplified models that attempt to catch only the essence of a particular technique. In some instances simplified models succeed to an astonishing degree, the best example being the description of ion zone motion in the process known as isotachophoresis, where the description of zone to zone concentration changes and boundary motion was initiated by Kohlrausch in 1897. Despite their successes, contemporary versions of that theory give little insight into the development and structure of the boundaries. Furthermore, because most such models are couched in very specific terms, relaxation of the constraints is difficult. Nevertheless, it is reasonable to expect that most electrophoretic processes can be described with a model based on a small number of axioms implemented using digital computation methods. The purpose of this and the following paper is to present such a model and describe its application to several electrophoretic processes. We are particularly interested in the processes called moving boundary electrophoresis (MBE), zone electrophoresis (ZE), isotachophoresis (ITP), and isoelectric focusing (IEF). As will be explained in Part II, these classical modes of electrophoresis can all be derived, at least in principle, from constraints first considered by Hittorf (1853) and Kohlrausch (1897).

Previous Mathematical Models

The classical modes are distinguished by the structure and motion of the electrolyte boundaries. In MBE, ZE, and ITP the boundaries move, and separation of sample constituents is based on mobility differences. The composition pattern may evolve to a migrating steady state form, as in ITP, or change continually, as in ZE. On the other hand, separation in IEF is based on differences between the isoelectric points of the sample constituents, which results in the formation of a stationary, steady state structure. It is impractical here to attempt a comprehensive review of previous theoretical work so we will limit our discussion to two representative processes, ITP and IEF. Only that work dealing with the salient features will be mentioned; the reader is referred to reviews of electrophoresis for additional material—see, for example, Bier (1959, 1967); Everaerts et al., (1976); Deyl (1979); and Righetti et al., (1979).

Kohlrausch (1897) clearly established many characteristics of electrophoretic processes, especially isotachophoresis, even though he dealt only with fully ionized solutes and ignored diffusion in his description of boundary migration. A more general, multicomponent, system was analyzed by Dole (1945), who also worked with fully ionized solutes and ignored diffusion. Alberty (1950) followed up by treating systems with weak acids and bases, but the inclusion of diffusion has always been predicated on the notion of complete ionization (MacInnes and Longsworth, 1932; Konstantinov and Oshurkova, 1966; Martin and Everaerts, 1967.) Despite the approximate nature of these models they do show how the thickness of the boundary region changes with mobility and field strength. However, explicit expressions for composition and field strength as functions of position in the column are not readily found and this tends to limit the utility of the solutions. To display the boundary structure clearly, a complete, explicit solution for a three component fully ionized system is given in the Appendix. This provides a useful tool for testing numerical schemes designed for more complex systems.

The theory of isoelectric focusing is more recent and has a different history. Svensson (1961) developed a simple theory based on a steady state analysis of a system whose pH and conductivity are known at the outset. This theory was adequate to explain IEF in systems that employ synthetic carrier ampholyte mixtures (e.g., ampholine), since they tend to generate nearly linear pH profiles. More recent work deals with somewhat more complicated chemistry, but even though numerical methods are employed to solve the differential equations, linear pH profiles are assumed (Cann and co-workers, 1977, 1978, 1979, 1980; Park et al. (1978) Weiss et al., 1974). Consequently, the results are mainly applicable to experiments employing synthetic ampholytes, whose composition and electrokinetic properties are largely unknown. Interest in generating pH gradients with well-defined ampholytes and buffers has fostered renewed efforts to develop a theory free of the earlier constraints (Caspers and Chrambach, 1977; Chrambach and Nguyen, 1977; Nguyen and Chrambach, 1976, 1977). Until recently the most advanced model capable of handling multiampholyte systems was that of Almgren (1971), who neglected diffusional contributions to the current and based his approach on rather restrictive assumptions regarding ion mobilities, namely, equal mobilities for all the ions except hydroxyl and hydrogen. Thus the applicability of Almgren's model is limited and it cannot handle situations where steep gradients are present. These restrictions were removed in the model developed for IEF by Palusinski et al. (1981b) wherein assumptions regarding the shapes of the pH or the conductivity profiles are avoided. (See also Palusinski et al., 1981a; Bier et al., 1981; and Bier et al. (1983). The current model is based on this work, expanded so as to cover a wide variety of electrophoretic modes.

An Outline of This Report

The model describes the chemical reactions, conservation of the individual species, and the relation between charge and potential. An explanation of how the chemical reactions are taken into account is given first. Then conservation relations are described and simplified, taking advantage of the speed of the chemical reactions as compared to the transport processes so as to adapt the model to electrophoretic processes involving weak electrolytes. After this, analytical results pertaining to ITP are described. In Part II the numerical treatment of the equations is presented, as are tests for stability, convergence, and effects due to changes in the boundary conditions.

THE MATHEMATICAL MODEL

As noted earlier, most previous models have been based on approximations peculiar to the chemical features of a given process and are difficult to adapt to situations where the constraints are different. Such simplifications are avoided here. In the model, chemical species are transported by convection, diffusion, and migration in the electric field. Although diffusion and electromigration are handled directly within the model, convective velocity fields are not. These must be supplied by consideration of the relevant fluid mechanics. Velocity fields due to electroosmosis, for example, would follow from solutions of the Navier-Stokes equations augumented to account for electrical forces. However, in the applications described later, convective motion is absent and attention is focused on transport by diffusion and electromigration. Effects due to Joule heating are also supressed in the applications and the systems deemed isothermal. Such simplifications are appropriate when dealing with electrophoresis in thin layers or columns with gels or other packings. The results illustrate clearly the interplay between reaction, diffusion, and electromigration free of complications arising from flow or heat transfer. Effects of this sort are important, however, and warrant attention in future work. We begin by describing the way chemical reactions are modeled.

Chemical Reactions

The general model is based on dissociation-association reactions involving water

$$H_2O \rightleftarrows H^+ + OH^- \tag{1}$$

and ampholytes where

$$Am_i^o \rightleftarrows H^+ + Am_i^- \tag{2}$$

and

$$Am_i^+ \rightleftarrows Am_i^o + H^+. \tag{3}$$

The symbols used to represent concentrations are: $[H_2O] = \hat{n}_0$, $[H^+] = \hat{n}_1$, $[OH^-] = \hat{n}_2$, $[Am_i^o] = \hat{n}_{3i}$, $[Am_i^+] = \hat{n}_{3i+1}$, $[Am_i^-] = \hat{n}_{3i+2}$, each measured in moles per unit volume. The concentrations are made dimensionless using one of the initial concentrations n_r as a reference. Dimensionless concentrations are defined as $n_o = \hat{n}_o/n_r$, $n_1 = \hat{n}_1/n_r$, $n_2 = \hat{n}_2/n_r$, $n_{3i} = \hat{n}_{3i}/n_r$, $n_{3i+1} = \hat{n}_{3i+1}/n_r$, and $n_{3i+2} = \hat{n}_{3i+2}/n_r$.

Mass action kinetics are assumed, so the rates of production of the anionic forms, for example, are written in dimensionless form as

$$R_{3i+2} = \frac{\hat{k}_{3i+2}^{(f)}}{k_r} n_{3i} - \frac{\hat{k}_{3i+2}^{(r)} n_r}{k_r} n_1 n_{3i+2} \tag{4}$$

where k_r is one of the forward reaction rate constants.

It should be noted that the rates of ionic reactions of this sort are fast compared to the other rate processes, and we will use this property to simplify the development later. For the present, we simply note that at equilibrium, where the rates of forward and reverse reactions balance, the familiar equilibrium relations are obtained in dimensionless form as

$$K_o = n_1 n_2 / n_o \tag{5}$$

for water and

$$K_i^{(-)} = n_1 n_{3i+2} / n_{3i} \tag{6}$$

$$K_i^{(+)} = n_1 n_{3i} / n_{3i+1} \tag{7}$$

for the ampholytes. Note that the equilibrium constants are dimensionless, being scaled by the reference concentration n_r.

It should also be clear that weak or strong acids can be accommodated by appropriate treatment of the equilibrium equations. Multiple ionization, for example, is readily accommodated by adding new species and the corresponding equilibrium relations. For more complex electrolytes such as proteins, experimental data can be utilized in the form of tables or empirical formulas.

Certain elementary species preserve their identity and the net rate of production of any such species must be zero. Thus, for the rate of production of hydroxyl in both of its forms, we have

$$R_0 + R_2 = 0. \tag{8}$$

Similarly for hydrogen

$$R_0 + R_1 + \sum_{i=1}^{I} (R_{3i} + 2R_{3i+1}) = 0, \tag{9}$$

and each of the ampholytes

$$R_{3i} + R_{3i+1} + R_{3i+2} = 0. \tag{10}$$

The rate of production of charge is also nil but this relation is not an independent one since it is obtained from a linear combination of Eqs. 8, 9, and 10 as

$$\sum_{k=1}^{N} z_k R_k = 0 \tag{11}$$

where $N = 2 + 3I$.

Conservation Relations

To express the conservation equations in dimensionless form the following reference scales are introduced: a characteristic length inferred from the initial or boundary conditions, x_r; a mobility, Ω_r, one of the ionic mobilities; a production rate, $R_r = k_r n_r$; an electrical potential, $\phi_r = RT/e$; and time, $t_r = ex_r^2/RT\Omega_r$. These scales are introduced via transformations of the form $\hat{x} \rightarrow x_r x$ where the new (transformed) variables are dimensionless. The conservation relation for each species reads

$$\frac{\partial}{\partial t} n_i = -\nabla \cdot f_i + \alpha^{-1} R_i \tag{12}$$

with α denoting the dimensionless group $RT\Omega_r/ex_r^2 k_r$, and f_i representing the dimensionless flux in dilute systems as

$$f_i = -z_i \Omega_i n_i \nabla\phi - \Omega_i \nabla n_i + Pevn_i. \tag{13}$$

Here v stands for the vector of local velocities and Pe for the Peclet number, $ev_r x_r/RT\Omega_r$, where v_r is the reference velocity. Henceforth we will deal with systems in which the solvent, water, is in excess and its concentration unvarying. Thus, the rate of generation, R_o, is nil and Eq. 8 shows that R_2 must also vanish. It follows that the vector, v, represents the velocity of the solvent.

The balance between charge and potential, Poisson's equation, takes the form

$$\beta \nabla^2 \phi = -\sum_{k=1}^{N} z_k n_k \tag{14}$$

with $\beta = \epsilon RT/e^2 x_r^2 n_r$.

At this point we have only to specify the initial and boundary conditions to complete the problem statement. However, the equations would be difficult if not impossible to solve due to disparities in several of the time scales and so some simplifications are in order.

The description of transport in systems with partially ionized solutes differs from the treatment of systems with strong electrolytes due to the way generation terms are handled. With strong electrolytes the amount of undissociated solute is vanishingly small and the generation terms in the species balances can be ignored (see Newman, 1973, for an extensive treatment of such problems). In the situations under study here the dissociation-recombination reactions are extremely rapid but the concentrations of the individual ionic species are tied to the concentrations of undissociated solutes. Thus, although the reactions appear to be at equilibrium on time scales characteristic of diffusion, we cannot ignore accumulation or transport processes on the grounds that their rates are small. To do so produces a model that is underdetermined. The rates of transport and accumulation are clearly constrained by the reaction rates and the model must take this into account consistently. By forming linear combinations of the constraints embodied in Eq. 8 through 10 with the species balances, a set of conservation relations is produced which describe the total concentrations of various species. For example, hydrogen appears as hydrogen ions in ampholyte species and as part of the water molecule. Thus, a balance on the total concentration of hydrogen species can be formed from adding Eq. 9 to the individual balances for hydrogen ions, i.e., Eq. 12 with $i = 1$ and $i = 3, \ldots 3I + 1$.

The following equations are obtained:

Hydrogen species

$$\frac{\partial}{\partial t}\left[n_1 + \sum_{i=1}^{I} (n_{3i} + 2n_{3i+1})\right] = \nabla \cdot \left[f_1 + \sum_{i=1}^{I} (f_{3i} + 2f_{3i+1})\right]; \quad (15)$$

Ampholyte species

$$\frac{\partial}{\partial t}[n_{3i} + n_{3i+1} + n_{3i+2}] = -\nabla \cdot [f_{3i} + f_{3i+1} + f_{3i+2}],\ i = 1, 2, \ldots, I. \quad (16)$$

These relations replace $I + 1$ of the individual species balances embodied in Eq. 12.

At this point it is helpful to review the equations that make up the model. There are $3I + 3$ dependent variables: hydrogen and hydroxyl ions, $3I$ ampholyte species, and the potential gradient. One set of independent equations consists of $2I$ equations of the form of Eq. 12 for the charged ampholyte species, another set of I equations of the form of Eq. 16 for the ampholyte totals. Eq. 15, which replaces the individual hydrogen ion balance, and Eq. 14, which relates the potential gradient to the ion densities. This form is well suited for simplification since we can take advantage of the reaction speed without sacrificing the number of independent relations.

To simplify the model for situations where the reactions are near equilibrium we take advantage of the smallness of α and β. A characteristic time for diffusion is x_r^2/D_r where D_r is a characteristic molecular diffusivity, namely, $RT\Omega_r/e$ in the present circumstances. A characteristic reaction time is k_r^{-1} and the ratio of these two times (reaction time/diffusion time), α, is very small. The second parameter involves two length scales. The expression $\epsilon RT/e^2 n_r$ denotes an electrical length scale associated with the net free charge, sometimes called the Debye length κ^{-1}. In highly conducting ionic solutions of the sort under study, this length is of the order of a few nanometers, whereas x_r, the characteristic length scale that arises from the initial conditions or boundary conditions, may be of the order of several millimeters. Thus the parameter β, which is the ratio of the squares of these two scales, i.e., $\beta = (\kappa x_r)^{-2}$, is small and the system behaves as though electrically neutral on the length scale of interest (Newman, 1973). It should be noted, however, that by reducing the order of the system of differential equations we lose the ability to study any thin transition regions near the boundaries of the system.

Now, to simplify the model in a self-consistent fashion we set the parameter α equal to zero in Eq. 12 so as to reduce these equations to the equilibrium relations for water and the ampholyte species. Upon setting β to zero we obtain the relation for electrical neutrality. This can be viewed as part of a process of constructing a perturbation expansion where, to leading order, we have local chemical equilibrium and charge neutrality. Expressions for higher order terms in the perturbation expansion would follow from a more detailed analysis.

One more rearrangement is in order before summarizing the model. To obtain the more familiar expression for the conservation of current we add the ion balances weighted according to the ion valences and use Eqs. 11 and 14, with $\beta = 0$, yielding

$$0 = \nabla \cdot \sum_{k=1}^{N} z_k f_k \quad (17)$$

as the balance relation for charge transport. This expression may be used in place of any of the other balance expressions and we choose to replace the balance for hydrogen, Eq. 15, with Eq. 17.

The simplified or local equilibrium model consists of the following equations:

- An equilibrium expression for water, Eq. 5
- Equilibrium expressions for the ampholytes, Eqs. 6 and 7
- A balance equation for the charge transport, Eq. 17
- Balance equations for each amphoteric species, Eq. 16 with $i = 1, 2, \ldots I$
- The charge neutrality expression obtained from Eq. 14, namely,

$$\sum_{k=1}^{N} z_k n_k = 0 \quad (18)$$

The local equilibrium model consists of $2I + 2$ equations representing the ionic equilibria and charge neutrality and $I + 1$ partial differential equations that describe the transport of the ampholyte species and current. Recall that there are now only $3I + 3$ dependent variables since the water is in excess.

The model just described enables us to study a variety of electrophoretic processes in detail using numerical methods to solve the equations. These matters will be taken up in Part II. However, analytical results derived from the model furnish insight into some situations. Before turning to the numerical results we illustrate this facility with the process known as isotachophoresis.

ISOTACHOPHORETIC REGULATING FUNCTIONS AND BOUNDARY THICKNESS

Some of the characteristics of isotachophoresis (ITP) set it apart from other well-known modes of electrophoresis. In ITP a mixture (the sample ions) is inserted between what are called the leading and terminating electrolytes in a column. Upon application of an electric field, a set of contiguous zones is formed, all moving at the same velocity. Except for transition regions at the zone boundaries, the composition of a given zone is uniform but compositions vary from one zone to the next. Several questions come to mind. How fast do the zones move? In what manner does the composition vary from zone to zone? How thick are the transition regions? Answers to the first two queries can be set out using analytical results derived from the mathematical model, whereas a detailed response to the third generally requires numerical computations. A simple three-component system will be analyzed below to provide insight into the distinctive features of ITP.

The simplest configurations for ITP are gel columns, small bore capillaries, or long narrow gaps between closely spaced flat plates. In such configurations we can take the convective velocity to be zero so that transport is by diffusion and electromigration. Then in a coordinate system moving with the band pattern mentioned above, the structure appears stationary and the transition zones are analogous to one-dimensional shock waves studied in gas dynamics. To study the structure of a single boundary imagine a system wherein three ampholytes, Am_1, Am_2, and Am_3, are distributed throughout a long column. Part of the column contains Am_1, in water; the other part is filled with a solution of Am_3. Am_2 is present throughout the column. The mobility of Am_1 is greater than that of Am_3. Upon application of a current, the boundary between Am_1 and Am_3 will move (and adjust) until a steady state is attained. In this state the boundary translates with a constant velocity.

First, to ascertain the speed of translation we transform to a new coordinate system moving with a (dimensionless) speed, v, relative to the old system and in which the concentration fields are stationary. The coordinate transformation is

$$\begin{aligned} \tau &= t \\ u &= x - vt \end{aligned} \quad (19)$$

In this reference frame the ampholyte balances, Eq. 21, read

$$v \frac{\partial}{\partial u}(n_{3i} + n_{3i+1} + n_{3i+2}) = \frac{\partial}{\partial u}(f_{3i} + f_{3i+1} + f_{3i+2}) \quad i = 1, 2, \ldots I. \tag{20}$$

Next, upon integration across the boundary, taking into account the way the fluxes are represented (cf. Eq. 13), the valences, and the fact that concentration gradients away from the boundary are negligible, we find the jump in composition between the two uniform regions to be

$$v\Delta\{n_{3i} + n_{3i+1} + n_{3i+2}\} = -\Delta\left\{[\Omega_{3i+1}n_{3i+1} - \Omega_{3i+2}n_{3i+2}]\frac{\partial \phi}{\partial u}\right\} \tag{21}$$

A similar procedure with the relation for the current, Eq. 17, yields

$$\eta = -\sum_{k=1}^{N} z_k^2 \Omega_k n_k \frac{\partial \phi}{\partial u} d - \sigma \frac{\partial \phi}{\partial u} \tag{22}$$

where η represents the constant (dimensionless) current. In the situation under study the ampholyte compounds denoted by the index $i = 1$ and $i = 3$ disappear across the boundary, so

$$v = \left(\frac{\Omega_4 n_4 - \Omega_5 n_5}{n_3 + n_4 + n_5}\right)_a \frac{\eta}{\sigma_a} = \left(\frac{\Omega_{10} n_{10} - \Omega_{11} n_{11}}{n_9 + n_{10} + n_{11}}\right)_b \frac{\eta}{\sigma_b} \tag{23}$$

Here the compositions for Am_1 are those ahead of the boundary and those for Am_3 are from the region behind the boundary. The concentrations denoted as n_3, n_4, n_5 are unaltered from those given at the outset but n_9, n_{10}, n_{11} are changed by application of the current. The conductivity, σ, is evaluated either for the region ahead of the front, σ_a, or the region behind the front, σ_b. The relation shows that the boundary speed is proportional to the current applied and the ion mobilities.

The change in composition across the boundary is governed in part by one of the well known regulating functions first derived by Kohlrausch (1897) for strong electrolytes. These were extended by Dole (1945) to multicomponent systems involving boundaries moving in both directions with an intervening stationary boundary, and by Alberty (1950) to simple weak acids and bases. For our problem involving ampholytes, Eq. 23 discloses yet another regulating function

$$\frac{(n_9 + n_{10} + n_{11})_b}{(n_3 + n_4 + n_5)_a} = \frac{(\Omega_{10} n_{10} - \Omega_{11} n_{11})_b}{(\Omega_4 n_4 - \Omega_5 n_5)_a} \frac{(\sigma)_a}{(\sigma)_b}, \tag{24}$$

Four additional relations are needed to specify the composition behind the front: the equilibria expressed by Eqs. 5, 6, and 7; and Eq. 18, the expression for charge neutrality. Solution of these five relations sets the composition of ampholyte species, n_9, n_{10}, and n_{11}, along with the amounts of hydrogen and hydroxyl ions.

Equations 23 and 24 depict two features of the isotachophoretic process: the concentration jump across the boundary and its velocity. Since the theory is easy to apply to simple ionic species, relations of this form can be used to determine mobilities of monovalent and divalent ions (see Everaerts et al., 1976, for an extensive discussion).

The third basic feature of isotachophoresis is the shape of the zone boundary. We have calculated boundary shapes for the transient and steady state numerically and these results will be discussed in Part II. Nevertheless, considerable insight can be obtained from the simplest possible isotachophoretic boundary involving three ions. A complete analytical solution is outlined in the Appendix for the steady state translation; two results of that analysis are discussed here. First, it is shown in the Appendix that the electroneutrality approximation is more than satisfactory even in the boundary region where concentrations vary rapidly. The argument parallels that already mentioned in connection with Eq. 11 and 18, namely, that the intrinsic length scale for the ionic charge cloud is much smaller than the length scale involving the current. In the example cited their ratio is 10^{-5}, and since the square of this ratio governs the extent of disequilibrium, the electroneutrality approximation can be employed with confidence in similar situations. Once this approximation is invoked, it is shown that the length scale for the boundary between two ionic species denoted by the subscripts 1 and 3 is $RT\hat{n}_2^a\hat{\Omega}_1\hat{\Omega}_3/\hat{\eta}(\hat{\Omega}_1 - \hat{\Omega}_3)$. The symbol $\hat{n}_2^a$ is the concentration of the common counterion ahead of the boundary. It follows that the mobility of the leading ion, $\hat{\Omega}_1$, must exceed that of the trailing ion, $\hat{\Omega}_3$, in order for the front to persist. Furthermore, the greater this difference, the sharper the transition. Larger currents also enhance the transition, whereas increases in the counterion concentration spread the boundary. These quantitative arguments, derived from an exact solution to a simple problem, carry over to more complicated systems as will be illustrated in part II using numerical results for weakly ionizing species.

CONCLUDING REMARKS

The model presented here treats the chemical processes as reactions at equilibrium and accounts for convection, diffusion, and electromigration of individual species. When applied to the process known as ITP, analytical results are obtained. The real strength of the model, however, lies in its ability to track the behavior of complicated processes including ampholytes and other weak electrolytes. These features will be studied in detail for other modes of electrophoresis in Part II, where certain aspects of the numerical algorithm used to implement the model will be examined.

Experimental validation studies are now in progress and will be reported separately.

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NOTATION

D_r = characteristic diffusivity, m^2/s
d_1, d_2, d_3 = constants of integration
e = molar charge, coulombs/ mol
$\hat{f}_i, f_i$ = vector of fluxes of the ith species, $mol/m^2 \cdot s$, dimensionless form
I = total number of ampholyte species
k_r = characteristic reaction rate, s^{-1}
$\hat{k}_i^{(f)}, k_i^{(f)}$ = forward reaction rate constant, s^{-1}, dimensionless form
$\hat{k}_i^{(r)}, k_i^{(r)}$ = reverse reaction rate constant, $m^3/mol \cdot s$, dimensionless form
$\hat{K}_o, K_o$ = equilibrium constant for water, mol/m^3, dimensionless form
$\hat{K}_i^{(-)}, K_i^{(-)}$ = constant describing the equilibrium between the neutral and negatively charged species of the ith ampholyte, dimensionless form
$\hat{K}_i^{(+)}, K_i^{(+)}$ = constant describing the equilibrium between the neutral and the positively charged species of the ith ampholyte, dimensionless form
ℓ = electrical length scale, m

n_r = characteristic concentration, mol/m^3
$\hat{n}_o, n_o$ = water concentration, mol/m^3, dimensionless concentration
$\hat{n}_1, n_1$ = hydrogen concentration, mol/m^3, dimensionless concentration
$\hat{n}_2, n_2$ = hydroxyl ion concentration, mol/m^3, dimensionless concentration
$\hat{n}_i, n_i$ = concentration of the ith species, mol/m^3, dimensionless concentration
N = total number of ionic species
Pe = Peclet number, $Pe = ex_r v_r/RT\Omega_r$
R = gas constant, kg · m^2/s^2 · K
$\hat{R}_i, R_i$ = rate of production of the ith species, mol/m^3 · s, dimensionless form
t_r = characteristic time, s
$\hat{t}, t$ = time, s, dimensionless form
T = temperature, K
$\hat{u}, u$ = spatial coordinate, m, dimensionless form
$\hat{v}, v$ = vector of fluid velocities, m/s, dimensionless form
x_r = characteristic distance, m
$\hat{x}, x$ = vector of spatial coordinates, m, dimensionless form
z_i = valence of the ith species

Greek Letters and Symbols

α = dimensionless constant
β = dimensionless constant
$\gamma = (1 + \Omega_1)\Omega_3/(1 + \Omega_3)\Omega_1$
$\delta = \Omega_1\Omega_3/(\Omega_1 - \Omega_3)$
Δ = denotes a difference taken across an isotachophoretic boundary
ϵ = permittivity of the solvent ($\epsilon = \epsilon_o\epsilon_r$), C/V · m
ϵ_o = permittivity of vacuum, C/V · m
ϵ_r = dielectric constant for water
$\zeta = \exp(-u/\delta)$
$\hat{\eta}, \eta$ = current density, A/m^2, dimensionless form
κ^{-1} = Debye length, m
$\nu = \gamma/2(1 - \gamma)$
σ = dimensionless conductivity
τ = dimensionless coordinate
$\hat{\phi}, \phi$ = electric potential, volts, dimensionless form
ϕ_r = characteristic potential, volts
Ω_r = characteristic mobility, m^2/V · s
$\hat{\Omega}_i, \Omega_i$ = mobility of the ith species, m^2/V · s, dimensionless form
∇ = denotes a gradient

APPENDIX: BOUNDARY STRUCTURE IN ISOTACHOPHORESIS WITH FULLY IONIZED SOLUTES

The front structure can be established analytically for the situation depicted schematically in Figure A1 where the anions concentrations are $\hat{n}_1 = [A^-]$ and $\hat{n}_3 = [C^-]$ with $\hat{n}_2 = [B^+]$ as the common counterion. Water is treated as a solvent and its ionization ignored. We start with the equations in dimensional form to illustrate the analysis with a concrete example. The conservation equations for a one-dimensional system without flow are

$$\frac{\partial}{\partial \hat{t}}\hat{n}_i = \frac{\partial}{\partial \hat{x}}\left[z_i\hat{\Omega}_i\hat{n}_i\frac{\partial\hat{\phi}}{\partial\hat{x}} + \frac{RT}{e}\hat{\Omega}_i\frac{\partial\hat{n}_i}{\partial\hat{x}}\right], \qquad i = 1, 2, 3 \quad \text{(A1)}$$

and

$$\epsilon\frac{\partial^2\hat{\phi}}{\partial\hat{x}^2} = -e\sum_{k=1}^{3} z_k\hat{n}_k \quad \text{(A2)}$$

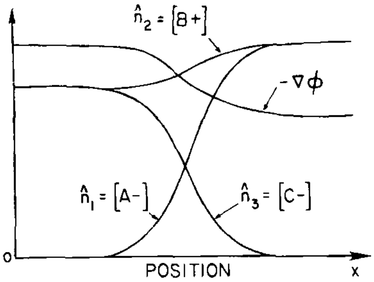

Figure A1. The structure of an isotachophoretic boundary. $\hat{n}_1$, $\hat{n}_2$, $\hat{n}_3$ are the concentrations of the leading ion [A^-], the counterion [B^+], and the terminating ion [C^-], respectively; $\nabla\phi$ is the potential gradient.

After the steady current, $\hat{\eta}$, has been established the boundary evolves to a stationary front when viewed from a coordinate system moving with velocity, $\hat{v}$. In the moving coordinate system where $\hat{u} = \hat{x} - \hat{v}\hat{t}$, the steady fields are described by solutions to

$$-\hat{v}\frac{\partial}{\partial\hat{u}}\hat{n}_i = \frac{\partial}{\partial\hat{u}}\left[z_i\hat{\Omega}_i\hat{n}_i\frac{\partial\hat{\phi}}{\partial\hat{u}} + \frac{RT}{e}\hat{\Omega}_i\frac{\partial\hat{n}_i}{\partial\hat{u}}\right] \quad \text{(A3)}$$

and

$$\epsilon\frac{\partial^2\hat{\phi}}{\partial\hat{u}^2} = -e\sum_{k=1}^{3} z_k\hat{n}_k \quad \text{(A4)}$$

Far ahead of the front the concentrations asymptote to $\hat{n}_1^a = \hat{n}_2^a$ and $\hat{n}_3^a = 0$, while far behind the front *$\hat{n}_1^b = 0$ and $\hat{n}_2^b = \hat{n}_3^b$*. To simplify the model we take advantage of the fact that the length scale based on the current, $\ell = RT\Omega_2\hat{n}_2^a/\hat{\eta}$, is much larger than the scale based on the intrinsic properties of the ionic solution, $\kappa^{-1} = \sqrt{\epsilon RT/e^2\hat{n}_2^a}$, and transform the problem as follows:

$$\hat{n}_i = \hat{n}_2^a n_i, \; \hat{\phi} = \frac{RT}{e}\phi, \; \hat{u} = \frac{RT\hat{\Omega}_2\hat{n}_2^a}{\hat{\eta}}u,$$

Now with $\hat{v} = \hat{\eta}/e\hat{n}_2^a$ the problem reads

$$\frac{\partial n_i}{\partial u} = \frac{\partial}{\partial u}\left[z_i\Omega_i n_i\frac{\partial\phi}{\partial u} + \Omega_i\frac{\partial n_i}{\partial u}\right] \quad \text{(A5)}$$

$$\beta\frac{\partial^2\phi}{\partial u^2} = -\sum_{k=1}^{3} z_k n_k \quad \text{(A6)}$$

with

$$n_1, n_2 \rightarrow 1; n_3 \rightarrow 0 \quad \text{for } u \rightarrow \infty \quad \text{(A7)}$$

$$n_1 \rightarrow 0; n_2, n_3 \rightarrow \gamma \quad \text{for } u \rightarrow -\infty \quad \text{(A8)}$$

Here we have introduced the notation

$$\beta^d \epsilon RT/(e^2 n_2^a \ell^2) = (\kappa\ell)^{-2} \quad \text{(A9)}$$

The mobilities have also been scaled on $\hat{\Omega}_2$, the mobility of the common counterion. For typical conditions (Table 1), we find $\ell \doteq 10^{-4}$ m and $\kappa^{-1} \doteq 10^{-9}$ m so that β is very small.

Next we take advantage of the fact that $\beta \ll 1$ to replace Eq. A6 with the condition of electrical neutrality. Then the equations can be integrated once to give

$$-n_i = z_i\Omega_i n_i\frac{\partial\phi}{\partial u} + \Omega_i\frac{\partial n_i}{\partial u} + d_i, \; i = 1, 2, 3 \quad \text{(A10)}$$

and

$$\sum_{i=1}^{3} z_i n_i = 0 \quad \text{(A11)}$$

There are six parameters to be fixed: d_1, d_2, d_3, $(\partial\phi/\partial u)_a$. $(\partial\phi/\partial u)_b$, and γ. Since the net flux of species A^- and C^- is zero,

TABLE 1. CHARACTERISTIC MAGNITUDES AND CONSTANTS

Mobility, Ω_r	10^{-8}(m/s)/(V/m)
Concentration, n_r	10 mol/m^3
Current density, η_r	1 A/m^2
Column length, x_r	10^{-2} m
Forward reaction rate, k_r	10^5 s^{-1}
Universal gas constant, R	8.314(kg · m^2)/(s^2 · K · mol)
Molar charge, e	96,500 C/mol
Permittivity constant, $\epsilon = \epsilon_o \epsilon_r$	$80 \times 8.854 \cdot 10^{-12}$ c/(v · m)
(ϵ_o, permittivity of vacuum; ϵ_r, water dielectric constant)	
Temperature, T	298 K
Potential, $\phi_r = (RT)/e$	$2.567 \cdot 10^{-2}$ V
Time, $t_r = (ex_r^2)/(RT\Omega_r) = x_r^2/(\phi_r \Omega_r)$	$3.89 \cdot 10^5$ s
Dimensionless time scaling constant $\alpha = (RT\Omega_r)/(ex_r^2 k_r) = k_r^{-1} t_r$	$2.567 \cdot 10^{-11}$
Debye length, κ^{-1} $(\epsilon RT)/(e^2 n_r)$	$4.339 \cdot 10^{-9}$ m
Dimensionless length scaling constant, $\beta = (\epsilon RT)/(e^2 n_r x_r^2) = (\kappa x_r)^{-2}$	$1.883 \cdot 10^{-13}$
Diffusivity, $D_r = (RT\Omega_r)/e = \phi_r \hat{\Omega}_r$	$2.567 \cdot 10^{-10}$ m^2/s
Electrical length scale, $\ell = (RT\hat{\Omega}_2 \hat{n}_2^a)/\hat{\eta}_r$	$2.478 \cdot 10^{-4}$ m

$d_1 = d_3 = 0$. By inspecting the behavior of the equations far from the front we find:

$$(\partial\phi/\partial u)_a = \Omega_1^{-1},\ (\partial\phi/\partial u)_b = \Omega_3^{-1},\ d_2 = -(1+\Omega_1)/\Omega_1$$

and

$$\gamma = (1+\Omega_1)\Omega_3/(1+\Omega_3)\Omega_1.$$

Note that the expression for γ is one form of regulating function for the concentration jump across the front. To find expressions for the dependent variables we proceed as follows.

First, upon elimination of $\partial\phi/\partial u$ from the first and third members of Eq. A10 we can deduce that if the origin ($u = 0$) is set at the place where $n_1 = n_3$ then

$$n_3 = n_1 \exp(-u/\delta) \qquad \text{(A12)}$$

where

$$\delta \overset{d}{=} \Omega_1\Omega_3/(\Omega_1 - \Omega_3). \qquad \text{(A13)}$$

This furnishes a dimensionless scale for the decay process, and upon reverting to dimensional variables we find the decay scale is

$$\frac{\hat{\Omega}_1\hat{\Omega}_3}{(\hat{\Omega}_1 - \hat{\Omega}_3)}\,\frac{RT\hat{n}_2^a}{\hat{\eta}}.$$

For the conditions listed in Table 1 this works out to be roughly 100 μm. Although this expression depicts the effects of current and mobility qualitatively, the parent expression, Eq. A13, shows only that the concentration ratio changes on the length scale given above, so we need to complete the solution of the problem.

To obtain explicit formulas we use the electroneutrality condition to eliminate the potential gradient from Eq. A5 and obtain

$$\frac{\partial}{\partial u}[n_1 + n_2 + n_3] + n_1\Omega_1^{-1} + n_2 + n_3\Omega_3^{-1} = [1+\Omega_1]/\Omega_1 \qquad \text{(A14)}$$

Since $n_2 = n_1 + n_3$ and $n_1 = n_3 \exp(u/\delta)$ we can obtain a single differential equation and then integrate it. The final expression for n_3 is

$$n_3 = \gamma + \frac{2-\gamma}{2(1-\gamma)}\,\frac{\zeta^{(1+\nu)}}{\sqrt{(1+\zeta)}}\int_\infty^\zeta \frac{\mu^{-2-\nu}}{\sqrt{(1+\mu)}}\,d\mu \qquad \text{(A15)}$$

where $\zeta = \exp(-u/\delta)$, and $\nu = \gamma/2(1-\gamma)$. The potential is

$$\frac{d\phi}{du} = \frac{1}{\Omega_3} + \frac{1}{n_3}\frac{dn_3}{du} \qquad \text{(A16)}$$

These expressions give n_3 and $d\phi/du$ explicitly in terms of position and are of practical as well as theoretical importance since they can be used to test numerical schemes designed for systems with more complicated chemistry.

The result given here for the concentration ratio, Eq. A13, was first reported by Konstantinov and Oshurkova (1966), but the explicit formulae for concentrations and potential appear to be new. This result also suggests a straightforward way of accounting for weak violations of electrical neutrality using perturbation methods. If we regard the expressions for n_3 and $d\phi/du$ as leading terms in expansions of the form

$$n_3 \sim n_3^{(0)} + \beta n_3^{(1)} + \cdots, \qquad \text{(A17)}$$

then a sequence of nonhomogeneous ordinary differential equations is generated for the higher order terms. These could be solved to provide asymptotic expansions valid for small values of β Coxon and Binder (1974) attempted to include the effects of charge disequilibrium using an ad hoc numerical scheme, but due to the structure of their method it is not clear whether it would succeed even when there is electrical neutrality.

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