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Transport of solutes across aqueous phase interfaces by electrophoresis. Mathematical modeling

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ABSTRACT

Polarizations of solutes undergoing electrophoresis occur at the interfaces of aqueous two-phase systems. The first mathematical model of this situation is presented, and is shown to predict results qualitatively similar to our experimental work. The numerical simulation of electrophoresis is based on an earlier single-phase model, extended with additional equations and boundary conditions needed in the two-phase case. Results from the simulation of some simple model systems are presented and discussed.

INTRODUCTION

We have previously shown that the electrophoresis of solutes across the interface in aqueous two-phase systems could be strongly influenced by the partitioning of the solutes in the systems [1]. Unexpected polarization was noted as proteins or certain dyes were transported across the interface from preferred to non-preferred phase. The polarization was directional, in that movement from non-preferred to preferred phase appeared unaffected by the phase boundary. The problem is of considerable importance. Similar phenomena were reported by Maxwell in 1892 [2] for organic–organic interfaces. Nernst and Riesenfeld [3] noted the occurrence of polarizations at organic–aqueous interfaces in 1902. We believe our investigations to be the first report on these polarizations in aqueous–aqueous phase systems. This sort of behavior at interfaces can be used as the basis for novel separations. It can also help clarify other examples of interphase transport in aqueous systems, such as transport across cell membranes or within an electrochromatography column. Modeling was undertaken because of this practical importance, and also since these results were not accounted for by any current theory of electrophoresis. Using simple systems, our model demonstrates some of the essential characteristics of the experimental results.

The framework of our theory for electrophoretic transport is provided by Bier and co-workers [4–8], which we expand to account for the special case of a two-phase system. The model for electrophoresis in single phase systems is based on a set of unsteady state mass balances for each species i composing the complete system. The system is assumed isothermal with transport in one dimension only. A generation term R_i (mol/m^3 · s) enables the interconversion between neutral and charged species of a given molecule,

$$\frac{\partial c_i}{\partial t} = -\frac{\partial F_i}{\partial x} + R_i \tag{1}$$

where c_i is the concentration of i (mol/m^3) and t is time (s). F_i represents the flux of i in mol/m^2 · s given by

$$F_i = -c_i z_i \Omega_i \frac{\partial \Phi}{\partial x} - \frac{RT}{e}\Omega_i \frac{\partial c_i}{\partial x} \tag{2}$$

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Flux by both electrophoresis and diffusion is allowed, all other mechanisms are neglected. The left hand term in the flux equation describes electrophoretic motion, with z_i representing the charge on i, Ω_i a mobility factor in $m^2/V \cdot s$, and $\partial\Phi/\partial x$ the gradient in applied potential (V/m). The right hand term in this equation characterizes diffusion. R is the gas constant in V · Coulombs/K · mol, T the absolute temperature in K and e the molar charge of 96 500 Coulomb/mol.

The generation term R_i is assumed to follow mass action kinetics, represented by equations of the form

$$R_{A^-} = k_f c_{HA} - k_r c_{H^+} c_{A^-} \quad (3)$$

where k_f and k_r are forward and reverse reaction rate constants, respectively, in appropriate units. They describe an illustrative reaction such as, for example

$$HA \rightleftharpoons H^+ + A^- \quad (4)$$

In addition to the set of partial differential equations (PDEs) resulting from describing the transport of each species i, a system of algebraic relationships must be simultaneously solved in order to fully describe the problem. Certain elementary species are conserved. For example, the rate at which water disappears from the system must be matched by the rate at which hydroxyl ion appears. Linear combinations of other species yield similar relationships. These may be summed to yield

$$\sum_i z_i R_i = 0 \quad (5)$$

The rate of charge generation within the system must be zero everywhere except at the electrodes. Therefore, the divergence of the sum of the fluxes of charged species is zero,

$$-\nabla \cdot \sum_i e z_i F_i = 0 \quad (6)$$

Also, we assume that electroneutrality prevails on the scale of interest,

$$\sum_i e z_i c_i = 0 \quad (7)$$

It is generally necessary to solve the equations numerically, because of the complex nature of the system. All the classically known modes of electrophoresis could be described with this model, by proper choice of initial and boundary conditions for the PDEs [4].

MODEL FOR ELECTROPHORESIS IN TWO-PHASE SYSTEMS

Our model for two-phase electrophoresis begins with similar equations written for each phase of the two-phase system. Two additional boundary conditions are required at the interface in order to complete the description of this system. The work of Davies [9] and the text of Crank [10] involving diffusion only across the interface of two-phase systems suggested two different possibilities.

One assumption is instantaneous equilibrium across the phase boundary. If the two-phase system is considered infinite in extent with the interface at $x = 0$, phase a encompasses $x < 0$ and phase b extends from $x > 0$. Then

$$K = \frac{c_{i,b}}{c_{i,a}} \quad \text{for} \quad x = 0 \quad (8)$$

where K is the equilibrium partition coefficient of solute i.

The second alternative for this boundary condition is to assume that there is significant resistance to transport. Then, a mass transfer expression best defines the flux at the interface

$$F_i = \alpha(K c_{i,a} - c_{i,b}) \quad \text{for} \quad x = 0 \quad (9)$$

where α is the mass transfer coefficient (m/s).

For either possibility, the second boundary condition at the interface states that all mass flowing out of phase a must flow into phase b. The fluxes across the phase boundary must match

$$F_{i,a} = F_{i,b} \quad \text{for} \quad x = 0 \quad (10)$$

To complete either description of the two-phase system, the remaining boundary and initial conditions must be stated

$$c_{i,a} = c_{i,-\infty} \quad \text{for} \quad x = -\infty \quad (11)$$

$$c_{i,b} = c_{i,+\infty} \quad \text{for} \quad x = +\infty \quad (12)$$

$$c_{i,a} = c_{i,-\infty} \quad \text{for} \quad t = 0 \quad (13)$$

$$c_{i,b} = c_{i,+\infty} \quad \text{for} \quad t = 0 \quad (14)$$

The new boundary conditions at the interface must be substituted into the finite difference approx-

imations for the PDEs at the appropriate spacial grid points. For example, consider an illustrative 50-point grid. Concentrations are estimated at the grid points from fluxes, which are calculated between grid points. The interface is located at the center of the grid, between points 25 and 26. The flux associated with transport across the interface is defined as flux 25.

All concentrations in the model, except those on either side of the interface (25 and 26) are calculated by the normal finite difference approximation

$$\frac{c_i' - c_i}{\Delta t} = -\left(\frac{F_i - F_{i-1}}{\Delta x}\right) \quad (15)$$

where the subscript i now refers to position in the grid. The prime (') indicates a quantity being calculated at the next time point in the simulation from current time point data.

To model the case of equilibrium across the interface, eqn. 15 must be written for grid points 25 and 26:

$$\frac{c_{25}' - c_{25}}{\Delta t} = -\left(\frac{F_{25} - F_{24}}{\Delta x}\right) \quad (16)$$

$$\frac{c_{26}' - c_{26}}{\Delta t} = -\left(\frac{F_{26} - F_{25}}{\Delta x}\right) \quad (17)$$

By use of the boundary condition given by eqn. 10, the common flux 25 is eliminated between these equations. The equilibrium expression across the interface, eqn. 8, is then used to substitute for the new concentration at grid point 26:

$$\frac{\Delta x}{\Delta t}(c_{25} - c_{25}') + F_{24} = \frac{\Delta x}{\Delta t}(Kc_{25}' - c_{26}) + F_{26} \quad (18)$$

This can be solved for the new concentration at point 25

$$c_{25}' = \frac{c_{25} + c_{26}}{1 + K} + \frac{\Delta t}{\Delta x}\left(\frac{F_{24} - F_{26}}{1 + K}\right) \quad (19)$$

The equilibrium equation is then used once more to obtain

$$c_{26}' = Kc_{25}' \quad (20)$$

These last two equations replace eqn. 15 at grid points 25 and 26 for simulations where instantaneous equilibrium is assumed across the interface. Calculation for concentration values at all other spacial grid points are made with eqn. 15.

Alternatively, one may assume there is mass transfer resistance at the interface, not instantaneous equilibrium. To model this case, the mass transfer expression, eqn. 9, is substituted for eqn. 2 for flux 25 at the interface. Eqn. 2 is retained to calculate all other fluxes.

The solution of the models for two-phase systems now follows easily from the single-phase model. All equations are put into non-dimensional form. The dimensionless form of eqn. 6 for conservation of charge is integrated to yield

$$\sum_i z_i F_i = \theta \quad (21)$$

where F_i is now a dimensionless flux and θ is the dimensionless current. An initial component distribution is input into the simulation package. Equilibrium expressions derived from equations of the type shown by eqn. 3 are combined with the requirement for electroneutrality, eqn. 7. From this, pH and concentrations for ionizable species are obtained. Conductivity can then be calculated, which when combined with the applied current, yields the gradient in the potential between pairs of grid points. Fluxes for species are figured from eqn. 2 or 9, as appropriate. Eqn. 1, 19, or 20 is finally solved, as needed, to obtain concentrations at the new step forward in time. The Runga–Kutta–Fehlberg method was used for integration in the examples which follow. The process is then essentially repeated, until the simulation time is finished. Complete details regarding the solution are available in the previously cited references [4–8].

RESULTS

The results presented here are from models in which the following simplifying assumptions are made. Only one component, the one of interest, is considered to be partitioned at the interface. All the other components in the simulation behave as though electrophoresis is taking place in a bulk phase. Furthermore, unless specified otherwise, each component has the same electrophoretic mobility in either phase. Although this is not generally true in reality, it simplifies the interpretation of results since a change in mobility at the interface in and of itself will cause other polarization effects. We have previously demonstrated experimentally that for the

special case of equal electrophoretic velocity in either phase, polarizations still occur at the interface of two-phase systems [1].

The two models, with either instantaneous equilibrium or mass transfer resistance at the interface, have been run for a number of cases. To verify that each was working correctly, the limiting cases for bulk transport by diffusion only with a single solute was tested. Both models were found to be in excellent agreement with the analytical solutions published by Crank [10]. Also, as a further check of the reliability of numerical solution, the amount of solute which accumulates at the interface with electrophoresis in the complete model compares well with the amount calculated for the case of an impermeable wall at the interface.

The simulations used either hemoglobin or a strong base as the partitioned component of interest. The base simplifies the interpretation of results. The values for the partition coefficient and the mass transfer coefficient were those given by Shanbhag [11], for hemoglobin in a system similar, but not identical, to the two-phase system examined experimentally. The partitioned solute was present as a minor component of the system, over 3000 times more dilute than the next most dilute component. This reduces the interaction between the components. The remainder of the system was a buffer, to minimize the effect of pH. The system initially begins at its equilibrium distribution and the figures show a concentration profile calculated after the electric field has been applied for 1 min. Pertinent data for the simulations are summarized in Table I.

TABLE I

PARAMETERS FOR SIMULATIONS

Concentration profiles are shown 1 min after electrophoresis begins from equilibrium conditions.

Simulation space = 0.005 m divided into 200 grid points

Initial current density = 20 A/m^2

For equilibrium model simulations

Run assuming constant current

Supporting Buffer

0.01 M acetate ion, pK = 4.76, $\Omega = 4.12 \cdot 10^{-8}$ m^2/V · s.
0.0063 M ammonium ion, pK = 9.25, $\Omega = 6.39 \cdot 10^{-8}$ m^2/V · s.
pH = 5.0

Hemoglobin, diffusion coefficient = $6.8 \cdot 10^{-7}$ cm^2/s

pH	Charge
3.0	68.5
3.5	43.5
4.5	25.5
6.0	10.25
8.0	−10.25
9.0	−20.5
10.0	−30.75
11.0	−50.0
11.5	−63.5

For mass transfer model simulations

Run assuming constant voltage drop across separation space

Supporting buffer

0.01 M cacodylate ion, pK = 6.21, $\Omega = 2.31 \cdot 10^{-8}$ m^2/V · s.
0.0086 M Tris ion, pK = 8.30, $\Omega = 2.41 \cdot 10^{-8}$ m^2/V · s.
pH = 7.0

Fig. 1 reports the results of a simulation with instantaneous equilibrium at the interface. Two differet values of the equilibrium partition coefficient K are examined. In both cases, phase a is the preferred phase, so that electrophoresis is from preferred into non-preferred phase. Material in both these simulations tends to concentrate on the left side of the interface. As the partitioning between the phases becomes greater (the partition coefficient decreases from a value of 1), the observed polarization increases.

Fig. 2 also demonstrates the results of simulations

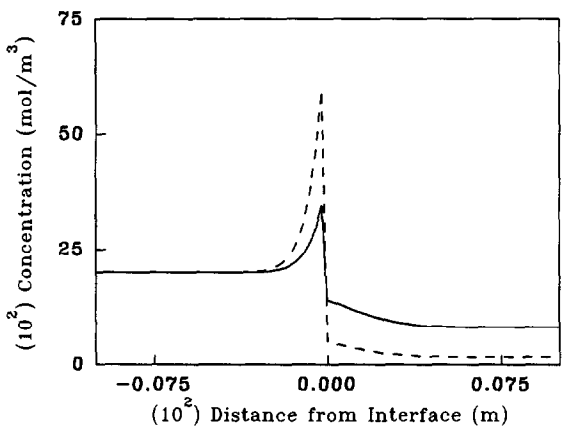

Fig. 1. Concentration profile after 1 min of electrophoresis, with equilibrium for two different partition coefficients. For K = 0.41 (———), $c_{i,-\infty} = c_{i,a,t=0} = 0.20$ mol/m^3; $c_{i,\infty} = c_{i,b,t=0} = 0.082$ mol/m^3. For K = 0.082 (– – –), $c_{i,\infty} = c_{i,b,t=0} = 0.0164$ mol/m^3. The partitioned species is a strong base, with $\Omega = 1.0 \cdot 10^{-8}$ m^2/V · s.

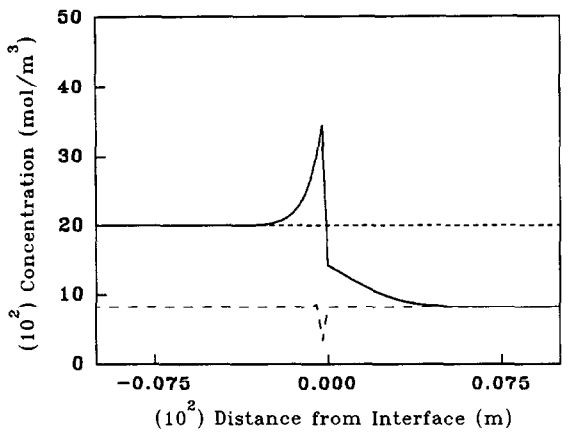

Fig. 2. Concentration profile after 1 min of electrophoresis, for the equilibrium model for three cases of directional transport. Transport from preferred to non-preferred phase (———), K = 0.41; $c_{i,-\infty} = c_{i,a,t=0} = 0.20$ mol/m³; $c_{i,\infty} = c_{i,b,t=0} = 0.082$ mol/m³. Transport from non-preferred to preferred phase (– – –), K = 2.439; $c_{i,-\infty} = c_{i,a,t=0} = 0.082$ mol/m³; $c_{i,\infty} = c_{i,b,t=0} = 0.20$ mol/m³. Neither phase preferred (-----), K = 1; $c_{i,-\infty} = c_{i,a,t=0} = c_{i,\infty} = c_{i,b,t=0} = 0.20$ mol/m³. Partitioned species is a strong base, as in Fig. 1.

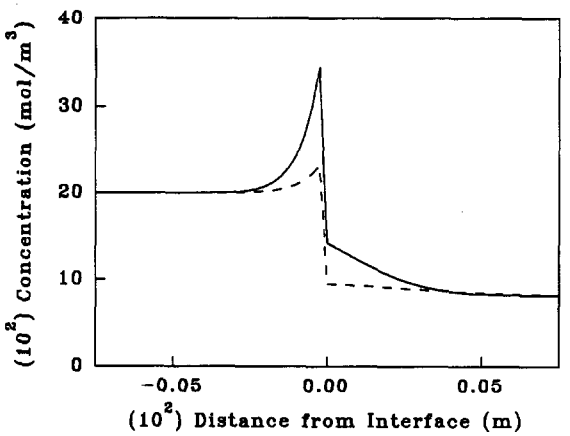

Fig. 3. Concentration profile after 1 min of electrophoresis for equilibrium model; effect of a mobility difference. With the same mobility in either phase (———), $\Omega = 1 \cdot 10^{-8}$ m²/V · s. For differing mobility (– – –), $\Omega = 1 \cdot 10^{-8}$ m²/V · s in phase a; $2 \cdot 10^{-8}$ m²/V · s in phase b. For both simulations, K = 0.41; $c_{i,-\infty} = c_{i,a,t=0} = 0.20$ mol/m³; $c_{i,\infty} = c_{i,b,t=0} = 0.082$ mol/m³. Partitioned species is a strong base.

with equilibrium at the interface. For the simulation where K is 0.41, the solute is being transported from preferred to non-preferred phase, and concentration occurs at the interface. If K is changed to 2.439 (1/0.41), the solute is electrophoresed from non-preferred to preferred phase. In this case, this model predicts a small dilution to occur at the interface. Finally, if K is set equal to 1, neither phase is preferred, and the solute is evenly distributed between the phases at equilibrium. The model predicts that the interface has no observable effect on the transport of the solute under these conditions and no polarization is calculated for the interface.

Fig. 3 examines a more complex situation, where the partitioned species has two times the mobility in phase b compared with phase a. This is compared with a similar simulation, with the same mobility in both phases. Equilibrium is assumed at the interface, with K = 0.41. The amount of polarization at the interface decreases with increased mobility in phase b.

Behavior of the protein hemoglobin is examined in Fig. 4, in a simulation with equilibrium assumed at the interface. A comparison run is made with a strong base. For both simulations, K is set at 0.41. The protein is also predicted to concentrate at the interface, when electrophoresis is from preferred to non-preferred phase. The irregularity to the left of the concentrated zone is due to the difficulty in numerically simulating the sharp gradient in concentration in that neighborhood.

In Fig. 5, concentration profiles from simulations

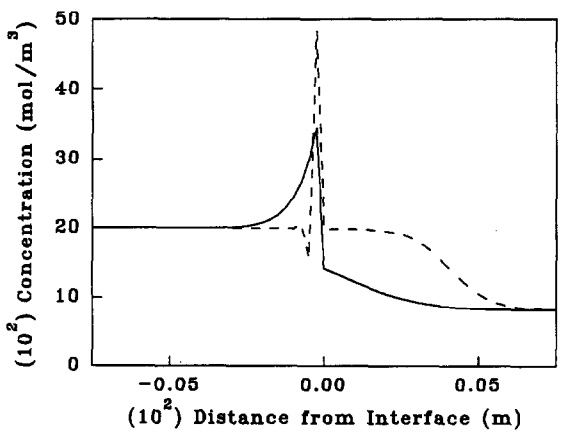

Fig. 4. Concentration profile after 1 min of electrophoresis for equilibrium model; effect of the nature of the partitioned solute. Strong base (———), $\Omega = 1 \cdot 10^{-8}$ m²/V · s; hemoglobin (– – –). For both simulations, K = 0.41; $c_{i,-\infty} = c_{i,a,t=0} = 0.20$ mol/m³; $c_{i,\infty} = c_{i,b,t=0} = 0.082$ mol/m³.

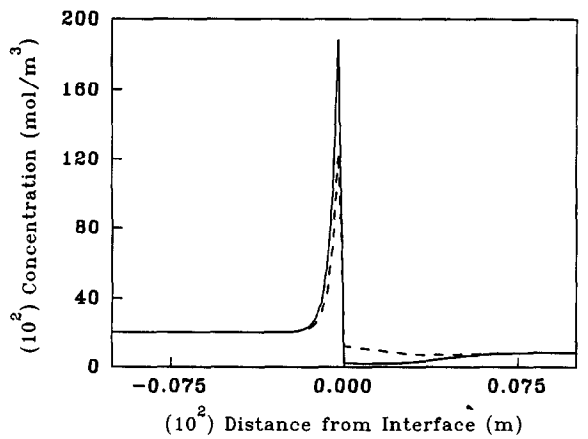

Fig. 5. Concentration profile after 1 min of electrophoresis for mass transfer model for two different mass transfer coefficients. For low mass transfer case (———), $\alpha = 2.29 \cdot 10^{-7}$ m/s, for high mass transfer case (– – –), $\alpha = 2.29 \cdot 10^{-6}$ m/s. For both cases, $K = 0.41$; $c_{i,-\infty} = c_{i,a,t=0} = 0.20$ mol/m^3; $c_{i,\infty} = c_{i,b,t=0} = 0.082$ mol/m^3. Partitioned species is a strong base, as in Fig. 1.

which use a mass transfer resistance model at the interface are shown. Two sets of values for these coefficients are compared, a low interfacial mass transfer rate and a high rate. As expected, the case with a lower mass transfer rate demonstrates a greater concentration of the strong base at the interface for equivalent electric fields applied for the same amount of time.

DISCUSSION

Both models presented here qualitatively demonstrate the most startling effect of our electrophoresis experiments in two-phase systems, the polarization at the interface when electrophoresis is from preferred to non-preferred phase.

The equilibrium model predicts dilution at the interface when transport is from non-preferred to preferred phase. It also calculates no observable polarizations when $K = 1$. Both of these results were observed experimentally, as reported in our earlier work. The polarization increases as the distribution of the solute between the phases at equilibrium becomes more dissimilar.

Similar results can also be obtained from the resistance model. However, few experimental values are available for mass transfer coefficients at these interfaces, and our attempts at measuring them proved unsuccessful. We have concentrated on the equilibrium model, as it provides a simpler explanation of the phenomenon.

The versatility of the model is demonstrated by the simulation which contains protein and the simulation with the change in solute mobility at the interface. Weak electrolytes, peptides, and other amphoteric substances may also be studied. It also allows modeling of a number of other situations, such as partitioning of the buffer salts in addition to the solute partitioning. The behavior of all these systems is made more complex as the concentrations of solute and buffer become comparable and the system is rendered more interactive. Further numerical simulations are underway to explore these situations.

Comparisons of these numerical solutions to our experimental work are limited because of the difficulties in measuring concentration as a function of position in two-phase systems. As noted above, both models are qualitatively in agreement with the experiments in some respects. However, the width of the polarized zone is calculated to be narrower than that observed in our experiments.

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