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Nonequilibrium thermodynamics of membrane-confined electrophoresis

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Abstract

Membrane-confined electrophoresis (MCE) is an electrophoretic transport method in which macromolecules in solution are confined within a cuvette through which a current flows. Small ions that can permeate the membranes permit current flow. The method is the electrophoretic analog to analytical ultracentrifugation. Systems in the MCE instrument are described by nonequilibrium thermodynamics. This description forms the basis of a program, implemented using finite element methods, that can model transport processes in such systems over an extended time, from arbitrary starting conditions to steady state. Issues relevant to the analysis of systems in which macromolecular species are involved in mass–action associations are discussed. Particular attention is given to steady-state electrophoresis, from which measurements of reduced molecular charge are sought. The relationship of such measurements to valence is discussed.

Keywords: Membrane-confined electrophoresis; Steady-state electrophoresis; Nonequilibrium thermodynamics; Macromolecular interactions; Finite element simulation

1. Introduction

1.1. The instrument

Macromolecular charge influences macromolecular structure and function in solution. Membrane-confined electrophoresis (MCE) is a method of investigating solution properties, such as electrophoretic mobility, that involve macromolecular charge. It is an electrophoretic transport method in which macromolecules in solution are confined within a cuvette through which a current, i, flows [1,2]. (See Section 5 for the typical units used for current and other parameters.) The four rectangular walls of the cuvette are joined at right angles to each other to form a box with open, rectangular ends (Fig. 1). The cuvette is oriented so that the current flows through it vertically. The top and bottom of the cuvette are sealed with semipermeable membranes. The x-axis of the cuvette is defined to be directed vertically downward, with its origin at the top membrane. Thus, the positive x-axis is parallel to, and in the same direction as, the electrophoretic transport of positively charged species when the electric field vector, $\vec{E}$, is also

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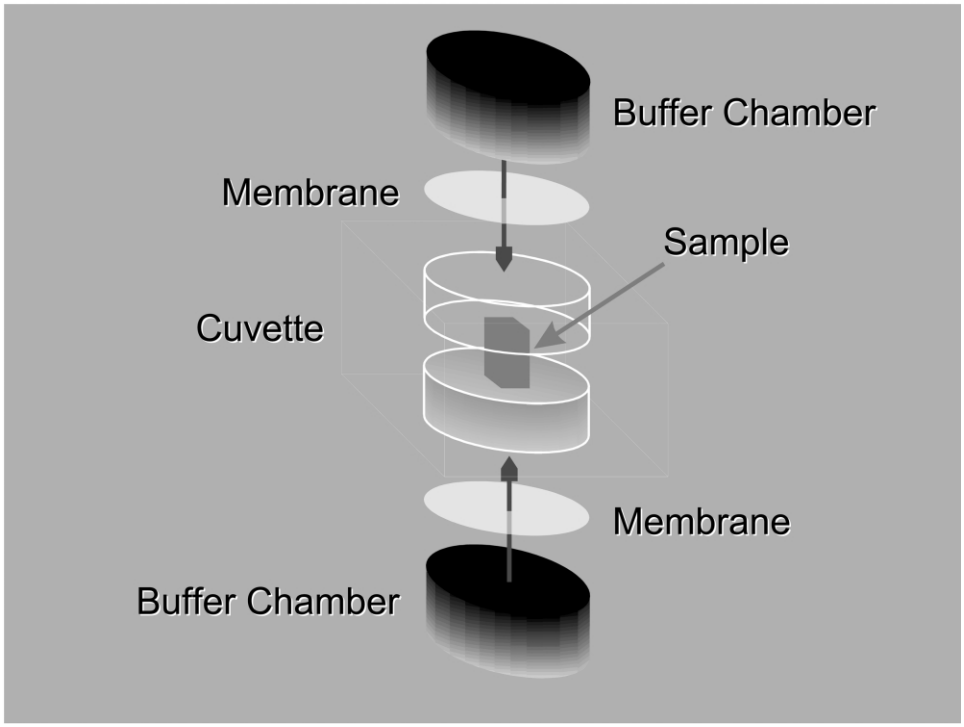

Fig. 1. Cuvette of the MCE instrument.

directed downward. The plane cross-sectional area, A, of the cuvette is perpendicular to the x-axis, and independent of the vertical position, x. Furthermore, the current density is assumed to be invariant with position over any horizontal cross-section. This, plus the invariance of A with x, leads to the expectation that the current density should be independent of position in the cuvette.

For simplicity, the inner surfaces of the cuvette walls and the membranes are chosen as the boundaries of the system in MCE. The system typically contains a solution composed of some species that can permeate the membranes, and other species that cannot. Among the usual membrane-permeant species are a solvent such as water; small ions, such as K^+ and Cl^-; and a buffer, such as Tris, to control the pH. Among the usual membrane-impermeant (membrane-confined) species are macro-ions, such as proteins or polynucleotides. Buffer solutions are maintained above the top membrane and below the bottom membrane (Fig. 2). These buffer solutions are typically identical to each other, and contain all the solution components found in the system with the exception of the membrane-confined species. The solution composition outside the system is kept constant with time by constantly pumping fresh buffer solutions past the outside of the membranes. Electrodes located downstream from the membranes produce the electrical current through the cuvette. The products of electrochemical reactions at the electrodes are flushed to waste. The temperature of the system is held constant by a temperature-controlled, recirculating waterbath connected to water jackets that surround the cuvette [1]. At the start of a typical MCE experiment, the solution inside the system is in dialysis equilibrium with the surrounding buffer, and there are no concentration or temperature gradients inside the system.

The data obtained from the MCE instrument are light intensities. These intensities are obtained for fixed points along the x-axis of the system using

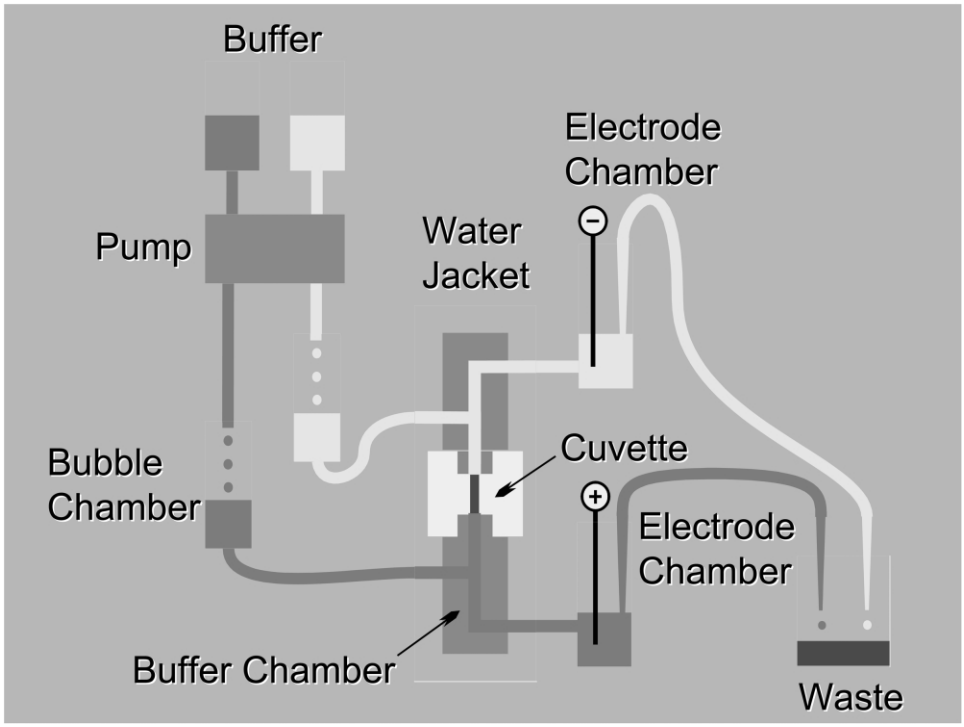

Fig. 2. Buffer flow through the MCE instrument.

a photodiode array imaging system (Fig. 3). Typically, data that are proportional to the concentration of membrane-confined species, and insensitive to the concentration of membrane-permeant species, are sought. To obtain such data, the intensities are converted to optical densities using a set of reference intensities obtained at a current sufficient to concentrate the membrane-confined species at the membranes, in general, or, preferably and more typically, at the lower membrane alone [1]. The wavelength of the light source is chosen so that, in most cases, the optical densities are only affected by the concentrations of the membrane-confined species. The current used to obtain a set of reference intensities is generally within the range of the currents used in moving boundary experiments, in which one or more fairly sharp concentration gradients (Fig. 4) move rapidly through the system [2]. Lower currents are used in steady-state experiments to eventually produce broad, time-invariant macro-ion concentration gradients (Fig. 5) in the system [2]. As will be shown in Section 3, different parameters relevant to charge are obtained from the different types of experiments. The apparent electrophoretic mobility, discussed in Section 2.3, is the natural parameter of charge for moving boundary experiments, and the reduced molecular charge, discussed in Section 2.4, is the natural parameter of charge for steady-state experiments.

1.2. The system

A system reaches equilibrium when its properties cease to change with time, and there are no flows of energy or matter through the system. If its properties are invariant with time, but there are flows of energy or matter through it, a system is described as being at steady state [3]. A flow of electrical current through the system in the MCE instrument results in a flow of mass (that of the membrane-permeant ions) into and out of the system. Furthermore, as an electrical current passes

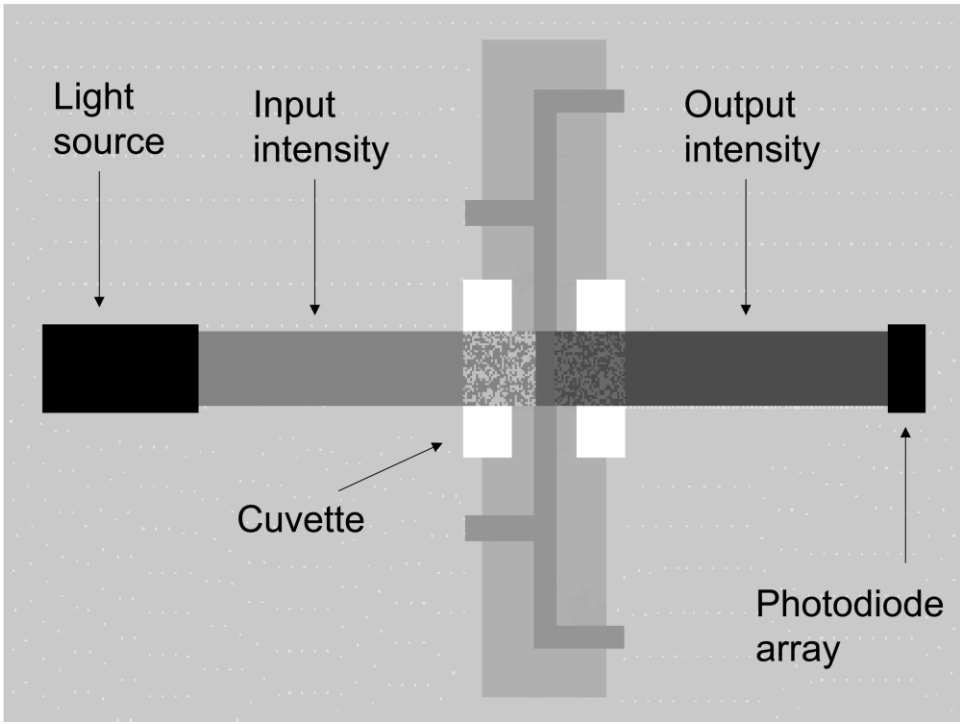

Fig. 3. Light intensity measurement in the MCE instrument.

through the system, power is dissipated, and in the process of maintaining a constant system temperature, heat flows from the system to the surroundings. (At the currents used in MCE experiments, the flow of heat is negligible [1,4,5].) Due to the current flowing through it, a system in the MCE instrument can never achieve equilibrium, but it can reach steady state. The appropriate thermodynamic description of such a system is one based on nonequilibrium thermodynamics, the theoretical basis of which was greatly advanced by Onsager [6,7].

The nonequilibrium thermodynamic description of MCE closely resembles that of analytical centrifugation. However, as systems ultimately reach steady state rather than equilibrium in MCE, there are complicating flow effects present in MCE at steady state that are absent in sedimentation at equilibrium. Calculating, on the basis of theory, the contribution of these flow effects to measurements of the sort made by MCE and other electrophoretic methods is beyond the scope of this paper. Approaches for such calculations [8–10] consider systems that are microscopic in scale, making them ill suited for describing systems macroscopically. In contrast, the nonequilibrium thermodynamic formalism is a purely macroscopic, phenomenological approach, making it well suited for describing transport processes throughout an entire system in the MCE instrument.

In 1989, Godfrey applied nonequilibrium thermodynamics to the problem of steady-state MCE in nonassociating systems [4]. More generally, nonequilibrium thermodynamics can describe an associating or nonassociating system during MCE, whether it is at steady state or not, provided that, as discussed in Section 2.2, certain conditions are met. A nonequilibrium thermodynamic description of the system forms the basis of a program that models transport processes in an entire system over an extended time, from arbitrary starting conditions to steady state. That program is imple-

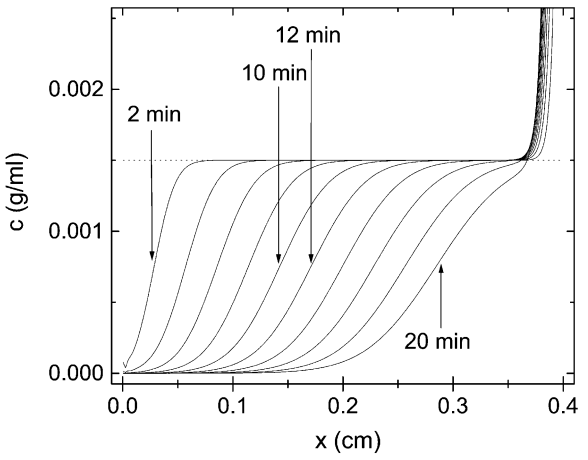

Fig. 4. Simulation of boundary velocity experiment, c_{mc} vs. x: (·····), at $t=0$; (——), from 2 to 20 min in 2 min steps. The model interacting system is $2A+B+X^{-1} \leftrightarrows A_2BX$, where A, B and A_2BX are membrane-confined species, while X^{-1} is a membrane-permeant ion. The reaction is divided into two steps: $2A+B \leftrightarrows A_2B$, for which the equilibrium constant is $K_1=2\times10^8$; and $A_2B+X^{-1} \leftrightarrows A_2BX$, for which the equilibrium constant is $K_2=300$. Thus, $K^*_2=K_2c_X$ describes the equilibrium for the apparent reaction, $A_2B \leftrightarrows A_2B^*$. The reaction kinetics are essentially instantaneous. An additional membrane-permeant ion, P^{+1}, is present. The parameters describing the various species are as follows: in g/mol, $M_A=6000$, $M_B=8000$, $M_X=35.453$, $M_P=39.0983$, $M_{A_2B}=20\,000$, $M_{A_2BX}=20\,035.453$; in proton equivalents, $z_A=+4$, $z_B=+8$, $z_X=-1$, $z_P=+1$, $z_{A_2B}=+16$, $z_{A_2BX}=+15$; in cm^2/s, $D_A=1.5\times10^{-6}$, $D_B=1.4\times10^{-6}$, $D_X=16.18\times10^{-6}$, $D_P=16.18\times10^{-6}$, $D_{A_2B}=1.0\times10^{-6}$, $D_{A_2BX}=1.1\times10^{-6}$; in cm^{-1}, $w_A=150$, $w_B=175$, $w_X=-190$, $w_P=190$, $w_{A_2B}=260$, $w_{A_2BX}=195$. No apparent nonideality was included in the model, so $w_j=w^0_j$, $D^*_j=D_j=k_BT/f_j$, and f_j is independent of concentration for each species. The concentrations in the plateau, in g/ml, were approximately $c_A=1.88073\times10^{-4}$, $c_B=8.40977\times10^{-5}$, $c_X=3.54530\times10^{-3}$, $c_P=3.90983\times10^{-3}$, $c_{A_2B}=5.95345\times10^{-4}$ and $c_{A_2BX}=6.33605\times10^{-4}$. Using the plateau concentrations of the membrane-permeant species, applying Eqs. (4), (19) and (21), and using an estimate of $\kappa=0.01186$ siemens/cm (obtained by treating the solution as if it were 0.1 M KCl at 293.15 K), yields $E=5$ V/cm. (This calculation of E neglects the contribution of membrane-confined species.) Using Eq. (68) to analyze the applicable boundaries, $u^*_w=4.70676\times10^{-5}\pm10^{-7}$ cm^2/V s is obtained, which is in agreement with the direct calculation of u^*_w from the plateau concentrations and the apparent electrophoretic mobilities, which were calculated as $u^*_j=(k_BT/f_jE)w_j$.

mented using finite element methods [11], and its essential features are described in Section 3.3. Systems in which membrane-confined species are involved in mass–action associations are discussed in Section 3.1.

2. Theory

2.1. Flux, current and electric field

The net transport of matter in the MCE system can be described in terms of the molar flux of each species in the system. The molar flux vector, $\vec{J}_j$, of species j is

$$\vec{J}_j=\frac{c_j\vec{v}_j}{M_j}, \tag{1}$$

where M_j is the molar mass, c_j is the mass concentration and $\vec{v}_j$ is the transport velocity vector of species j.

It is assumed that the electric fields present in the system are weak enough, and the thermal motion of each species is great enough, that, on average, all species are randomly oriented. As a

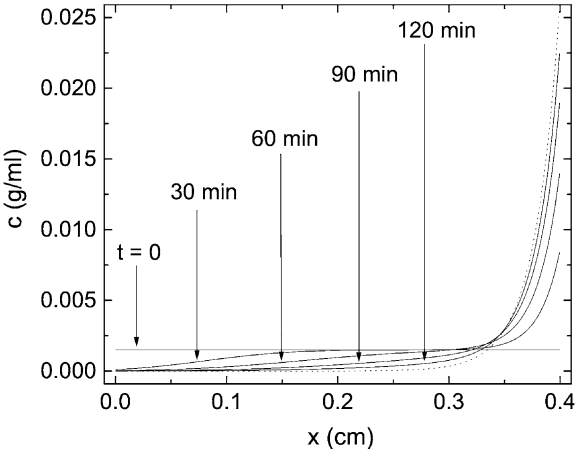

Fig. 5. Simulation of the approach to steady state, c_{mc} vs. x: (——), from 0 to 2 h in 30 min steps; (·····) at 36 h (steady state). The system is the same as that in Fig. 4, except that E, and thus each w_j, is divided by 5. Also, the plateau concentrations of Fig. 4 are the initial concentrations for this figure.

result, no net transport along the *y*- or *z*-axis is expected to result from forces directed along the *x*-axis. In the absence of turbulent flow, then, $\vec{J}_j$ and $\vec{v}_j$ have no component along the *y*- or *z*-axis of the system. In the normal operation of the MCE instrument, no turbulent flows are expected, so that only the *x*-components of these vectors need to be considered. Thus, $\vec{J}_j = \hat{e}_x (J_j)_x$, where $\hat{e}_x$ is the unit vector in the direction of the positive *x*-axis, and $(J_j)_x$ is the *x*-component of $\vec{J}_j$. This allows the vector notation to be dropped, so that $J_j = (J_j)_x$ can be used in place of $\vec{J}_j$. Similarly, $v_j = (v_j)_x$ can be used in place of $\vec{v}_j$.

Chemical potential gradients in any of the species, the electric potential gradient acting on the charge of any of the species, and solvent flux determine $\vec{J}_j$. Chemical potential gradients in charged species are constrained by the requirement of charge neutrality [12], which is described by

$$\sum_j z_j \frac{c_j}{M_j} = 0, \tag{2}$$

where z_j, the valence of species *j*, is a signed parameter. Charge neutrality holds from macroscopic volumes down to some minimum volume that includes some minimum number of charged particles. Such minimum volumes can become polarized by transport processes, however, giving rise to the *asymmetry effect*, discussed in Section 2.2.

In the MCE instrument, *A*, *i* and κ, the conductivity of the solution, are exploited to control $\vec{E}$. In general, $\vec{E} = -\varpi \vec{\nabla} \Psi$, where Ψ is the electric potential and ϖ is a conversion constant. The design of the MCE instrument, however, results in $\vec{\nabla}\Psi$ having no component along the *y*- or *z*-axis of the system. Consequently, $(\partial\Psi/\partial x)_{t,y,z} = \nabla\Psi_x$ and $E = E_x$ can be used in place of $\vec{\nabla}\Psi$ and $\vec{E}$, respectively. Thus, at any given time, *t*,

$$E = -\varpi \left(\frac{\partial \Psi}{\partial x} \right)_{t,y,z}. \tag{3}$$

Other than *A*, the most easily controlled variable in an MCE experiment is *i*, which is equal to the sum of the currents of all species. The current is really a vector, $\vec{i}$. In the MCE instrument, however, $\vec{i}$ has no component along the *y*- or *z*-axis of the system, so that $i = i_x$ can be used in place of the vector. During an experiment, *i* is typically held constant with time. Due to conservation of current, and because there are no sources or sinks of current within the system, $\vec{\nabla} \cdot \vec{i} = 0$ everywhere at all times in a typical experiment. Provided that, as should be the case in the MCE instrument, each $\vec{J}_j$ is everywhere perpendicular to the horizontal cross-section,

$$i = \frac{AF}{\Theta} \sum_j z_j J_j, \tag{4}$$

where *F* is the Faraday, and Θ is a conversion constant. The sum is taken over all species. The current carried by species *j* is $i_j = (AF/\Theta) z_j J_j$. As each product, $z_j J_j$, is signed, each i_j is a signed parameter. In the absence of overwhelming, opposing chemical potential gradients, species that differ with respect to the sign of z_j will differ with respect to the sign of J_j. Consequently, when no such chemical potential gradients are present, the sign of i_j is the same for all charged species.

A particle of species *j* has a charge, Q_j, equal to $z_j e$, where *e* is the elementary charge. As $F = N_A e$, where N_A is Avogadro's number, Eq. (4) could be written in terms of Q_j rather than z_j. Thus, Q_j can be defined as the charge that a particle would transfer if it were functioning as a current carrier. This definition of Q_j is in accord with the particle being defined as including any material that remains bound to it despite the forces present in the system. The particle may include noncovalently bound material, such as a layer of water [13]. Any bound material that is charged will contribute to Q_j [13]. The boundary between material that is bound to a particle and material that is free to move relative to the particle can be defined as the surface of shear [13]. Thus, Q_j can be considered to be the net charge within the surface of shear of a particle of species *j* [13].

2.2. Nonequilibrium thermodynamic description of MCE

The formalism of nonequilibrium thermodynamics applies when fluxes can be expressed as linear functions of the forces present [14,15]. The forces that give rise to fluxes in the MCE instrument are assumed to be small enough such that this formalism is applicable. Given this assumption, and denoting the solvent as species n, the equation for J_j^s, the molar flux of species j in the solvent frame of reference, can be written as

$$J_j^s = \sum_{k=1}^{n-1} L_{j,k} X_k = J_j - \frac{c_j}{M_j}\frac{M_n}{c_n} J_n = J_j - \frac{c_j}{M_j} v_n, \qquad (5)$$

where $L_{j,k}$ is the phenomenological coefficient linking the transport of species j to X_k [15], and X_k is the conjugate molar force of J_k [16]. (A conjugate force is assigned to each flux through the system [16].) The sum is taken over all linearly independent forces [16]. Each conjugate molar force is really a vector, $\vec{X}_k = -\vec{\nabla} U_k$, where U_k is the total molar potential of solute species k. In the MCE instrument, however, $\vec{X}_k$ has no component along the y- or z-axis of the system, so that $X_k = (X_k)_x$ can be used in place of the vector. There is a total of n conjugate molar forces, but X_n, that of the solvent, has been expressed in terms of the others in Eq. (5). The bulk fluid velocity is described by v_n, the nonvector representation of the velocity of the solvent flow through the system. In the absence of any forces other than that associated with solvent flux, $X_{k\neq n} = 0$, and $v_j = v_n$, from which it follows, in such cases, that $J_j = (c_j/M_j)v_n$.

The phenomenological coefficients are functions of system properties, such as temperature, pressure and the concentrations of solutes, but are independent of the magnitudes of any forces present, provided that those forces are sufficiently small [15]. The phenomenological coefficients pertaining to coupled fluxes are the $L_{j,k}$ terms for which $k \neq j$. By a statistical mechanical treatment of microscopically reversible processes, which assumes the absence of magnetic fields or Coriolis forces in the system, Onsager showed that these cross terms are symmetric, so that the reciprocal relations are $L_{j,k} = L_{k,j}$ for all j and k [6,7,14]. Denoting any magnetic fields or Coriolis forces by G, the most general expression of the reciprocal relations is $L_{j,k}(G) = L_{k,j}(-G)$, where $L_{j,k}(G)$ is $L_{j,k}$ in the presence of G, and $L_{k,j}(-G)$ is $L_{k,j}$ in the presence of $-G$ [7]. In the MCE instrument, G is negligible. A large body of experimental evidence suggests that the applicability of Onsager's reciprocal relations is broader than that might be expected, given that their theoretical basis deals only with processes that are close to equilibrium [16].

The dissipation function,

$$\begin{aligned}\Phi &= \sum_{j=1}^{n} J_j X_j + \sum_{g=1}^{r} J_g^r A_g = \sum_{j=1}^{n-1} J_j X_j + J_n X_n + \sum_{g=1}^{r} J_g^r A_g \\ &= \sum_{j=1}^{n-1} J_j^s X_j + \sum_{g=1}^{r} J_g^r A_g, \end{aligned} \qquad (6)$$

measures the local rate of free energy dissipation per unit volume [16]. This equation is used to determine the proper fluxes and forces to include in Eq. (5). In the MCE instrument, all significant fluxes are either those of particles or those of chemical reactions. The summation indexed by j gives the contribution of particle fluxes to Φ. The summation indexed by g gives the contribution of independent chemical reaction fluxes to Φ. Each term in that summation is the product of the molar reaction flux, J_g^r, of reaction g, times the molar affinity, A_g, of reaction g.

The total number of all possible fluxes is $n+r$, where n is the number of possible molar fluxes, and $r \leqslant n-1$ is the number of independent chemical reaction fluxes [14]. The total number of all possible forces is also $n+r$. As J_g^r and A_g are scalars, their tensorial order is 0. As J_j and X_j are really vectors, their tensorial order is 1. Given that reaction fluxes are not expected to produce molar fluxes when the system is isotropic, it is assumed that the Curie–Prigogine principle [14,16] applies in the MCE system, with some possible exceptions that are discussed in Section 3.1.1. Accordingly, there is assumed to be no coupling between fluxes and forces of different tensorial order, with the

result that no phenomenological coefficients link the molar affinities of any chemical reactions to the molar flux of species j in Eq. (5).

Without the r molar affinities to contend with in Eq. (5), there remain n conjugate molar forces, one of which may be expressed in terms of the others, so that only $n-1$ linearly independent forces appear in Eq. (5). The Gibbs–Duhem relation is used to express X_n in terms of the other conjugate molar forces, each of which is associated with one solute species.

It is assumed that the system develops gradients in the electric potential and chemical potentials only, and that these gradients are non-zero only in the direction of the x-axis. Thus, $\vec{X}_k = -Fz_k\vec{\nabla}\Psi - \vec{\nabla}\mu_k$, where μ_k is the chemical potential of species k. To write X_k, $(\partial\Psi/\partial x)_{t,y,z}$ can be used in place of $\vec{\nabla}\Psi$, and as $\vec{\nabla}\mu_k$ has no component along the y- or z-axis, $(\partial\mu_k/\partial x)_{t,y,z}$ can be used in its place, where $(\partial\mu_k/\partial x)_{t,y,z} = (\nabla\mu_k)_x$. Furthermore, as system properties do not vary with y or z in the system, y and z do not need to be held constant in the derivatives, so that $(\partial\mu_k/\partial x)_{t,y,z} = (\partial\mu_k/\partial x)_t$ and $(\partial\Psi/\partial x)_{t,y,z} = (\partial\Psi/\partial x)_t$. Thus, $X_k = -Fz_k(\partial\Psi/\partial x)_t - (\partial\mu_k/\partial x)_t$.

Eqs. (1) and (5) can be combined to obtain an expression for $L_{j,j}$. If $X_{k\neq j}=0$, $J_j = L_{j,j}X_j = (c_j/M_j)v_j$. As $X_j = N_A f_j v_j$ in the absence of the transport of other species,

$$L_{j,j} = \frac{c_j}{M_j f_j N_A}. \tag{7}$$

The hydrodynamic frictional coefficient, f_j, is that which applies to species j in the absence of the transport of other species [15]. Nevertheless, f_j is dependent on solute concentrations and other system properties [15], and can thus be viewed as affected by hydrodynamic nonideality. Even when the solvent (typically water) is not treated explicitly as a species, it is treated implicitly through the hydrodynamic frictional coefficients of the solutes.

Solving Eq. (5) for J_j in terms of the sum over k and the solvent flux, separating the terms for species j from the rest of the terms in the sum over k, using Eq. (7) to substitute for $L_{j,j}$, and substituting $-Fz_j(\partial\Psi/\partial x)_t - (\partial\mu_j/\partial x)_t$ for X_j, gives the molar flux of species j as

$$J_j = -\frac{c_j}{M_j}\left(\frac{Fz_j}{N_A f_j}\left(\frac{\partial\Psi}{\partial x}\right)_t + \frac{1}{N_A f_j}\left(\frac{\partial\mu_j}{\partial x}\right)_t\right) + \sum_{k\neq j} L_{j,k}X_k + \frac{c_j}{M_j}v_n. \tag{8}$$

The sum excludes species j and n, but includes all other species.

The chemical potential of species j is a function not only of c_j, but also of the absolute temperature, T, the pressure, P, and the concentrations of all other solute species. The gradient in the chemical potential of species j can be written, then, as

$$\left(\frac{\partial\mu_j}{\partial x}\right)_t = \left(\frac{\partial\mu_j}{\partial T}\right)_{t,P,c}\left(\frac{\partial T}{\partial x}\right)_t + \left(\frac{\partial\mu_j}{\partial P}\right)_{t,T,c}\left(\frac{\partial P}{\partial x}\right)_t + \sum_{q=1}^{n-1}\left(\frac{\partial\mu_j}{\partial c_q}\right)_{t,T,P,c_{i\neq q}}\left(\frac{\partial c_q}{\partial x}\right)_t, \tag{9}$$

where the subscript c means that the concentrations of all species are held constant. As $(\partial T/\partial x)_t = 0$, $(\partial\mu_j/\partial T)_{t,P,c}(\partial T/\partial x)_t = 0$.

For the second term on the right-hand side of Eq. (9), a standard thermodynamic relation yields $(\partial\mu_j/\partial P)_{t,T,c} = (M_j\bar{v}_j)$, where $\bar{v}_j$ is the partial specific volume of the system (the solution) with respect to species j. If the solution can be considered an incompressible fluid, and any fluid flow through the system can be treated as steady and nonviscous, then the flow is described by Bernoulli's equation, which can be written as $P = P_0 + \rho(g(x-x_0) + v_n^2/2)$, where g is the gravitational acceleration, and P_0 is the reference pressure at the reference position x_0 when $v_n = 0$. (The choice of x_0 is arbitrary, and it is usually assigned a value of zero. The position of the upper membrane is the uppermost possible position of x_0.) As J_n and c_n are nearly invariant with x, and A is invariant with x, $(\partial v_n/\partial x)_t$ is either zero or negligible throughout the system. Furthermore, as ρ is dominated by the solvent density, $(\partial\rho/\partial x)_t$ is expected to be negligible throughout the system.

Finally, due to the short length of the system, $(\partial g/\partial x)_t$ is essentially zero throughout the system. To a close approximation, then, $(\partial P/\partial x)_t = \rho g$, so that $(\partial \mu_j/\partial P)_{t,T,c}(\partial P/\partial x)_t = M_j \bar{\upsilon}_j \rho g$. While this may appear to be significant, a comparison of this term with its counterpart in sedimentation shows that, due to the small change in height from one end of the cuvette to the other, $M_j \bar{\upsilon}_j \rho g$ is too small to have a detectable effect on any concentration gradient in species j in the MCE system unless $M_j \bar{\upsilon}_j \rho$ exceeds approximately 10^7 g/mol. (The typical cuvette height is 0.4 cm.) As a result, $(\partial \mu_j/\partial P)_{t,T,c}(\partial P/\partial x)_t$ generally makes no discernable contribution to $(\partial \mu_j/\partial x)_{t,y,z}$, and is treated as equal to zero.

The third term on the right-hand side of Eq. (9) is the sole, significant contributor to $(\partial \mu_j/\partial x)_t$. This term can be made more tractable by representing the activity of species j as $c_j \gamma_j$, where γ_j, the activity coefficient of species j, is a function of T, P and the concentrations of all of the solutes [17]. In the limit as the total concentration of all the solutes approaches zero, and T and P approach their standard state values, γ_j approaches 1 [17]. As $\mu_j = \mu_j^0 + RT \ln c_j \gamma_j$, where R is the ideal gas constant, and μ_j^0, the standard state chemical potential of species j, is a constant, $(\partial \mu_j/\partial c_q)_{t,T,P,c_{i\neq q}} = RT(\partial \ln c_j \gamma_j/\partial c_q)_{t,T,P,c_{i\neq q}}$. With $(\partial \ln c_j/\partial c_q)_{t,T,P,c_{i\neq q}} = 1/c_j$ for $q=j$, and $(\partial \ln c_j/\partial c_q)_{t,T,P,c_{i\neq q}} = 0$ for all $q \neq j$,

$$\sum_{q=1}^{n-1}\left(\frac{\partial \mu_j}{\partial c_q}\right)_{t,T,P,c_{i\neq q}}\left(\frac{\partial c_q}{\partial x}\right)_t = RT\left(\frac{\partial \ln c_j}{\partial x}\right)_t\left(1 + c_j\sum_{q=1}^{n-1}\left(\frac{\partial \ln \gamma_j}{\partial c_q}\right)_{t,T,P,c_{i\neq q}}\left(\frac{\partial c_q}{\partial c_j}\right)_t\right). \quad (10)$$

Analogous expressions can be written for each $(\partial \mu_k/\partial x)_t$, where $k \neq j$.

The diffusion coefficient, $D_{j,k}$, is the coefficient of $(1/M_k)(\partial c_k/\partial x)_t$ that makes $D_{j,k}(1/M_k)(\partial c_k/\partial x)_t$ equal to $L_{j,k}(\partial \mu_k/\partial x)_t$ [12,16]. Thus,

$$D_{j,k} = \frac{L_{j,k}RTM_k}{c_k}\left(1 + c_k\sum_{q=1}^{n-1}\left(\frac{\partial \ln \gamma_k}{\partial c_q}\right)_{t,T,P,c_{k\neq q}}\left(\frac{\partial c_q}{\partial c_k}\right)_t\right) \equiv \frac{L_{j,k}RTM_k}{c_k}\Xi_{j,k}, \quad (11)$$

where $\Xi_{j,k}$ describes the contribution that species k makes to the thermodynamic nonideality of the system [18]. For $k=j$, Eqs. (7) and (11) give $D_{j,j} = (k_B T/f_j)\Xi_{j,j}$, where k_B is the Boltzmann constant. Thus, $D_{j,j}$ is directly proportional to $\Xi_{j,j}$. In addition, $D_{j,j}$ is inversely proportional to the effect of hydrodynamic nonideality on f_j [15].

Defining the electrophoretic mobility coefficient, $u_{j,k}$, as

$$u_{j,k} = \frac{L_{j,k}Fz_kM_k}{\varpi c_k}, \quad (12)$$

gives $u_{j,j} = Fz_j/N_A f_j \varpi$. Replacing all remaining X_k with $-Fz_k(\partial \Psi/\partial x)_t - (\partial \mu_k/\partial x)_t$, using Eq. (3) to replace $(\partial \Psi/\partial x)_t$ with $-E/\varpi$, using Eq. (11) to substitute for all $L_{j,k}(\partial \mu_k/\partial x)_t$, employing Eq. (12), and rearranging, Eq. (8) becomes

$$J_j = \frac{c_j}{M_j}\left(u_{j,j}E - D_{j,j}\left(\frac{\partial \ln c_j}{\partial x}\right)_t + \upsilon_n\right) + \sum_{k\neq j}\frac{c_k}{M_k} \times\left(u_{j,k}E - D_{j,k}\left(\frac{\partial \ln c_k}{\partial x}\right)_t\right). \quad (13)$$

2.3. The diminished valence and apparent electrophoretic mobility

Defining the diminished valence [4] of species j as

$$z_j^* = z_j + \frac{\varpi f_j N_A}{EF}\upsilon_n + \frac{M_j f_j N_A}{c_j}\sum_{k\neq j}L_{j,k}z_k, \quad (14)$$

and defining the apparent diffusion coefficient of species j as

$$D_j^* = D_{j,j} + M_j\sum_{k\neq j}\frac{D_{j,k}}{M_k}\left(\frac{\partial c_k}{\partial c_j}\right)_t, \quad (15)$$

results in

$$J_j = \frac{c_j}{M_j}\left(\frac{Fz_j^* E}{N_A f_j \varpi} - D_j^*\left(\frac{\partial \ln c_j}{\partial x}\right)_t\right). \quad (16)$$

As can be seen from substitution of D^*_j with its full expression given by Eq. (15), $D^*_j(\partial \ln c_j/\partial x)_t$ may not be zero when $(\partial \ln c_j/\partial x)_t$ is zero if concentration gradients in species other than j are present. Similarly, as shown by substituting z^*_j with its full expression given by Eq. (14), $Fz^*_jE/N_A f_j \varpi$ will be zero when E is zero only if, in the limit as E approaches zero, v_n approaches zero. The latter substitution also shows that, due to the third term on the right-hand side of Eq. (14), for $|E|>0$, z^*_j need not be zero even when z_j and v_n are zero.

The diminished valence might be viewed as something akin to the buoyant molar mass in analytical centrifugation. The buoyant molar mass is a natural variable obtained from the analysis of equilibrium sedimentation data, and is strongly dependent on the properties of the system, in that it represents a molecular parameter, M_j, multiplied by a system factor, $(1-\bar{v}_j\rho)$. Similarly, z^*_j is not strictly a molecular parameter, as it is strongly influenced by various system properties. Additionally, z^*_j is strongly influenced by fluxes through the system.

The expectation that $|z^*_j|<|z_j|$, at least in the case of $v_n=0$, gives z^*_j its name. Two effects tend to make $|z^*_j|$ less than $|z_j|$. One, variously referred to as the *electrophoretic effect*, *electrophoretic friction*, or *coupled ion flows*, results from the fluxes of species other than j [13,15,19]. In particular, momentum transfer to the solvent from counterion fluxes, which are typically opposite in direction from the central ion flux, are expected to produce solvent flows opposing the electrophoretic transport of the central ion. (The central ion is taken here to be a particle of species j, and a counterion is thus any species k for which $z_k/|z_k| = -z_j/|z_j|$.) The other effect, typically called the *asymmetry effect* or the *ion relaxation effect*, results from the accumulation of an excess of counterions in the wake of the central ion [12,13,19]. This asymmetric counterion distribution about the central ion results in a local polarization, which creates an induced electric field. Due to the orientation of the polarization, the induced electric field is opposite in direction to E, and thus opposes the electrophoretic transport of the central ion.

The first term on the right-hand side of Eq. (14) is the valence, z_j, and as such, is an electrostatic term. The second term on the right-hand side of Eq. (14) is a hydrodynamic term consisting of the contribution of solvent flow to z^*_j. The sign of this hydrodynamic term is determined by v_n/E. The last term on the right-hand side of Eq. (14) is an electrohydrodynamic term that sums the contributions of electrophoretic fluxes in species other than j or the solvent to z^*_j. This electrohydrodynamic term accounts for the electrophoretic and asymmetry effects, and is therefore expected to be opposite in sign from, but lower in magnitude than z_j.

The apparent electrophoretic mobility, u^*_j, is equal to $Fz^*_j/N_A f_j \varpi$, and thus has the same sign as z^*_j. Replacing $Fz^*_j/N_A f_j \varpi$ with u^*_j in Eq. (16) gives

$$J_j = \frac{c_j}{M_j}\left(u_j^* E - D_j^*\left(\frac{\partial \ln c_j}{\partial x}\right)_t\right). \quad (17)$$

This equation shows that the molar flux of species j, in the absence of concentration gradients, is equal to $(c_j/M_j)u^*_jE$.

Using Eq. (17) to substitute for J_j in Eq. (4), and solving the resulting equation for E gives

$$E = \frac{i + \dfrac{AF}{\Theta}\displaystyle\sum_j \frac{z_j D_j^*}{M_j}\left(\frac{\partial c_j}{\partial x}\right)_t}{\dfrac{AF}{\Theta}\displaystyle\sum_j \frac{z_j c_j u_j^*}{M_j}}, \quad (18)$$

provided that E is everywhere perpendicular to A, and does not vary across A. The denominator is equal to $A\kappa$ [19], so that Eq. (18) can be written as

$$E = \frac{i}{A\kappa} + \frac{\dfrac{F}{\Theta}\displaystyle\sum_j \frac{z_j D_j^*}{M_j}\left(\frac{\partial c_j}{\partial x}\right)_t}{\kappa}. \quad (19)$$

This equation holds even if κ varies with x.

As A is fixed and i can be held constant, E can be controlled to some extent by controlling κ. As κ is a function of Γ, κ can be controlled to the extent that Γ can be controlled. In general, κ increases as Γ increases, but κ is not linearly proportional to Γ [19]. For example, in a sufficiently dilute, aqueous solution of a uni-univalent salt at a fixed temperature, κ can be estimated by $\kappa = q_1\Gamma - q_2\Gamma^{3/2}$, which is a rearrangement of the Onsager equation. The coefficients q_1 and q_2 can be calculated [19], or can be obtained by fitting experimentally determined conductivity data. As $d\kappa/d\Gamma = q_1 - 3q_2(\Gamma^{1/2})/2$, $d\kappa/d\Gamma$ is diminished from q_1 by $3q_2(\Gamma^{1/2})/2$. The source of this diminution is thought to be an increase in flow effects, and thus a decrease in the apparent electrophoretic mobility of each ion, as Γ increases.

In some instances, Γ may vary with x and t, resulting in variations of κ and E with x and t. Neglecting the second term on the right-hand side of Eq. (19), this can be estimated by $E = E_0\kappa_0/\kappa$, where κ is a function of x and t, such that $\kappa_0 = \kappa$ at x_0 and some reference time. For steady-state experiments, infinite time, t_∞, is a practical reference time, in which case, at $x = x_0$ and $t = t_\infty$, $E = E_0$. (When the system is at steady state, the time elapsed since the start of the experiment can be considered to be t_∞.) For moving boundary experiments, the time at which current flow is started, $t_0 = 0$, is the most practical reference time, in which case, at $x = x_0$ and $t = t_0$, $E = E_0$. In either case, $E_0 \approx i/A\kappa_0$. In the simplest systems, Γ is not expected to vary significantly with x or t, with the result that, for all practical purposes, $\kappa \approx \kappa_0$, and $E \approx E_0$, at all x and all t.

Along gradients in Γ, liquid junction (diffusion) potentials, can arise from local polarization [19]. The development of diffusion potentials would cause E to vary with x and t. In the case of a uni-univalent electrolyte for which the cation and anion have closely matched transference numbers, υ of the cation is approximately equal to $-\upsilon$ of the anion. (Representing the transference number of current-carrying species j by τ_j, $\upsilon_j = i\tau_j M_j \Theta / FAz_j c_j$ [19].) For such an electrolyte, little, if any, polarization is expected to occur in the system [19]. Also, for any electrolyte, such polarization is minimized if the system is designed so that gradients in the mean ionic activity (the geometric mean of the individual ion activities) are negligible [19].

2.4. Reduced molecular charge

In analogy with the reduced molecular mass of equilibrium sedimentation [20], a reduced molecular charge may be defined for electrophoresis. Just as the reduced molecular mass is a natural parameter associated with the analysis of equilibrium sedimentation data, the reduced molecular charge is a natural parameter associated with the analysis of steady-state MCE data. The reduced molecular mass, however, is a flow-independent parameter. In contrast, the reduced molecular charge is a flow-dependent parameter. Nevertheless, a flow-independent term can be identified within the reduced molecular charge.

The reduced molecular charge, w_j, is defined by $w_j = FEz^*_j/RT\varpi$. The sign of w_j is determined by Ez^*_j. The three parts of w_j are equal to $FE/RT\varpi$ times the three parts of z^*_j, giving

$$w_j = \frac{FE}{RT\varpi} z_j + \frac{f_j}{k_B T} \upsilon_n + \frac{M_j f_j FE}{c_j k_B T \varpi} \sum_{k \neq j} L_{j,k} z_k. \qquad (20)$$

Each term on the right-hand side is analogous to its counterpart in Eq. (14). Thus, the first term on the right-hand side, $FEz_j/RT\varpi$, is an electrostatic term equal to the flow-independent reduced molecular charge, W_j, the sign of which is determined by Ez_j. The second term on the right-hand side is a hydrodynamic term resulting from the contribution of solvent flow to w_j. The sign of the hydrodynamic term is determined by υ_n. The last term on the right-hand side is an electrohydrodynamic term that is expected to be opposite in sign from, but lower in magnitude than W_j. Choosing an experimental design that ensures no significant variation in Γ with x can minimize the variation of z^*_j, and thus w_j, with x.

Solvent flux is expected to occur in certain systems, such as one in which the membrane-permeant electrolyte is uni-univalent and consists of a cation and anion with matched transference numbers but different degrees of solvation [19]. Charge-neutral, membrane-confined species can be

used to measure bulk fluid velocities of any significance [21]. Once measured, v_n can be incorporated into the data analysis [21]. Experiments have shown that for KCl, at the magnitudes of the currents used in MCE, v_n is negligible in the absence of an externally applied pressure gradient across the system [21]. (KCl is a uni-univalent, current-carrying electrolyte with relatively closely matched transference numbers.) Thus, with a judicious choice of electrolyte, v_n can be rendered negligible, in which case, typically, z^*_j will have the same sign as z_j, and w_j will have the same sign as W_j.

Using Eq. (20), Eq. (16) can be written as

$$J_j = \frac{c_j}{M_j}\left(\frac{k_B T}{f_j} w_j - D_j^*\left(\frac{\partial \ln c_j}{\partial x}\right)_t\right). \quad (21)$$

Comparison with Eq. (17) shows that $(k_B T/f_j)w_j = u^*_j E$. The advantage of w_j over u^*_j is that f_j must be known to calculate z^*_j from u^*_j, while z^*_j can be obtained from w_j even if f_j is unknown.

The mass flux of species j is

$$I_j = c_j\left(\frac{k_B T}{f_j} w_j - D_j^*\left(\frac{\partial \ln c_j}{\partial x}\right)_t\right). \quad (22)$$

Thus, $I_j = M_j J_j$. Each mass flux is really a vector, $\vec{I}_j$. Like $\vec{J}_j$, however, $\vec{I}_j$ has no component along the y- or z-axis of the system, so that $I_j = (I_j)_x$ can be used in place of the vector. Eq. (22) is used to write the mass conservation law [14] (continuity equation) that is needed to develop the equations for the finite element simulation program described in Section 3.3.

2.5. Multiple membrane-confined species

The presence of multiple membrane-confined species may result from mass–action association, in which different membrane-confined species constitute a single component. Alternatively, different membrane-confined species may be separate components. In either case, the different membrane-confined species will tend to differ with respect to w, f or D^*.

The total molar flux, J, is the sum of all the individual molar fluxes, and the total mass flux, I, is equal to the sum of all the individual mass fluxes. These total fluxes include the fluxes of both membrane-confined and membrane-permeant species. It is often useful to sum only the fluxes of membrane-permeant species, because membrane-permeant species usually do not contribute to the data acquired in MCE experiments. Nevertheless, the parameter w of each membrane-confined species must implicitly incorporate the effects of the fluxes of membrane-permeant species on that parameter. Furthermore, the parameters w, f and D^* of each membrane-confined species must implicitly incorporate the effects of the concentrations of membrane-permeant species on those parameters.

Using Eq. (21), and indexing all membrane-permeant species by h, J_{mc}, the total molar flux of membrane-confined species, is

$$J_{mc} = \sum_{j \neq h} \frac{c_j}{M_j}\left(\frac{k_B T}{f_j} w_j - D_j^*\left(\frac{\partial \ln c_j}{\partial x}\right)_t\right). \quad (23)$$

The summation is from $j=1$ to $j=m$, where m is the number of membrane-confined species. Consequently, $h > m$ for all h.

Because mass, rather than the number of moles, is conserved in all interacting systems, it is more convenient to work with mass flux, rather than molar flux. Using Eq. (22), I_{mc}, the total mass flux of membrane-confined species, is

$$I_{mc} = \sum_{j \neq h} c_j\left(\frac{k_B T}{f_j} w_j - D_j^*\left(\frac{\partial \ln c_j}{\partial x}\right)_t\right). \quad (24)$$

The advantage of working with I_{mc} instead of J_{mc} is most apparent when considering systems at steady state. This is because mass conservation ensures that $I_{mc} = 0$ at all x in all systems at steady state. In contrast, molarity may not be conserved in reacting systems, with the result that $J_{mc} = 0$ at all x only in special cases of systems at steady state.

3. Applications

3.1. Steady-state MCE

3.1.1. Characteristics of the system at steady state

At steady state, though mass may flow through the system, concentration cannot change with time anywhere in the system. As a result, for each membrane-permeant species, $dI_h/dx=0$ at all x at steady state. Eqs. (21) and (22) show that if $dI_h/dx=0$, $dJ_h/dx=0$, and Eq. (4) shows that if $dJ_h/dx=0$, $di_h/dx=0$. Consequently, $dJ_h/dx=0$ and $di_h/dx=0$ everywhere for each membrane-permeant species at steady state.

If, in addition to I_{mc} being zero, $J_{mc}=0$ at all x at steady state, then each I_j, J_j, v_j and i_j will be zero everywhere. If membrane-confined species self-associate, or associate with other species, however, J_{mc} may not be zero everywhere at steady state, with the result that individual values of I_j, J_j, v_j and i_j may be non-zero and may vary with x. Nevertheless, the net contribution of J_{mc} to i must be zero everywhere at steady state. Otherwise, with current conservation making i invariant with x, the net contribution of the molar flux of the membrane-permeant species to i would have to vary with x at steady state, which is not possible, given that di_h/dx must be zero everywhere for each membrane-permeant species. The only possible exception would be if $J_{mc}\neq 0$, but $dJ_{mc}/dx=0$, at steady state, which would permit each di_h/dx to be zero throughout the system, while allowing some of the current to be carried by membrane-confined species. As current conservation would require i_h in the system to be different from i_h outside the system in this case, making di_h/dx non-zero at the boundaries, it is unlikely that a stable, steady state condition of this sort could be established.

Suppose, for example, that in the chemical reaction $\alpha A+\beta B \leftrightarrows A_\alpha B_\beta$, two different membrane-confined species, A and B, associate to form the membrane-confined species $A_\alpha B_\beta$. Unless the reduced molecular charge scales with stoichiometry, which in this example would mean that $\alpha w_A+\beta w_B=w_{A\alpha B\beta}$, J_{mc} will not be zero at all x at steady state. Nevertheless, valence must scale with stoichiometry, which in this same example means that $\alpha z_A+\beta z_B=z_{A\alpha B\beta}$. Applying Eq. (4) to this example, conservation of current will require that $(z_A J_A+z_B J_B+z_{A\alpha B\beta}J_{A\alpha B\beta})AF/\Theta=0$ at all x at steady state. This does not necessarily mean that $J_A+J_B+J_{A\alpha B\beta}=0$ at all x at steady state. It does mean that, for some systems, some or all molar fluxes of membrane-confined species might differ from what they would be if unconstrained by the requirement of current conservation. Such adjustments of the system could be considered an electrohydrodynamic imposition of nonideality, and might alter chemical equilibria, in addition to affecting the molar fluxes of membrane-confined species.

If membrane-confined species associate with membrane-permeant species, current conservation again requires that the net contribution of the molar flux of the membrane-confined species to i will be zero everywhere at steady state. Modifying the previous example, suppose the chemical reaction is $\alpha A+\beta B+\chi X^{\pm\zeta} \leftrightarrows A_\alpha B_\beta X_\chi$, where A, B and $A_\alpha B_\beta X_\chi$ are membrane-confined species, while X is a membrane-permeant ion of valence $z_X=\pm\zeta$. Conservation of current will require that, at steady state, $(z_A J_A+z_B J_B+z_X J_X+z_{A\alpha B\beta X_\chi}J_{A\alpha B\beta X_\chi})AF/\Theta=z_X J_X AF/\Theta$ at all x. Again, for some systems, chemical equilibria and the molar fluxes of membrane-confined species might be affected by the requirement of current conservation. Put another way, the constraint of current conservation will result in molar fluxes and reaction fluxes becoming coupled in a steady-state system unless the requirement that the net contribution of J_{mc} to i be zero everywhere can be met in the absence of such coupling.

For neutral, membrane-permeant species, such as the typical solvent, H_2O, $dI_h/dx=0$ at all x at steady state. Whether or not I_h is zero at steady state depends on the nature of the system. If there is solvent flux through the system, the mass flux of the solvent will be non-zero and invariant with x at steady state.

In addition to current conservation, there is the constraint of charge neutrality throughout all MCE systems. To maintain charge neutrality, with charged, membrane-confined species present in the system, the molar concentration of membrane-permeant cations will generally differ from that of

membrane-permeant anions. Also, the presence of concentration gradients in charged, membrane-confined species, together with the requirement of charge neutrality, will cause concentration gradients in membrane-permeant ions. Nevertheless, the current, and thus the molar flux, of each membrane-permeant ion will be invariant with x at steady state. To maintain both constant molar flux and charge neutrality, the velocities of membrane-permeant ions must vary with x at steady state if concentration gradients exist in charged, membrane-confined species.

Applying Eq. (24) to the membrane-confined species at steady state, for which $I_{mc}=0$, and dividing both sides of the equation by k_BT, results in

$$\sum_{j\neq h}\frac{c_j}{f_j}\left(w_j-\frac{f_jD_j^*}{k_BT}\frac{\mathrm{d}\ln c_j}{\mathrm{d}x}\right)=0, \tag{25}$$

where an ordinary derivative has replaced the partial derivative because the gradient in the logarithm of each c_j is time-independent at steady state. The apparent thermodynamic nonideality is given by $f_jD^*{}_j/k_BT$.

3.1.2. Apparent nonideality at steady state

Carefully chosen assumptions about nonideality have yielded useful equations for a number of methods, including sedimentation [20,22] and MCE [4]. Two assumptions regarding nonideal behavior at steady state are made here to obtain, at least for some types of systems, relatively simple, analytical solutions of Eq. (25). First, the apparent thermodynamic nonideality is assumed to be adequately described by a virial expansion of $f_jD^*{}_j/k_BT$,

$$\frac{f_jD_j^*}{k_BT}=1+M_jc_j\sum_{k=1}^{\infty}\frac{k+1}{k}B_k\frac{\mathrm{d}c_{mc}^k}{\mathrm{d}c_j}, \tag{26}$$

where c_{mc}, the total concentration of membrane-confined species, is

$$c_{mc}=\sum_{j\neq h}c_j, \tag{27}$$

and where B_1 is the second virial coefficient of the apparent thermodynamic nonideality of the system, B_2 is the third virial coefficient, and so on. Second, the effects of electrohydrodynamic nonideality on w_j are assumed to be adequately described by a virial expansion of w_j,

$$w_j=w_j^0+M_j\sum_{k=1}^{\infty}\frac{k+1}{k}b_k\frac{\mathrm{d}c_{mc}^k}{\mathrm{d}x}. \tag{28}$$

where b_1 is the second virial coefficient of the electrohydrodynamic nonideality of the system, b_2 is the third virial coefficient, and so on.

Substitution of Eqs. (26) and (28) into Eq. (25) results in

$$\sum_{j\neq h}\frac{c_j}{f_j}\left(w_j^0-\Xi_j^*\frac{\mathrm{d}\ln c_j}{\mathrm{d}x}\right)=0. \tag{29}$$

where

$$\Xi_j^*=1+M_jc_j\sum_{k=1}^{\infty}\frac{k+1}{k}B_k^*\frac{\mathrm{d}c_{mc}^k}{\mathrm{d}c_j} \tag{30}$$

is the contribution of species j to the apparent nonideality of the system, w_j^0 is the reduced molecular charge of species j in the limit as c_{mc} approaches zero, and $B^*{}_k=B_k+b_k$ is the $(k+1)$th virial coefficient of the apparent nonideality of the system. The series in the summation can be terminated prior to $k=q$ by setting all $B^*{}_{k>(q-1)}=0$. The use of Eq. (30) results in the apparent nonideality being treated in the same way as thermodynamic nonideality is treated in NONLIN, the nonlinear least-squares analysis program that is widely used to analyze equilibrium sedimentation data [20]. Eq. (30) must account for any variation of w_j and $f_jD^*{}_j/k_BT$ with x, including any such variation that might result from the coupling of reaction fluxes and molar fluxes at steady state. Furthermore, if f_j is treated as a constant in Eq. (29), then any variation in that parameter with x must be accounted for by Eq. (30).

3.1.3. Nonassociating systems when $J_{mc}=0$ at steady state

For nonassociating, membrane-confined species, when $I_{mc}=0$, $J_{mc}=0$. In general, at steady state,

both I_{mc} and J_{mc} will be zero only when each I_j and each J_j is zero. In such cases, each term in the summations of Eqs. (23) and (24), and thus each term in the summation of Eq. (29), equals zero. An individual term in Eq. (29), then, reduces to

$$w_j^0 - \Xi_j^* \frac{\mathrm{d} \ln c_j}{\mathrm{d}x} = 0. \tag{31}$$

This equation can be solved by integration with respect to x,

$$w_j^0 \int_{x_0}^{x} \mathrm{d}x = \int_{c_{0_j}}^{c_j} \mathrm{d} \ln c_j + M_j \sum_{k=1}^{\infty} \frac{k+1}{k} B_k^* \int_{c_{0_{mc}}{}^k}^{c_{mc}{}^k} \mathrm{d}c_{mc}^k, \tag{32}$$

where use has been made of $c_j(\mathrm{d}c_{mc}^k/\mathrm{d}c_j)(\mathrm{d} \ln c_j/\mathrm{d}x) = \mathrm{d}c_{mc}^k/\mathrm{d}x$. Solving Eq. (32) for c_j produces

$$c_j = c_{0_j} \mathrm{e}^{w_j^0(x-x_0) - M_j \sum_{k=1}^{\infty} \frac{k+1}{k} B_k^* (c_{mc}^k - c_{0_{mc}}^k)}, \tag{33}$$

where c_{0_j} is c_j at x_0 and $c_{0_{mc}}$ is c_{mc} at x_0. Using

$$a_{0_j}^* = c_{0_j} \mathrm{e}^{M_j \sum_{k=1}^{\infty} \frac{k+1}{k} B_k^* c_{0_{mc}}^k} \tag{34}$$

to represent the apparent activity of species j at x_0 yields

$$c_j = a_{0_j}^* \mathrm{e}^{w_j^0(x-x_0) - M_j \sum_{k=1}^{\infty} \frac{k+1}{k} B_k^* c_{mc}^k}. \tag{35}$$

Application of Eq. (27) gives the total concentration of all nonassociating, membrane-confined species,

$$c_{mc} = \sum_{j \neq h} a_{0_j}^* \mathrm{e}^{w_j^0(x-x_0) - M_j \sum_{k=1}^{\infty} \frac{k+1}{k} B_k^* c_{mc}^k}. \tag{36}$$

In this equation, and in the following equations for which c_{mc} is the sole concentration variable, optical density measurements of the sort described in Section 1.1 can replace c_{mc} if the ratio of the molar extinction coefficient to the molar mass is the same for each membrane-confined species.

3.1.4. Self-associating systems when $J_{mc} = 0$ at steady state

At steady state, Eqs. (35) and (36) also apply to systems in which membrane-confined species self-associate, and to systems in which different membrane-confined species associate with each other, provided that the reduced molecular charge scales with stoichiometry. In such cases, $J_{mc} = 0$ when $I_{mc} = 0$.

For self-associating systems in which the kinetics of the reaction are fast enough to ensure chemical equilibrium at all x at steady state,

$$c_{mc} = \sum_{j \neq h} K_j c_1^j, \tag{37}$$

applies. If $J_{mc} = 0$ when $I_{mc} = 0$ in such systems at steady state, Eq. (37) permits the total concentration of a component comprising self-associating, membrane-confined species to be written as

$$c_{mc} = \sum_{j \neq h} K_j \left[a_{0_1}^* \mathrm{e}^{w_1^0(x-x_0) - M_1 \sum_{k=1}^{\infty} \frac{k+1}{k} B_k^* c_{mc}^k} \right]^j, \tag{38}$$

where K_j is the equilibrium constant (see relevant footnote in Section 5) for the reaction linking the monomer with the j-mer, c_1 is the concentration of the monomer, w_1^0 is the reduced molecular charge of the monomer in the limit as c_{mc} approaches zero, M_1 is the molar mass of the monomer, and $a^*{}_{0_1}$ is the apparent activity of the monomer at x_0. The reduced molecular charge of the j-mer in the limit as c_{mc} approaches zero is jw_1^0, the molar mass of the j-mer is jM_1, and the apparent activity of the j-mer at x_0 is $K_j(a^*{}_{0_1})^j$. For $j = 1$, $K_1 = 1$. The highest order polymer is the m-mer. The reaction describing m-mer formation is $m\mathrm{A} \leftrightarrows \mathrm{A}_m$, where A is the monomer. If no polymer of order H is formed, where $1 < H < m$, then $K_H = 0$. Eq. (38) is the MCE equivalent of the equation on which the analysis of equilibrium sedimentation data by NONLIN is based [20].

3.1.5. Hetero-associating systems when $J_{mc}=0$ at steady state

For hetero-associating systems in which two different membrane-confined species associate with each other, and where the kinetics of the reaction are fast enough to ensure chemical equilibrium at all x at steady state,

$$c_{\mathrm{mc}}=\sum_{j\neq h}K_j c_1^{\alpha_j}c_2^{\beta_j} \tag{39}$$

applies. If $J_{\mathrm{mc}}=0$ when $I_{\mathrm{mc}}=0$ in such systems at steady state, Eq. (39) permits the total concentration of a component comprising hetero-associating, membrane-confined species to be written as

$$c_{\mathrm{mc}}=\sum_{j\neq h}K_j a_{0_1}^{*\alpha_j}a_{0_2}^{*\beta_j}\mathrm{e}^{(\alpha_j w_1^0+\beta_j w_2^0)(x-x_0)-(\alpha_j M_1+\beta_j M_2)\sum_{k=1}^{\infty}\frac{k+1}{k}B_k^* c_{\mathrm{mc}}^k}, \tag{40}$$

where K_j is the equilibrium constant for the reaction linking monomer 1 and monomer 2 with heteropolymer j, c_1 is the concentration of monomer 1, c_2 is the concentration of monomer 2, $a^*_{0_1}$ is the apparent activity of monomer 1 at x_0, $a^*_{0_2}$ is the apparent activity of monomer 2 at x_0, M_1 is the molar mass of monomer 1, M_2 is the molar mass of monomer 2, w_1^0 is the reduced molecular charge of monomer 1 in the limit as c_{mc} approaches zero, w_2^0 is the reduced molecular charge of monomer 2 in the limit as c_{mc} approaches zero, α_j is the stoichiometry of monomer 1 in heteropolymer j and β_j is the stoichiometry of monomer 2 in heteropolymer j. The reduced molecular charge of heteropolymer j in the limit as c_{mc} approaches zero is $\alpha_j w_1^0+\beta_j w_2^0$, the molar mass of heteropolymer j is $\alpha_j M_1+\beta_j M_2$, and the apparent activity of heteropolymer j at x_0 is $K_j(a^*_{0_1})^{\alpha j}(a^*_{0_2})^{\beta j}$. For $j=1$ and $j=2$, $K_j=1$, while $\alpha_1=1$, $\beta_1=0$, $\alpha_2=0$ and $\beta_2=1$. The highest order heteropolymer is m, and applies to the reaction $\alpha_m\mathrm{A}+\beta_m\mathrm{B}\rightleftarrows\mathrm{A}_{\alpha m}\mathrm{B}_{\beta m}$, where A is monomer 1 and B is monomer 2. If no heteropolymer of order H is formed, where $2<H<m$, then $K_H=0$.

For steady-state systems in which membrane-confined species self-associate, or steady-state systems in which different membrane-confined species associate with each other, Eqs. (35) and (36) are inapplicable if reduced molecular charge does not scale with stoichiometry. In such cases, $J_{\mathrm{mc}}\neq 0$ when $I_{\mathrm{mc}}=0$, and resort must be made to Eq. (29), instead.

3.1.6. Self-associating systems when $J_{mc}\neq 0$ at steady state

Eq. (29) can be applied to a two-species, self-associating system, such as $j\mathrm{A}\leftrightarrows\mathrm{A}_j$, with reaction kinetics that are fast enough to ensure chemical equilibrium, described by Eq. (37), at all x at steady state. The result, in terms of the gradient in the logarithm of the monomer concentration when $I_{\mathrm{mc}}=0$ but $J_{\mathrm{mc}}\neq 0$, is

$$\frac{\mathrm{d}\ln c_1}{\mathrm{d}x}=\frac{f_j+f_1 Yj K_j c_1^{j-1}}{f_j\Xi_1^*+f_1 jK_j c_1^{j-1}\Xi_j^*}w_1^0, \tag{41}$$

where c_1 is the concentration of the monomer, A; w_1^0 is the reduced molecular charge of A in the limit as c_{mc} approaches zero; Y is a dimensionless coefficient that would be equal to 1 if the reduced molecular charge scaled with stoichiometry; $Yjw_1^0=w_j^0$ is the reduced molecular charge of the j-mer, A_j, in the limit as c_{mc} approaches zero; f_1 is the frictional coefficient of A; and f_j is the frictional coefficient of A_j. The equilibrium constant is $K_j=c_j/c_1^j$. Thus,

$$M_1 c_1\frac{\mathrm{d}c_{\mathrm{mc}}^k}{\mathrm{d}c_1}=M_j c_j\frac{\mathrm{d}c_{\mathrm{mc}}^k}{\mathrm{d}c_j}=k\left(\sum_{j\neq h}K_j c_1^j\right)^{k-1}\left(\sum_{j\neq h}jK_j c_1^{j-1}\right). \tag{42}$$

Eq. (42) shows that $\Xi^*_1=\Xi^*_j$. As with the previous self-association problem, $K_1=1$, the highest order polymer is the m-mer, and if no polymer of order H is formed, where $1<H<m$, then $K_H=0$.

If $B^*_k=0$ for all k, and if the frictional coefficients can be considered independent of x, Eq. (41) can be integrated with respect to x and solved for c_1, and Eq. (37) used to obtain

$$c_{\mathrm{mc}}=\sum_{j\neq h}K_j\left(c_{0_1}\left[\frac{f_j+f_1 YjK_j c_1^{j-1}}{f_j+f_1 YjK_j c_{0_1}^{j-1}}\right]^{\frac{Y-1}{Y(j-1)}}\mathrm{e}^{w_1^0(x-x_0)}\right)^j. \tag{43}$$

Analytic solutions that do not assume $B^*_k = 0$ for all k exist, but are unwieldy.

When $Y = 1$, Eq. (43) is equal to Eq. (38) for the case of $B^*_k = 0$ for all k. Furthermore, given that $\Xi^*_1 = \Xi^*_j$, when $Y = 1$, Eq. (41) reduces to $(\mathrm{d}\ln c_1/\mathrm{d}x)\Xi^*_1 = w_1^0$, the solution of which yields Eq. (38). This shows that Eq. (38) can be obtained either by assuming that $J_{\mathrm{mc}} = 0$ when $I_{\mathrm{mc}} = 0$, which permits the use of Eq. (31) as a starting point; or by making no assumptions about J_{mc} when $I_{\mathrm{mc}} = 0$, thus requiring the use of Eq. (29) as a starting point, but assuming that reduced molecular charge scales with stoichiometry, which in this case means that $jw_1^0 = w_j^0$.

3.1.7. Hetero-associating systems when $J_{mc} \neq 0$ at steady state

Eq. (29) can also be applied to a three-species, hetero-associating system, such as $\alpha\mathrm{A} + \beta\mathrm{B} \leftrightarrows \mathrm{A}_\alpha\mathrm{B}_\beta$, with reaction kinetics that are fast enough to ensure chemical equilibrium, described by Eq. (39), at all x at steady state. The result, in terms of the gradient in the logarithm of the concentration of monomer 1 when $I_{\mathrm{mc}} = 0$ but $J_{\mathrm{mc}} \neq 0$, is

$$\frac{\mathrm{d}\ln c_1}{\mathrm{d}x} = \frac{(f_3 + f_1 K c_1^{\alpha-1} c_2^{\beta} \alpha Y) f_2 c_1 w_1^0 + (f_3 + f_2 K c_1^{\alpha} c_2^{\beta-1} \beta Y) f_1 c_2 w_2^0}{(f_3 \Xi_1^* + f_1 K c_1^{\alpha-1} c_2^{\beta} \alpha \Xi_3^*) f_2 c_1} - \frac{(f_3 \Xi_2^* + f_2 K c_1^{\alpha} c_2^{\beta-1} \beta \Xi_3^*) f_1 c_2 \dfrac{\mathrm{d}\ln c_2}{\mathrm{d}x}}{(f_3 \Xi_1^* + f_1 K c_1^{\alpha-1} c_2^{\beta} \alpha \Xi_3^*) f_2 c_1}, \tag{44}$$

where c_1 is the concentration of monomer 1, which is arbitrarily chosen to be A; c_2 is the concentration of monomer 2, which is B by the process of elimination; w_1^0 is the reduced molecular charge of A in the limit as c_{mc} approaches zero; w_2^0 is the reduced molecular charge of B in the limit as c_{mc} approaches zero; f_1 is the frictional coefficient of A; and f_2 is the frictional coefficient of B. The frictional coefficient of the heteropolymer, $\mathrm{A}_\alpha\mathrm{B}_\beta$, is f_3; the concentration of $\mathrm{A}_\alpha\mathrm{B}_\beta$ is c_3; and the reduced molecular charge of $\mathrm{A}_\alpha\mathrm{B}_\beta$ in the limit as c_{mc} approaches zero is $w_3^0 = Y(\alpha w_1^0 + \beta w_2^0)$, where Y is a dimensionless coefficient that would be equal to 1 if the reduced molecular charge scaled with stoichiometry. The equilibrium constant is $K = c_3/c_1^{\alpha} c_2^{\beta}$. For such a system,

$$\frac{\mathrm{d}c_{\mathrm{mc}}^k}{\mathrm{d}c_1} = k(c_1 + c_2 + K c_1^{\alpha} c_2^{\beta})^{k-1} \times \left(1 + \alpha K c_1^{\alpha-1} c_2^{\beta} + [1 + \beta K c_1^{\alpha} c_2^{\beta-1}] \frac{\mathrm{d}c_2}{\mathrm{d}c_1}\right). \tag{45}$$

Expressions in terms of c_1 and c_2 can also be obtained for $\mathrm{d}c_{\mathrm{mc}}^k/\mathrm{d}c_2$ and $\mathrm{d}c_{\mathrm{mc}}^k/\mathrm{d}c_3$.

Hidden in Eq. (40) is the assumption that $\Xi^*_1 = \Xi^*_2 = \Xi^*_{j>2}$, which means that Ξ^* of monomer 1, Ξ^* of monomer 2, and Ξ^* of any heteropolymer are all the same. With this and the additional assumption that $(\mathrm{d}\ln c_2/\mathrm{d}x)\Xi^*_2 = w_2^0$ when $Y = 1$, Eq. (44) reduces to $(\mathrm{d}\ln c_1/\mathrm{d}x)\Xi^*_1 = w_1^0$. Applying Eq. (39) to the case of $(\mathrm{d}\ln c_1/\mathrm{d}x)\Xi^*_1 = w_1^0$ and $(\mathrm{d}\ln c_2/\mathrm{d}x)\Xi^*_2 = w_2^0$ yields Eq. (40). This shows that Eq. (40) can result from two different approaches: either assuming that $J_{\mathrm{mc}} = 0$ when $I_{\mathrm{mc}} = 0$, which permits the use of Eq. (31) as a starting point; or by starting with Eq. (29), making no assumptions about J_1 when $I_{\mathrm{mc}} = 0$, but assuming both that $J_2 = 0$ when $I_{\mathrm{mc}} = 0$, and that the reduced molecular charge scales with stoichiometry.

If $\beta = 1$, c_2 can be expressed in terms of c_1 and c_{mc}, the expression being

$$c_2 = \frac{c_{\mathrm{mc}} - c_1}{1 + K c_1^{\alpha}}, \tag{46}$$

so that

$$\frac{\mathrm{d}c_2}{\mathrm{d}x} = \frac{1}{1 + K c_1^{\alpha}} \left(\frac{\mathrm{d}c_{\mathrm{mc}}}{\mathrm{d}x} - \frac{1 + K[\alpha c_{\mathrm{mc}} - (\alpha - 1)c_1] c_1^{\alpha-1}}{1 + K c_1^{\alpha}} \frac{\mathrm{d}c_1}{\mathrm{d}x}\right) \tag{47}$$

and

$$\frac{\mathrm{d}\ln c_1}{\mathrm{d}x}=\frac{\left(f_3+f_1Kc_1^{\alpha-1}\frac{c_{\mathrm{mc}}-c_1}{1+Kc_1^{\alpha}}\alpha Y\right)f_2c_1w_1^0+(f_3+f_2Kc_1^{\alpha}Y)f_1\frac{c_{\mathrm{mc}}-c_1}{1+Kc_1^{\alpha}}w_2^0}{\left(f_3\Xi_1^*+f_1Kc_1^{\alpha-1}\frac{c_{\mathrm{mc}}-c_1}{1+Kc_1^{\alpha}}\alpha\Xi_3^*\right)f_2c_1+(f_3\Xi_2^*+f_2Kc_1^{\alpha}\Xi_3^*)f_1}-\frac{\left(\frac{\mathrm{d}c_{\mathrm{mc}}}{\mathrm{d}x}-\frac{1+K[\alpha c_{\mathrm{mc}}-(\alpha-1)c_1]c_1^{\alpha-1}}{1+Kc_1^{\alpha}}\frac{\mathrm{d}c_1}{\mathrm{d}x}\right)}{\left(f_3\Xi_1^*+f_1Kc_1^{\alpha-1}\frac{c_{\mathrm{mc}}-c_1}{1+Kc_1^{\alpha}}\alpha\Xi_3^*\right)f_2c_1(1+Kc_1^{\alpha})}. \quad (48)$$

As c_{mc} includes c_2 in it, and c_2 cannot, in general, be expressed in terms of c_1, Eqs. (44) and (48) have no obvious solutions. Eq. (48) could potentially be used directly to analyze data, however, because c_{mc} is generally known, and the frictional coefficients are, at least in principle, measurable. In systems where c_2 and c_{mc} can be measured independently, data could be analyzed using Eq. (44) directly, given knowledge of the frictional coefficients.

3.1.8. Binding of membrane-confined and membrane-permeant species at steady state

Eq. (29) only includes the mass flux of membrane-confined species, so it does not adequately describe systems in which a membrane-confined species associates with a membrane-permeant ion. Some systems of this sort can be treated as an apparent chemical equilibrium, however, so that Eq. (29) becomes applicable, provided that the reaction kinetics are fast enough to ensure chemical equilibrium at all x at steady state. For example, for a reaction such as $\alpha\mathrm{A}+\chi\mathrm{X}^{\pm\zeta}\leftrightarrows\mathrm{A}_\alpha\mathrm{X}_\chi$, where A and $\mathrm{A}_\alpha\mathrm{X}_\chi$ are membrane-confined species, and X is a membrane-permeant ion of valence $z_{\mathrm{X}}=\pm\zeta$, the equilibrium constant is

$$K=\frac{c_{\mathrm{A}_\alpha\mathrm{X}_\chi}}{c_{\mathrm{A}}^{\alpha}c_{\mathrm{X}}^{\chi}}. \quad (49)$$

Provided that X freely permeates the membranes, however, its concentration will vary little with x, and the reaction can be treated as an apparent one, $\alpha\mathrm{A}\leftrightarrows\mathrm{A}^*_\alpha$, where A and A^*_α are membrane-confined species, with A^*_α being the apparent polymer. The equation for the apparent equilibrium constant, K^*, is

$$K^*\approx\frac{c_2}{c_1^{\alpha}}=\frac{c_{\mathrm{A}_\alpha{}^*}}{c_{\mathrm{A}}^{\alpha}}=Kc_{\mathrm{X}}^{\chi}=\frac{c_{\mathrm{A}_\alpha\mathrm{X}_\chi}}{c_{\mathrm{A}}^{\alpha}}, \quad (50)$$

where A is species 1 and A^*_α is species 2. This equation is an approximation because K^* is treated as a constant, while c_2/c_1^{α} is expected to vary slightly with x, even if X freely permeates the membranes.

For $\alpha\geqslant 2$, K^* can be used in place of K, and whichever of Eq. (38) or Eq. (43) is most appropriate can be used to describe the system. For $\alpha=1$, Eq. (29) leads to

$$\frac{\mathrm{d}\ln c_1}{\mathrm{d}x}=\frac{f_2+f_1YK^*}{f_2\Xi_1^*+f_1K^*\Xi_2^*}w_1^0, \quad (51)$$

where $w_2^0=Yw_1^0$. If Ξ^*_2 can be treated as equal to Ξ^*_1, and the frictional coefficients can be considered independent of x, this equation can be solved for c_1 by integrating with respect to x. Applying the relation $c_{\mathrm{mc}}=(1+K^*)c_1$ to that solution results in

$$c_{\mathrm{mc}}=(1+K^*)\left(a_{0_1}^*\mathrm{e}^{\left(\frac{f_2+f_1YK^*}{f_2+f_1K^*}\right)w_1^0(x-x_0)-M_1\sum_{k=1}^{\infty}\frac{k+1}{k}B_k^*c_{\mathrm{mc}}^k}\right). \quad (52)$$

It is also possible to divide $\alpha\mathrm{A}+\chi\mathrm{X}^{\pm\zeta}\rightleftarrows\mathrm{A}_\alpha\mathrm{X}_\chi$ into two steps, $\alpha\mathrm{A}\leftrightarrows\mathrm{A}_\alpha$ and $\mathrm{A}\alpha+\chi\mathrm{X}^{\pm\zeta}\rightleftarrows\mathrm{A}_\alpha\mathrm{X}_\chi$. The second step can be treated as $\mathrm{A}_\alpha\leftrightarrows\mathrm{A}_\alpha{}^*$, for which K^* is the apparent equilibrium constant. Eq. (29) can then be applied to species A, A_α and $\mathrm{A}_\alpha{}^*$, with the concentration of $\mathrm{A}_\alpha{}^*$ expressed as $K^*c_{\mathrm{A}\alpha}$.

Reactions such as $\alpha\mathrm{A}+\beta\mathrm{B}+\chi\mathrm{X}^{\pm\zeta}\rightleftarrows\mathrm{A}_\alpha\mathrm{B}_\beta\mathrm{X}_\chi$, where A, B and $\mathrm{A}_\alpha\mathrm{B}_\beta\mathrm{X}_\chi$ are membrane-confined species, while X is a membrane-permeant ion of valence $z_{\mathrm{X}}=\pm\zeta$, can be divided into two steps, $\alpha\mathrm{A}+\beta\mathrm{B}\rightleftarrows\mathrm{A}_\alpha\mathrm{B}_\beta$ and $\mathrm{A}_\alpha\mathrm{B}_\beta+\chi\mathrm{X}^{\pm\zeta}\rightleftarrows\mathrm{A}_\alpha\mathrm{B}_\beta\mathrm{X}_\chi$. Treating the second step as $\mathrm{A}_\alpha\mathrm{B}_\beta\leftrightarrows\mathrm{A}_\alpha\mathrm{B}_\beta{}^*$, Eq. (29) can then be applied to species A, B, $\mathrm{A}_\alpha\mathrm{B}_\beta$ and $\mathrm{A}_\alpha\mathrm{B}^*_\beta$, with K^*, the apparent equilibrium constant for the second step, used to express the

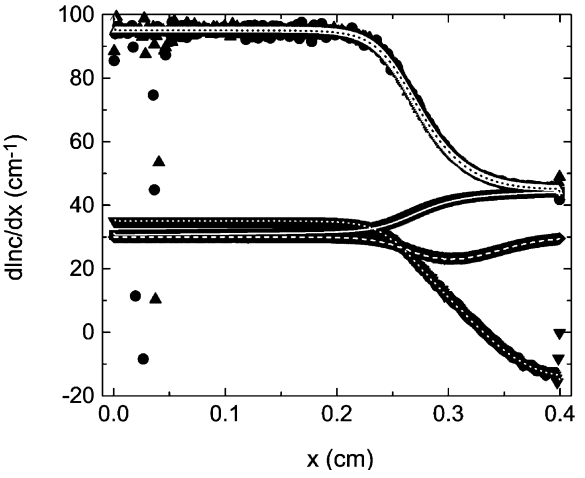

Fig. 6. Simulation of steady-state experiment, $\mathrm{d}\ln c/\mathrm{d}x$ vs. x. The system is the same as that in Fig. 5, but only the steady-state concentration applies to this figure. Denoting A as species 1, B as species 2, A_2B as species 3, and A_2BX in the form of A_2B^* as species 4, with $\alpha=2$, and with $\Xi^*_1=\Xi^*_2=\Xi^*_3=\Xi^*_4=1$, Eqs. (53)–(59) apply to this system. The results of calculations using these equations with the known parameters, plus c_{mc} and c_1, are compared with the results from finite element simulation. Results from finite element simulation: $\mathrm{d}\ln c_1/\mathrm{d}x$ (♦); $\mathrm{d}\ln c_2/\mathrm{d}x$ (▼); $\mathrm{d}\ln c_3/\mathrm{d}x$ (▲); $\mathrm{d}\ln c_4/\mathrm{d}x$ (●); $\mathrm{d}\ln c_{mc}/\mathrm{d}x$ (■). Results from calculations: white (----), $\mathrm{d}\ln c_1/\mathrm{d}x$; white (·····), $\mathrm{d}\ln c_2/\mathrm{d}x$; white (△), $\mathrm{d}\ln c_3/\mathrm{d}x$; black (·····), $\mathrm{d}\ln c_4/\mathrm{d}x$; white (——), $\mathrm{d}\ln c_{mc}/\mathrm{d}x=(c_1\,\mathrm{d}\ln c_1/\mathrm{d}x+c_2\,\mathrm{d}\ln c_2/\mathrm{d}x+c_3\,\mathrm{d}\ln c_3/\mathrm{d}x+c_4\,\mathrm{d}\ln c_4/\mathrm{d}x)/c_{mc}$, where, using $c_2=(c^*_{mc}-c_1)/(1+K_1c_1^\alpha)$, $c_3=c^*_{mc}-c_1-c_2$ and $c_4=c_{mc}-c^*_{mc}$.

concentration of $A_\alpha B^*_\beta$ as $K^*c_{A\alpha B\beta}$ (Fig. 6), provided, again, that the reaction kinetics are fast enough to ensure chemical equilibrium at all x at steady state.

The general description of the type of reaction illustrated by Figs. 4–6 is $\alpha A+B+X^{-1}\leftrightarrows A_\alpha BX$, where A, B and $A_\alpha BX$ are membrane-confined species, while X is a membrane-permeant ion. The reaction is divided into two steps: $\alpha A+B\rightleftarrows A_\alpha B$, for which K_1 is the equilibrium constant; and $A_\alpha B+X^{-1}\rightleftarrows A_\alpha BX$, for which K_2 is the equilibrium constant. Thus, $K^*_2=K_2c_X$ describes the equilibrium for the apparent reaction, $A_\alpha B\leftrightarrows A_\alpha B^*$. Denoting A as species 1, B as species 2, $A_\alpha B$ as species 3 and $A_\alpha BX$ in the form of $A_\alpha B^*$ as species 4, the equation for $\mathrm{d}\ln c_1/\mathrm{d}x$ in terms of c_1 and c_{mc} is

$$\frac{\mathrm{d}\ln c_1}{\mathrm{d}x}=\frac{\left(f_3+f_1K_1c_1^{\alpha-1}\dfrac{c^*_{mc}-c_1}{1+K_1c_1^\alpha}\alpha Y_p\right)f_2c_1w_1^0+(f_3+f_2K_1c_1^\alpha Y_p)f_1\dfrac{c^*_{mc}-c_1}{1+K_1c_1^\alpha}w_2^0}{\left(f_3\Xi^*_1+f_1K_1c_1^{\alpha-1}\dfrac{c^*_{mc}-c_1}{1+K_1c_1^\alpha}\alpha\Xi^*_p\right)f_2c_1}-\frac{(f_3\Xi^*_2+f_2K_1c_1^\alpha Y_p)f_1\left(\dfrac{\mathrm{d}c^*_{mc}}{\mathrm{d}x}-\dfrac{1+K_1[\alpha c^*_{mc}-(\alpha-1)c_1]c_1^{\alpha-1}}{1+K_1c_1^\alpha}\dfrac{\mathrm{d}c_1}{\mathrm{d}x}\right)}{\left(f_3\Xi^*_1+f_1K_1c_1^{\alpha-1}\dfrac{c^*_{mc}-c_1}{1+K_1c_1^\alpha}\alpha\Xi^*_p\right)f_2c_1(1+K_1c_1^\alpha)},\quad(53)$$

where, with $c^*_{mc}=c_1+c_2+c_3=c_{mc}-c_4$,

$$c_2=\frac{c^*_{mc}-c_1}{1+K_1c_1^\alpha}\quad(54)$$

and

$$c^*_{mc}=\frac{c_{mc}(1+K_1c_1^\alpha)+K^*_2K_1c_1^{\alpha+1}}{1+K_1c_1^\alpha(1+K^*_2)}.\quad(55)$$

The reduced molecular charge coefficient of stoichiometry is

$$Y_p=\frac{w_3^0}{\alpha w_1^0+w_2^0}\left(1+\frac{f_3}{f_4}K^*_2\frac{w_4^0}{w_3^0}\right),\quad(56)$$

and

$$\Xi^*_p=\left(\Xi^*_3+\frac{f_3}{f_4}K^*_2\Xi^*_4\right)\quad(57)$$

is the polymeric apparent nonideality function. The expression for c_2 is used to obtain $\mathrm{d}\ln c_2/\mathrm{d}x$ in terms of c_{mc} and c_1. Species 3 and 4 can be described in terms of species 1 and 2, resulting in

$$\frac{\mathrm{d}\ln c_4}{\mathrm{d}x}=\frac{\mathrm{d}\ln c_3}{\mathrm{d}x}=\alpha\frac{\mathrm{d}\ln c_1}{\mathrm{d}x}+\frac{\mathrm{d}\ln c_2}{\mathrm{d}x},\quad(58)$$

where

$$\frac{\mathrm{d}\ln c_2}{\mathrm{d}x}=\frac{1}{c^*_{\mathrm{mc}}-c_1}\times\left(\frac{\mathrm{d}c^*_{\mathrm{mc}}}{\mathrm{d}x}-\frac{\mathrm{d}c_1}{\mathrm{d}x}\right)-\frac{\alpha K_1 c_1^{\alpha-1}}{1+K_1c_1^{\alpha}}\frac{\mathrm{d}c_1}{\mathrm{d}x}. \quad (59)$$

3.1.9. Slow reaction kinetics at steady state

Although Eq. (36) applies to associating systems in which the reduced molecular charge scales with stoichiometry and the reaction kinetics are too slow to ensure chemical equilibrium at all x at steady state, its use cannot yield information about the kinetics. Such kinetic information might be obtainable by the application of numerical methods, however. Numerical methods would also be applicable to systems for which the reduced molecular charge does not scale with stoichiometry and the reaction kinetics are too slow to ensure chemical equilibrium at all x at steady state.

3.1.10. Applicability to equilibrium sedimentation

The steady-state equations above can be modified to apply to equilibrium sedimentation by replacing x with $r^2/2$, where r is the radial position; replacing c_{mc} with c_{mm}, the total concentration of macromolecules; and replacing w_j^0 with σ_j^0, the reduced molecular mass of species j in the limit as c_{mm} approaches zero. An underlying assumption of the resulting equations is that the kinetics of any reactions are fast enough to ensure chemical equilibrium at all r when the system becomes invariant with time. For an associating system in which the buoyant molar mass does not scale with stoichiometry, neither will the reduced molar mass, and the sedimentation analog of Eqs. (43) and (44) or Eq. (48) will apply. In such cases, letting J_{mm} represent the molar flux of macromolecules, and I_{mm} represent the mass flux of macromolecules, $I_{\mathrm{mm}}=0$ but $J_{\mathrm{mm}}\neq 0$ at equilibrium.

3.2. Boundary velocity MCE

3.2.1. Method

Boundary velocity MCE is a broad-zone method, similar to velocity sedimentation. The initial state of the system in a typical boundary velocity MCE experiment is one in which the concentration of each species is invariant with x. Compared to steady-state MCE, boundary velocity MCE is conducted at relatively high current.

Assuming that v_j is positive for all membrane-confined species, boundary velocity MCE produces one or more downward moving boundaries in the concentrations of those species. Behind the boundaries, a region where $c_{\mathrm{mc}}=0$ develops. Ahead of the boundaries is a plateau region, where $c_{\mathrm{mc}}>0$ and I_{mc} is invariant with x. Thus, the continuity equation gives $(\partial c_{\mathrm{mc}}/\partial t)_x=0$ in the plateau region. (This stands in contrast to the radial dilution that occurs in velocity sedimentation, where, as a consequence of the system being sector-shaped and the angular acceleration being proportional to r, the plateau concentration decreases exponentially with time [23,24].) Ahead of the plateau region, above the bottom membrane, $(\partial c_{\mathrm{mc}}/\partial t)_x>0$. (If v_j differs in sign for different membrane-confined species, some boundaries may move downward while others move upward.)

The transport velocity of species j is

$$v_j=u_j^*E-D_j^*\left(\frac{\partial\ln c_j}{\partial x}\right)_t \quad (60)$$

and the weight average velocity of membrane-confined species is

$$v_w=\frac{\sum_{j\neq h}c_jv_j}{\sum_{j\neq h}c_j}=u_w^*E-D_g^*\left(\frac{\partial\ln c_{\mathrm{mc}}}{\partial x}\right)_t, \quad (61)$$

where u^*_w is the weight average apparent electrophoretic mobility, and D^*_g, the gradient average apparent diffusion coefficient, is equal to

$$D_g^*=\frac{\sum_{j\neq h}D_j^*\left(\frac{\partial c_j}{\partial x}\right)_t}{\sum_{j\neq h}\left(\frac{\partial c_j}{\partial x}\right)_t}. \quad (62)$$

In the plateau region, where $(\partial\ln c_{\mathrm{mc}}/\partial x)_t=0$, $u^*_j=v_j/E$ and $u^*_w=v_w/E$.

The relationship between u^*_w and the reduced

molecular charge is $u^*_w = k_B T(w/f)_w/E$, where $(w/f)_w$ is the weight average ratio of the reduced molecular charge to the frictional coefficient. The relationship between w_w, the weight average reduced molecular charge, and the apparent electrophoretic mobility is $w_w = E(u^*f)_w/k_B T$, where $(u^*f)_w$ is the weight average product of the apparent electrophoretic mobility and the frictional coefficient.

3.2.2. Weight average apparent electrophoretic mobility measurements

In general,

$$I_{mc} = \sum_{j \neq h} c_j v_j = v_w \sum_{j \neq h} c_j = c_{mc} u^*_w E - D^*_g \left(\frac{\partial c_{mc}}{\partial x} \right)_t. \quad (63)$$

In the plateau region, $(\partial c_{mc}/\partial x)_t = 0$, so that $I_{mc} = c_p u^*_w E$, where c_p is c_{mc} in the plateau region. If v_w could be measured in the plateau region, then u^*_w could be obtained, given knowledge of E. Such measurements might be made by electrophoretic light scattering [25], but are not possible with the existing MCE instrument. With that instrument, however, a moving boundary approach can be taken to obtain v_w, and thus u^*_w.

An approach analogous to that taken by Goldberg in 1953 [22] to obtain the weight-average sedimentation coefficient, s_w, can be used to get u^*_w. The plateau region is preceded by a moving boundary that, due to diffusion, spreads with time. To measure v_w, a point in the boundary that has a velocity equivalent to v_w is followed. That point, x_e, is the equivalent point. If the boundary did not spread with time, it would remain infinitely sharp, and its position would be given by x_e, so that c_{mc} would equal zero in the region preceding x_e, and c_{mc} would equal c_p in the region following x_e. If x_0 is a point where $c_{mc} = c_0 = 0$, and x_p is a point where $c_{mc} = c_p$, then

$$c_p = \int_{x_0}^{x_p} \left(\frac{\partial c_{mc}}{\partial x} \right)_t dx. \quad (64)$$

From the definitions of c_p, x_e, x_0 and x_p, it follows that

$$c_p \int_{x_e}^{x_p} dx = \int_{x_0}^{x_p} c_{mc} dx. \quad (65)$$

The left-hand side of this equation is just $c_p x_p - c_p x_e$. Making use of $d(c_{mc}x)/dx = x(\partial c_{mc}/\partial x)_t + c_{mc}$, the right-hand side can be integrated by parts, giving

$$\int_{x_0}^{x_p} c_{mc} dx = \int_{c_0 x_0}^{c_p x_p} d(c_{mc}x) - \int_{x_0}^{x_p} x \left(\frac{\partial c_{mc}}{\partial x} \right)_t dx. \quad (66)$$

The first integral on the right-hand side of this equation is equal to $c_p x_p - c_0 x_0$, which reduces to just $c_p x_p$, because $c_0 = 0$. Thus, Eq. (65) can be rewritten as

$$c_p x_p - c_p x_e = c_p x_p - \int_{x_0}^{x_p} x \left(\frac{\partial c_{mc}}{\partial x} \right)_t dx. \quad (67)$$

Solving Eq. (67) for x_e yields

$$x_e = \frac{\int_{x_0}^{x_p} x \left(\frac{\partial c_{mc}}{\partial x} \right)_t dx}{c_p} = \frac{\int_{x_0}^{x_p} x \left(\frac{\partial c_{mc}}{\partial x} \right)_t dx}{\int_{x_0}^{x_p} \left(\frac{\partial c_{mc}}{\partial x} \right)_t dx}. \quad (68)$$

As this equation shows, x_e is the first moment of the boundary, which is the gradient average of x in the boundary. Letting x_0 equal the position of the membrane behind the plateau, the elapsed time, t_e, is the time required for the first moment of the boundary to travel from x_0 to x_e. Thus, $v_w = x_e/t_e$, so $u^*_w = x_e/Et_e$. Where $u^*_w = I_{mc}/Ec_p$, $I_{mc} = c_p x_e/t_e$, and it could be said that this approach for determining u^*_w relies on measuring I_{mc} in the plateau by examining the depletion of membrane-confined species in the region behind the plateau.

3.3. Finite element simulation program

For a solution that includes the membrane-permeant solutes X and P, plus the membrane-confined solutes A, B and $A_\alpha B_\beta X_\chi$, the sum of

the continuity equations of the solutes is

$$\sum_{j=1}^{n-1}\left(\frac{\partial c_j}{\partial t}\right)_x = -\sum_{j=1}^{n-1}\left(\frac{\partial I_j}{\partial x}\right)_t - \frac{k_f c_{\mathrm{A}}^{\alpha} c_{\mathrm{B}}^{\beta} c_{\mathrm{X}}^{\chi} - k_r c_{\mathrm{A}_\alpha \mathrm{B}_\beta \mathrm{X}_\chi}}{M_{\mathrm{A}_\alpha \mathrm{B}_\beta \mathrm{X}_\chi}} \sum_{j=1}^{n-1} \nu_j M_j, \tag{69}$$

where the mass–action association is described by $\alpha\mathrm{A}+\beta\mathrm{B}+\chi\mathrm{X} \leftrightarrows \mathrm{A}_\alpha\mathrm{B}_\beta\mathrm{X}_\chi$, for which k_f is the forward rate constant, and k_r is the reverse rate constant. For reactants, ν_j is the stoichiometry. For products, ν_j is the stoichiometry times -1. For all other species, ν_j is zero. The summation in the second term on the right-hand side is zero, as will be any such summation for any mass–action association [26], so that

$$\sum_{j=1}^{n-1}\left(\frac{\partial c_j}{\partial t}\right)_x = -\sum_{j=1}^{n-1}\left(\frac{\partial I_j}{\partial x}\right)_t. \tag{70}$$

Eq. (70) is the starting point for developing the simulation program described here. That program, implemented using finite element methods [11], permits the simulation of MCE from arbitrary starting conditions to steady state. For each species, the continuity equation, $(\partial c_j/\partial t)_x = -(\partial I_j/\partial x)_t$, is used to develop a set of equations that can be solved to obtain a set of concentrations at consecutive time steps. Eq. (22) is used for I_j, and coefficients of the concentration of each species are used to treat nonideality in w_j, D^*_j and f_j. The continuity equation is multiplied by a set of N functions of x indexed, say, by q, where $1 \leqslant q \leqslant N$. The concentration is approximated as the sum of another set of N functions of x, each element k of which, where $1 \leqslant k \leqslant \mathrm{N}$, is multiplied by a scalar coefficient, $c^*_k(t)$, that is a function of t and is invariant with x. These steps render the partial derivatives ordinary, which permits integrating the result with respect to x. (If w_j, f_j and D^*_j of each species can be treated as dependent only on $c^*_k(t)$ of the various species present, then w_j, f_j and D^*_j are independent of x, and give rise to no partial derivatives.) Integration yields a useful expression of the time derivatives of $c^*(t)$, the set of all $c^*_k(t)$ for some species. The utility of the integration step depends, in part, on the flux at the boundaries of the system being zero for each species.

The simulation handles two overlapping systems. The limited system is that within the cuvette, and is the sole system that directly applies to the flux of completely membrane-confined species. At the boundaries of the limited system, the flux of membrane-confined species is zero. The nearly infinite system includes the cuvette, and extends a virtually infinite distance above and below the cuvette. The nearly infinite system directly applies to the flux of species that freely permeate the membranes. At the boundaries of the nearly infinite system, the flux of membrane-permeant species is considered to be zero.

The nearly infinite system is meant to simulate the continuous supply of fresh buffer above and below the cuvette. Except in the vicinity of the distant boundaries of the nearly infinite system, the concentration of each membrane permeant species is considered to be invariant with x and t above the top membrane and below the bottom membrane. With this arrangement, only the cuvette and the regions immediately above and below it need to be handled explicitly when using the nearly infinite system.

The program employs a Crank–Nicholson approach [27] to express $\mathrm{d}c^*(t)/\mathrm{d}t$ as $\Delta c^*(t)/\Delta t$, where $\Delta c^*(t)$ and Δt are finite increments. The time step is Δt. The difference between the unknown $c^*(t+\Delta t)$ and the known $c^*(t)$ is $\Delta c^*(t)$. The result is solved for $c^*(t+\Delta t)$. Interactions between species are handled separately between time steps. It has been found that the program is made more robust by first calculating $c^*(t+\Delta t)$ starting from $c^*_1(t+\Delta t)$, then recalculating $c^*(t+\Delta t)$ starting from $c^*_N(t+\Delta t)$, and averaging the results.

For species that permeate the membranes with some difficulty, the percent of permeability is equal to the percent of time steps in which the infinite system is applied, while the limited system is applied in all other time steps. With such species, the application of each system is distributed as evenly as possible over the entire time course of the simulation.

The program has been used to check the validity of the equations relevant to the kinds of systems described in this paper (e.g. see Figs. 4 and 6). The program can also simulate sedimentation, to which no analog of the infinite system of MCE applies. For sedimentation, the applicable continuity equation is the Lamm equation, $(\partial c_j/\partial t)_r = -(1/r)(\partial I_j r/\partial r)_t$ [26], where $s_j\omega^2 r$ replaces $k_B T w_j/f_j$ in I_j, with ω being the angular velocity of the centrifuge rotor.

4. Conclusions

Being a numerical method, the finite element simulation program is a potential tool for analyzing data from complicated systems for which no analytic solution of the applicable differential equation can be found. This includes some MCE systems at steady state, some sedimenting systems at equilibrium, and many boundary velocity MCE and velocity sedimentation systems. The use of finite element simulations to fit velocity sedimentation data from interacting systems has been demonstrated for systems in which the reaction kinetics are fast enough to ensure chemical equilibrium throughout at all times [27]. This suggests that the same general approach to data analysis might be extendable to other types of complicated systems in both MCE and sedimentation. No method of analysis that has its basis in nonequilibrium thermodynamics, however, can independently yield information about molecular parameters, such as valence.

As discussed in Section 2.3, z^*_j is not a molecular parameter. Therefore, u^*_j, w_j and w_j^0 are not molecular parameters, and determining z_j from u^*_j, w_j or w_j^0 is complicated by the necessity to account for the electrophoretic effect and the asymmetry effect. For small, rigid proteins in some solvent conditions, at least, these complications appear to be surmountable, so that z_j can be obtained by comparing experimental electrophoretic measurements with corresponding results from either relatively simple calculations or detailed boundary element modeling [28].

The relatively simple calculations utilize Booth's or Henry's equation for the apparent electrophoretic mobility [15]. Henry's is the simplest, and can be written in terms of the valence and the diminished valence, giving

$$z_j^* = \frac{z_j H(\chi R_{E_j})}{1+\chi R_{E_j}}, \tag{71}$$

where R_{E_j} is the exclusion radius of species j, χ is the inverse Debye length (a function of T and Γ), and $H(\chi R_{E_j})$ is Henry's function, which increases sigmoidally from its lower limit of 1.0 to its upper limit of 1.5 as $\log(\chi R_{E_j})$ ranges from approximately -1 to 3. The Stokes radii of the spherical particles that are hydrodynamically equivalent to the central ion (species j) and its counterion (species c) are R_{S_j} and R_{S_c}, respectively. The sum of R_{S_j} and R_{S_c} is equal to R_{E_j}.

Use of Eq. (71) requires sufficient hydrodynamic data to determine R_{E_j} and sufficient electrophoretic data to determine z^*_j. For a membrane-confined, single-species component, in the limit as c_j approaches zero, $R_{S_j} = k_B T/6\pi\eta D^*_j$ [15], where η is the viscosity of the solution. (As the MCE instrument can be used to measure D^*_j of a membrane-confined, single-species component [29], both z^*_j and R_{S_j} could be obtained with data from this one instrument.) Tabulated values of D^*_c are generally used to estimate R_{S_c} as $k_B T/6\pi\eta D^*_c$. Efforts are under way to study the applicability of Henry's equation to experimental results with large, flexible proteins in solution.

5. List of parameters and constants

Due to the mixture of cgs and mks units commonly used in electrophoresis, the typical units of the parameters are given in parentheses. Similarly, the values and typical units of the constants are given in parentheses.

- γ: activity coefficient (dimensionless)
- Γ: ionic strength (mol/l)
- η: solution viscosity (g/cm s)
- Θ: Faraday conversion factor (299.7925×10^7 statcoulomb/Coulomb)
- κ: conductivity of the solution (Siemens/cm)
- μ: chemical potential (erg/mol)
- ν: signed stoichiometry used in the sum of the continuity equations (dimensionless)

Ξ_j: contribution of species j to the thermodynamic nonideality of the system (dimensionless)

Ξ^*_j: contribution of species j to the apparent nonideality of the system (dimensionless)

Ξ^*_p: polymeric apparent nonideality function (dimensionless)

ρ: solution density (g/cm^3)

σ: reduced molecular mass ($1/cm^2$)

τ: transference number (dimensionless)

$\bar{\upsilon}_j$: partial specific volume of the system with respect to species j (cm^3/g)

Φ: free energy dissipation function (erg/cm^3 s)

χ: inverse Debye length (1/cm)

Ψ: electric potential (statvolt)

ω: angular velocity (radian/s)

ϖ: electric field conversion factor (299.7925 V/statvolt)*

$a^*_{0_j}$: apparent activity of species j at x_0 (same dimensions as c)

A: cross-sectional area (cm^2)

A_g: molar affinity of reaction g (erg/mol)

b_k: $(k+1)$th virial coefficient of the electrohydrodynamic nonideality of the system ((mol/g)$(cm^3/g)^k$)

B_k: $(k+1)$th virial coefficient of the apparent thermodynamic nonideality of the system ((mol/g)$(cm^3/g)^k$)

B^*_k: $(k+1)$th virial coefficient of the apparent nonideality of the system ((mol/g)$(cm^3/g)^k$)

c: mass concentration (g/cm^3)

D: diffusion coefficient (cm^2/s)

e: elementary charge (4.8032354×10^{-10} statcoulomb)

$\hat{e}_x$: unit vector in the direction of the positive x-axis (dimensionless)

$\vec{E}$: electric field vector (V/cm)

f: hydrodynamic frictional coefficient (g/s)

F: Faraday (2.89253×10^{14} statcoulomb/mol)*

g: standard gravitational acceleration (980.7 cm/s^2)

G: magnetic field (T) or Coriolis force (dyne/g)

$H(\chi R_E)$: Henry's function (dimensionless)

$\vec{i}$: current vector (ampere)

$\vec{I}$: mass flux vector (g/cm^2 s)

$\vec{J}$: molar flux vector (mol/cm^2 s)

J^r_g: molar reaction flux of reaction g (mol/cm^3 s)

k_f: forward rate constant ($(cm^3/g)^n/s$, $n+1$ being the sum of the unsigned reactant stoichiometries)

k_r: reverse rate constant ($(cm^3/g)^m/s$, $m+1$ being the sum of the unsigned product stoichiometries)

k_B: Boltzmann constant (1.38066×10^{-16} erg/K)

K: equilibrium constant (treated as having the same dimensions as k_f/k_r)#

$L_{j,k}$: phenomenological coefficient linking the transport of species j to $\vec{X}_k$ (mol^2 s/g cm^3)

M: molar mass (g/mol)

N_A: Avogadro's number (6.022045×10^{23}/mol)

P: pressure ($dyne/cm^2$)

q_1: first coefficient of the Onsager equation ((l/mol)siemens/cm)

q_2: second coefficient of the Onsager equation $((l/mol)^{3/2}$siemens/cm)

Q: charge (statcoulomb)

r: radial position in a centrifuge rotor (cm^2)

R: ideal gas constant (8.31441×10^7 erg/mol K)*

R_E: exclusion radius (cm)

R_S: Stokes radius of a hydrodynamically equivalent spherical particle (cm)

s: sedimentation coefficient (s)

t: time (s)

T: absolute temperature (K)

u^*: apparent electrophoretic mobility (cm^2/V s)

U: total molar potential (erg/mol)

$\vec{v}$: transport velocity vector (cm/s)

w: reduced molecular charge (1/cm)

W: flow-independent reduced molecular charge (1/cm)

x: vertical position in an MCE cuvette (cm)

$\vec{X}_k$: conjugate molar force of the flux in species k (dyne/mol)

Y and Y_p: reduced molecular charge coefficients of stoichiometry (dimensionless)

z: valence (proton equivalent)

z^*: diminished valence (proton equivalent)

*With F and R in cgs units, $F/\varpi R = 11\,604.5$ K/V. This is also equal to the mks Faraday (96 484.56 coulomb/mol) divided by the mks ideal gas constant (8.31441 J/mol K).

#The equilibrium constant is typically expressed in terms of molar concentration, rather than mass concentration. For the most complicated reaction described in this paper, $\alpha A + \beta B + \chi X^{\pm\zeta} \leftrightarrows A_\alpha B_\beta X_\chi$, and neglecting nonideality, so that concentration can be substituted for activity in the expressions, K expressed in terms of mass concentration is given by $c_4/c_1^\alpha c_2^\beta c_3^\chi$, while K expressed in terms of molar concentration is given by $(c_4/c_1^\alpha c_2^\beta c_3^\chi)(M_1^\alpha M_2^\beta M_3^\chi/M_4)(1000\ \mathrm{cm}^3/\mathrm{l})^{1-\alpha-\beta-\chi}$, where the subscripts, 1, 2, 3 and 4, refer to species A, B, $X^{\pm\zeta}$ and $A_\alpha B_\beta X_\chi$, respectively. Strictly speaking, K, or any other quantity, must be made dimensionless before taking its logarithm. To make K dimensionless, the concentration of each species is first divided by the appropriate standard-state unit concentration, and K is then calculated using the resulting ratios in place of the corresponding concentrations.

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