Biophysical Chemistry 14 (1981) 389–397
Elsevier/North-Holland Biomedical Press

MATHEMATICAL MODEL FOR TRANSIENT ISOELECTRIC FOCUSING OF SIMPLE AMPHOLYTES

O.A. PALUSINSKI and M. BIER

Biophysics Technology Laboratory, University of Arizona, Tucson, AZ 85721, U.S.A.

and

D.A. SAVILLE

Department of Chemical Engineering, Princeton University, Princeton, NJ 08540, U.S.A.

Received 23 March 1981
Revised manuscript received 20 October 1981

A mathematical model describing transient processes in isoelectric focusing (IEF) of L biprotic ampholytes is presented. The model is a generalization of our previous research on steady state in IEF and consists of L nonlinear partial differential equations coupled with $2L+2$ algebraic equations. Constraints imposed by the mode of operation, viz., constant current, voltage or power, are described. Due to the nonlinearity of the equations, analysis of the model requires computer simulation. Model equations suitable for computer implementation are derived.

1. Introduction

Isoelectric focusing (IEF) is widely used for the analysis of complex protein mixtures because of its exquisite resolution. IEF is based on electrophoretic transport of sample components to their respective isoelectric points in a stable pH gradient. Most often, such gradients are generated naturally by the focusing of complex and chemically ill defined amphoteric mixtures (e.g., Ampholine).

A mathematical model has been recently developed which permits the computation of relevant steady-state parameters in IEF of electrochemically defined simple ampholytes and/or monovalent buffers. The model was formulated from fundamental principles describing the components' chemical equilibria, mass transport resulting from electromigration and diffusion, and electroneutrality [1]. The computer exercise of this model has rendered possible a rational selection of buffering components for experimental IEF. Several systems which have been developed and utilized for preparative IEF offer better resolution than that achievable using commercial ampholytes [2].

The approach to the steady state, particularly in low-voltage experiments, is long and complex. Since each experiment may require several days to achieve steady state [4,10,14], a model of the transient processes can be a useful tool for organizing experiments. This can be of great importance when working with materials that are unstable in the focusing milieu. Moreover, computer simulations can help conserve valuable materials which might otherwise be consumed in pilot experiments. The objective of this paper is to present an extension of the steady-state model which will encompass the transient states in IEF.

The mathematical description of transient states in IEF has previously received some attention. The analysis of Weiss et al. [3] relies on many simplifying assumptions. Two of these assumptions, namely: (a) a linear pH profile is preestablished, (b) the electrical field is uniform, are very restrictive. They are particularly inappropriate for systems employing ampholytes. Our steady-state model [1,2], as well as data from other laboratories [3–5], show conclusively that pH profiles are nonlinear. Moreover, Dishon and Weiss [6] have shown that pH profile nonlinearities may cause transient double peaks even with homogeneous samples. Descriptions of this behavior have appeared in the literature [7,8]. This underscores the weakness of the first assumption listed above.

Similar assumptions were made by Cann et al. [9,10]. Their model for transient IEF is based on transport in a uniform electric field ($\nabla\phi$). Computations using a numerical procedure with a fixed grid size revealed a high sensitivity of the solution to the prescribed field. This illustrates that the nonlinearity of the field cannot be neglected. These results were later compared with simulations using a distorted grid procedure [11]. Although there was good agreement in solute distributions, the responses to changes in the field were altered.

The numerous reports in the literature wherein the predicted behavior did not agree with experimental observations [5,7,12,12a,13] demonstrate the need for new theoretical developments.

In our mathematical model of transient IEF we eliminated the two assumptions (noted above as a and b) which render the existing theory incapable of explaining the observed phenomena. The pH gradient and electric field are not preset in our model, but are computed as part of the solution of the model equations.

The present model is based on our previous work [1,2,14,15], and the same simplifying assumptions are made: (1) The process is one dimensional (only the movements along the column axis are considered); (2) there is no macroscopic flow and no electroosmosis; (3) the system is isothermal and has uniform transport properties; (4) water is used as a solvent and only dilute solutions are considered; (5) the only electrode reactions are those generating H^+ and OH^-; (6) dissociation reactions are instantaneous; (7) the solution remains electrically neutral.

2. Chemical reactions

We shall consider here only simple ampholytes (A_j) which dissociate in water into single valence ions, i.e., A_j^+ and A_j^-. The approach proposed here can be easily extended to nonamphoteric buffers and to ampholytes characterized by more than two dissociation constants. The following notation for species concentration is used:

$$[H_2O] = n_0 M_0$$
$$[H^+] = M_1 = n_1 M_0$$
$$[OH^-] = M_2 = n_2 M_0$$

$$\left.\begin{aligned} [A_j] &= M_{3j} = n_{3j} M_0 \\ [A_j^+] &= M_{3j+1} = n_{3j+1} M_0 \\ [A_j^-] &= M_{3j+2} = n_{3j+2} M_0 \end{aligned}\right\} j = 1, 2, \ldots, L \qquad (1)$$

where M_0 is the reference concentration and L is the number of ampholytes in the system. The MKS system of units will be used. Thus, the M_i represent the concentrations in terms of mole/m^3 and the n_i are dimensionless variables. It is also worth noting that the system with L ampholytes will introduce $3L+3$ unknowns.

The water ionization process is characterized by the relation:

$$\tilde{K}_w = M_1 M_2$$

where $\tilde{K}_w$ is the water dissociation constant. Use of dimensionless variables results in the equation

$$n_1 n_2 = \frac{\tilde{K}_w}{M_0^2} = K_w \tag{2}$$

where K_w is the scaled water dissociation constant. The ampholyte dissociation reactions

$$A_j + H^+ \underset{C_{j2}}{\overset{C_{j1}}{\rightleftharpoons}} A_j^+$$

$$A_j^- + H^+ \underset{C_{j4}}{\overset{C_{j3}}{\rightleftharpoons}} A_j$$

can be described by the equilibrium equations

$$\begin{aligned} &M_{3j} M_1 - \tilde{K}_{j1} M_{3j+1} = 0 \\ &M_{3j+2} M_1 - \tilde{K}_{j2} M_{3j} = 0 \end{aligned} \tag{3}$$

where $\tilde{K}_{j1}$ and $\tilde{K}_{j2}$ denote dissociation constants. Use of dimensionless variables results in the equations

$$\begin{aligned} &n_{3j} n_1 - K_{j1} n_{3j+1} = 0 \\ &n_{3j+2} n_1 - K_{j2} n_{3j} = 0 \end{aligned} \tag{4}$$

where K_{j1} and K_{j2} denote scaled dissociation constants

$$K_{j1} = \frac{\tilde{K}_{j1}}{M_0}$$

$$K_{j2} = \frac{\tilde{K}_{j2}}{M_0}$$

3. Diffusion and electromigration

The flux of the i-th species caused by electromigration and diffusion is

$$-F_i = \Omega_i z_i M_i \frac{\partial \Phi}{\partial X} + \frac{RT}{e} \Omega_i \frac{\partial M_i}{\partial X} \tag{5}$$

where e is the molar charge or Faraday constant (96500 coulomb/mole), Φ the potential (V), R the universal gas constant (8.314 kg·m^2/s^2·K·mole), T the absolute temperature (K), X the distance from one end of the column (m), and Ω_i the mobility coefficient (m^2/V·s), z_i the valence, M_i the concentration (mole/m^3) of the i-th species. The Einstein relation between the mobility coefficient (Ω_i) and diffusion constant (D_i):

$$D_i = \frac{RT}{e} \Omega_i$$

illustrates how the mobility coefficient is defined.

The law of mass conservation of the i-th species in the absence of bulk flow is

$$\frac{\partial M_i}{\partial \tau} = -\frac{\partial F_i}{\partial X} + R_i \tag{6}$$

where R_i denotes the rate of production of the i-th species (mole/m^3·s), and τ is time (s).

The total production rate of the j-th ampholyte is zero

$$R_{3j}+R_{3j+1}+R_{3j+2}=0$$

and this results in L equations

$$\frac{\partial}{\partial\tau}(M_{3j}+M_{3j+1}+M_{3j+2})=\frac{\partial}{\partial X}[-F_{3j}-F_{3j+1}-F_{3j+2}];\quad j=1,2,\ldots,L. \tag{7}$$

There is no net production of charge at any point within the column, and this can be written as

$$\sum_{i=1}^{N} z_i R_i=0$$

where N is the number of species (for simple ampholytes $N=3L+2$). Taking into account eq. (6), the charge conservation equation can be expressed in the form

$$\frac{\partial}{\partial\tau}\sum_{i=1}^{N} z_i M_i=\frac{\partial}{\partial X}\sum_{i=1}^{N} z_i(-F_i) \tag{8}$$

The balance between charge and potential takes the form of the Poisson equation

$$\epsilon\frac{\partial^2\Phi}{\partial X^2}=-e\sum_{i=1}^{N} z_i M_i \tag{9}$$

where ϵ is the product of the dielectric constant and permittivity of free space which, for our work, is close to that for water, viz., $80\times 8.85\cdot 10^{-12}$ coulomb/V·m.

Using the species notation defined by eqs. (1) shows the valences to be:

$$\begin{aligned} &z_1=1,\ z_2=-1\\ &z_{3j}=0,\ z_{3j+1}=1,\ z_{3j+2}=-1;\quad j=1,2,\ldots,L. \end{aligned} \tag{10}$$

4. Simplifications of the mathematical model

The process description consists of eq. (2); $2L$ eq. (3); L eq. (7); eq. (8); and eq. (9). These involve $3L+2$ unknown concentrations, M_i, and the voltage, Φ. The number of equations is, therefore, consistent with the number of unknowns. To complete the description of the process the boundary conditions must be specified. Before specifying these conditions, however, it will be convenient to present the model equations in scaled form and discuss certain simplifications.

4.1. Scaling the equations

For any sort of consistent treatment it is necessary to express the equations in dimensionless form. We use eq. (1) and the following definitions

$$\begin{aligned} &\omega_i=\frac{\Omega_i}{\Omega_0};\ i=1,2,\ldots,N\\ &\phi=\frac{\Phi}{\Phi_0}\\ &x=\frac{X}{X_0}\\ &t=\frac{\tau}{T_0} \end{aligned} \tag{11}$$

where Ω_0, Φ_0, X_0 and T_0 are reference values for mobility, potential, length and time, respectively.

Using eq. (5) to eliminate F_i in eq. (7), making use of eq. (10), and scaling the resulting equations, yields L equations of the form

$$\frac{\partial}{\partial t}(n_{3j}+n_{3j+1}+n_{3j+2})=a\frac{\partial}{\partial x}\left[b(\omega_{3j+1}n_{3j+1}-\omega_{3j+2}n_{3j+2})\frac{\partial\phi}{\partial x}\right]+a\frac{\partial}{\partial x}\left(\omega_{3j}\frac{\partial n_{3j}}{\partial x}+\omega_{3j+1}\frac{\partial n_{3j+1}}{\partial x}+\omega_{3j+2}\frac{\partial n_{3j+2}}{\partial x}\right) \tag{12}$$

where a and b are the constants resulting from the scaling, viz.,

$$a=\frac{RT\,\Omega_0\,T_0}{eX_0^2},\; b=\frac{\Phi_0 e}{RT}.$$

Applying the scaling procedure to the charge conservation eq. (8) yields

$$\frac{\partial}{\partial t}\sum_{i=1}^{N} z_i n_i=a\frac{\partial}{\partial x}\sum_{i=1}^{N} z_i f_i \tag{13}$$

where

$$f_i=bz_i\omega_i n_i\frac{\partial\phi}{\partial x}+\omega_i\frac{\partial n_i}{\partial x}.$$

The scaled Poisson equation is

$$q\frac{\partial^2\phi}{\partial x^2}=-\sum_{i=1}^{N} z_i n_i \tag{14}$$

where q is the dimensionless constant resulting from scaling

$$q=\frac{\epsilon}{e}\frac{\Phi_0}{X_0^2\,M_0}$$

4.2. Electroneutrality approximation

We assume that the solution is electrically neutral, therefore the Poisson eq. (14) is replaced by

$$\sum_{i=1}^{N} z_i n_i=0 \tag{15}$$

often called the electroneutrality equation or electroneutrality approximation * [16–18]. Using eq. (15) in the charge conservation eq. (13) yields

$$\frac{\partial}{\partial x}\sum_{i=1}^{N} z_i f_i=0.$$

This equation can be integrated once, resulting in the relation determining the dimensionless current

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* The validity of the electroneutrality approximation is readily established for the equations under study by noting that here q is extremely small so that $|q\partial^2\phi/\partial x^2|\ll 1$. This argument also illustrates why the potential cannot be a linear function of position but must be established by solving the appropriate differential equations, not $\partial^2\phi/\partial x^2=0$.

density

$$\sum_{i=1}^{N} z_i f_i = -\eta.$$

Note that the dimensionless current density η can be a function of time. Taking into account the definitions of scaled fluxes f_i we obtain

$$b \sum_{i=1}^{N} \left[z_i^2 \omega_i n_i\right] \frac{\partial \phi}{\partial x} + \sum_{i=1}^{N} z_i \omega_i \frac{\partial n_i}{\partial x} = -\eta. \tag{16}$$

It is easy to verify that the current density J and the dimensionless current density η are related as follows

$$\eta = \frac{X_0}{\Omega_0 M_0 RT} J.$$

5. Boundary conditions

It is assumed that the only reactions occurring at the electrodes are those generating the H^+ and OH^-, which are the only current carriers. Thus, the ampholyte fluxes at the column ends are zero. The boundary conditions can therefore be expressed as $L+1$ relations

$$F_{3j} + F_{3j+1} + F_{3j+2} = 0; \quad j = 1, 2, \ldots, L$$

$$z_1 F_1 + z_2 F_2 = -J$$

which have to be enforced at $X=0$ and $X=X_{\max}$. Using eqs. (5) and (10) in the above and scaling the resulting equations we obtain these conditions in the form

$$b(\omega_{3j+1} n_{3j+1} - \omega_{3j+2} n_{3j+2}) \frac{\partial \phi}{\partial x} + \frac{\partial}{\partial x}(\omega_{3j} n_{3j} + \omega_{3j+1} n_{3j+1} + \omega_{3j+2} n_{3j+2}) = 0;$$
$$x = 0 \text{ and } \xi = \frac{X_{\max}}{X_0}, \quad j = 1, 2, \ldots, L. \tag{17}$$

and

$$b(\omega_1 n_1 + \omega_2 n_2) \frac{\partial \phi}{\partial x} + \omega_1 \frac{\partial n_1}{\partial x} - \omega_2 \frac{\partial n_2}{\partial x} = -\eta; \quad x = 0 \text{ and } \xi \tag{18}$$

There are also L initial conditions to be specified. These conditions are obtained by taking into account the fact that the total concentration of the j-th ampholyte (n_{tj}) is related to the concentrations of the subspecies by:

$$n_{tj} = n_{3j} + n_{3j+1} + n_{3j+2}.$$

Since the initial total concentration of each ampholyte in any experiment is known or can be easily determined (in most cases the ampholytes are initially uniformly distributed in the column), we can write

$$n_{3j}(x,0) + n_{3j+1}(x,0) + n_{3j+2}(x,0) = \psi_j(x), \quad j = 1, 2, \ldots, L \tag{19}$$

where $\psi_j(x)$ denotes the given initial (total) distribution of the j-th ampholyte.

6. Conditions imposed by the mode of column operation

The simplified mathematical model of transient IEF consists of $L+1$ partial differential eqs. (12) and (16), and of $2L+2$ nonlinear algebraic eqs. (2), (4) and (15). A particular solution of the model is determined by $2L+2$ boundary conditions, eqs. (17) and (18), and L initial conditions, eqs. (19). In general, there are three modes of controlling the focusing process: it can be run at constant current, constant voltage, or constant electrical power. The model presented above is complete for the case of constant current. In order to describe the other two cases, this model has to be augmented by adding an equation which determines the current density as a function of the specified voltage or power.

6.1. Constant voltage mode

Introducing the symbol κ for dimensionless conductivity

$$\kappa=b\sum_{i=1}^{N} z_i^2\omega_i n_i$$

we can write eq. (16) in the form

$$\frac{\partial\phi}{\partial x}=\frac{\eta}{\kappa}-\frac{\sum\limits_{i=1}^{N} z_i\omega_i\frac{\partial n_i}{\partial x}}{\kappa}. \tag{20}$$

This equation can be integrated along the column axis yielding the following relation between voltage difference applied to the column, $\phi(\xi)-\phi(0)=-\Delta$, and current density

$$\eta\int_0^{\xi}\frac{\mathrm{d}x}{\kappa}+\int_0^{\xi}\frac{1}{\kappa}\sum_{i=1}^{N}\left[\omega_i z_i\frac{\partial n_i}{\partial x}\right]\mathrm{d}x=\Delta. \tag{21}$$

Thus, eq. (21) is the equation which must be added to the model equations to describe operation with a fixed voltage difference, Δ.

6.2. Constant power mode

The relation between the power p (dimensionless) dissipated in the column and the current density can be obtained by integrating the local power $(-(\partial\phi/\partial x)\eta)$ dissipated along the column axis. Taking into account eq. (20) or (21) we obtain easily the following relation

$$\eta^2\int_0^{\xi}\frac{\mathrm{d}x}{\kappa}+\eta\int_0^{\xi}\frac{1}{\kappa}\sum_{i=1}^{N}\left[\omega_i z_i\frac{\partial n_i}{\partial x}\right]\mathrm{d}x=p \tag{22}$$

which is used to determine the current density for the case of constant power input.

7. Model transformation

The model of transient IEF in the form presented above is not suitable for numerical treatment, which is the only choice in further analysis because of the profusion of nonlinearities. The approach used in ref. [19] will be followed here with vectors $\boldsymbol{u}$, $\boldsymbol{v}$, $\boldsymbol{p}$, $\boldsymbol{s}$ and matrices $\boldsymbol{Q}$, $\boldsymbol{R}$, $\boldsymbol{D}(\boldsymbol{u})$ defined as before. Thus, we use the vector notation

$$\boldsymbol{u}^T=(n_3, n_6, n_9, \ldots, n_{3L})$$
$$\boldsymbol{v}^T=(n_1, n_2, n_4, n_5, \ldots, n_{3L+1}, n_{3L+2}). \tag{23}$$

Introducing a matrix $\boldsymbol{T}$ with entries

$$t_{ij}=\begin{cases}1; j=2i+1 \text{ or } j=2i+2\\ 0; j\neq 2i+1 \text{ and } j\neq 2i+2\end{cases} \tag{24}$$

and using the vector $\boldsymbol{p}$ and the matrices $\boldsymbol{Q}$ and $\boldsymbol{R}$ (defined in ref. [19]) we can write eq. (12) in the form

$$\frac{\partial \boldsymbol{u}}{\partial t}+\boldsymbol{T}\frac{\partial \boldsymbol{v}}{\partial t}=a\frac{\partial}{\partial x}\left[\boldsymbol{p}\frac{\partial \phi}{\partial x}+\boldsymbol{Q}\frac{\partial \boldsymbol{u}}{\partial x}+\boldsymbol{R}\frac{\partial \boldsymbol{u}}{\partial x}\right]. \tag{25}$$

Using the vector $\boldsymbol{s}$ (defined in ref. [19]) we write eq. (20) in the form

$$\frac{\partial \phi}{\partial x}=-\frac{\eta}{\kappa}-\frac{1}{\kappa}\boldsymbol{s}^T\frac{\partial \boldsymbol{v}}{\partial x}$$

which when used in eq. (25) yields

$$\frac{\partial \boldsymbol{u}}{\partial t}+\boldsymbol{T}\frac{\partial \boldsymbol{v}}{\partial t}=a\frac{\partial}{\partial x}\left[-\frac{1}{\kappa}\eta\boldsymbol{p}+\left(\boldsymbol{R}-\frac{1}{\kappa}\boldsymbol{p}\boldsymbol{s}^T\right)\frac{\partial \boldsymbol{v}}{\partial x}+\boldsymbol{Q}\frac{\partial \boldsymbol{u}}{\partial x}\right] \tag{26}$$

The elements of the vector $\boldsymbol{v}$ are related to the elements of the vector $\boldsymbol{u}$ by the algebraic relations eqs. (2), (4) and (15) and the vector $\boldsymbol{v}$ can be eliminated using these relations. Next the derivatives of the vector $\boldsymbol{v}$ can be eliminated using the relations

$$\begin{aligned}\frac{\partial \boldsymbol{u}}{\partial t}&=\boldsymbol{D}(\boldsymbol{u})\frac{\partial \boldsymbol{u}}{\partial t}\\ \frac{\partial \boldsymbol{v}}{\partial x}&=\boldsymbol{D}(\boldsymbol{u})\frac{\partial \boldsymbol{u}}{\partial x}\end{aligned} \tag{27}$$

where the matrix $\boldsymbol{D}(\boldsymbol{u})$ is given in ref. [19]. Elimination of the variables ($\boldsymbol{v}$) in eq. (26) (performed using eq. (27) and algebraic relations) yields a convenient form of the differential equations in the model

$$\boldsymbol{A}(\boldsymbol{u})\frac{\partial \boldsymbol{u}}{\partial t}=a\frac{\partial}{\partial x}\left[\boldsymbol{B}(\boldsymbol{u})\frac{\partial \boldsymbol{u}}{\partial x}-\frac{\eta}{\kappa}\boldsymbol{p}\right] \tag{28}$$

The square ($L\times L$) matrices $\boldsymbol{A}(\boldsymbol{u})$ and $\boldsymbol{B}(\boldsymbol{u})$ are

$$\begin{aligned}\boldsymbol{A}(\boldsymbol{u})&=\boldsymbol{I}+\boldsymbol{T}\boldsymbol{D}(\boldsymbol{u})\\ \boldsymbol{B}(\boldsymbol{u})&=\left(\boldsymbol{R}-\frac{1}{\kappa}\boldsymbol{p}\boldsymbol{s}^T\right)\boldsymbol{D}(\boldsymbol{u})+\boldsymbol{Q}\end{aligned} \tag{29}$$

where $\boldsymbol{I}$ is the unit matrix. An analogous variable elimination procedure applied to boundary conditions, eq. (17), yields

$$\boldsymbol{p}\frac{\partial \phi}{\partial x}+[\boldsymbol{Q}+\boldsymbol{R}\boldsymbol{D}(\boldsymbol{u})]\frac{\partial \boldsymbol{u}}{\partial x}=0;\ x=0 \text{ and } \xi \tag{30}$$

The potential gradient $\partial\phi/\partial x$ is determined by eq. (18), but n_1 and n_2 are functions of the vector $\boldsymbol{u}$. In the same manner the variables $n_{3j+1}(x,0)$ and $n_{3j+2}(x,0)$ in eq. (19) are eliminated and provide initial conditions for the entries of the $\boldsymbol{u}$ vector. When the column is operated under constant current, eq. (28) together with boundary conditions eqs. (18), (30) and initial conditions eq. (19) determine the model solution. In the case of constant voltage or constant power the current density (η) has to be determined by solving eq. (21) or (22).

8. Concluding remarks

The mathematical model of transient IEF is described by a set of nonlinear partial differential eqs. (28) with boundary conditions eqs. (18) and (30) and initial conditions eq. (19). The particular case of the steady state with current control has already been treated numerically [1,2,19] and computational experiments with the steady-state model showed good qualitative agreement [2,14] with laboratory experiments. Further model validation studies are currently being conducted. Certain numerical problems associated with the stiffness of the equations and high accuracy required when solving boundary problems numerically by 'shooting methods' had to be overcome and it is anticipated that similar difficulties will be present when solving the transient state model. Additional difficulties will be caused by the way the initial conditions are assigned. Another problem requiring attention is the determination of the current density when the column is operated with either constant voltage or constant power.

Acknowledgement

This work was supported in part by NASA Contract NAS8-32950 and NASA Grant NSG-7333.

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