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MATHEMATICAL MODELING AND COMPUTER SIMULATION OF ISOELECTRIC FOCUSING WITH ELECTROCHEMICALLY DEFINED AMPHOLYTES

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A mathematical model of isoelectric focusing at the steady state has been developed for an M-component system of electrochemically defined ampholytes. The model is formulated from fundamental principles describing the components' chemical equilibria, mass transfer resulting from diffusion and electromigration, and electroneutrality. The model consists of ordinary differential equations coupled with a system of algebraic equations. The model is implemented on a digital computer using FORTRAN-based simulation software. Computer simulation data are presented for several two-component systems showing the effects of varying the isoelectric points and dissociation constants of the constituents.

1. Introduction

In isoelectric focusing (IEF) a stable "natural" pH gradient is created by electrophoretic migration of carrier ampholytes to stationary zones in the separation column. Ampholytic sample components introduced in the system distribute themselves on the pH gradient so that they exhibit concentration maxima at their respective isoelectric points.

The initial theory of steady-state IEF is attributable to Svensson [1] and was developed in conjunction with the introduction of the technique. Svensson addressed the one-component system, i.e., the concentration distribution of a pure end-component, and also the concentration distribution of an ampholytic component added to a system whose pH and conductance are known as a function of the distance along the separation axis. Subsequent considerations of the theory of IEF have commonly assumed stable linear pH and conductivity gradients [2–6]. Such systems can be experimentally approximated as a result of Vesterberg's [7] development of a random mixture of carrier ampholytes, commercially introduced by LKB Produkter AB of Bromma, Sweden, under the tradename Ampholine. The wide applicability and ready availability of Ampholine and other similar products have assured the remarkable growth of various IEF techniques. Unfortunately, they have also dampened the stimulus for further development of the theory of steady state IEF, as their composition and electrochemical properties are unknown.

The most advanced model hitherto available is due to Almgren [8]. Unfortunately, some of the simplifying assumptions have limited the applicability of this model. These assumptions were: (i) the mobilities of all components of the mixture, except hydrogen and hydroxyl ions, are identical: the ratio of hydrogen and hydroxyl ion mobilities to mobilities of the remaining species is constant, i.e., 7 and 3 respectively; (ii) the current density is proportional to the conductivity and the voltage gradient, i.e., the contribution of diffusional current is neglected. This latter assumption might be justified in specific situations but is not valid in many practical situations, particularly involv-

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ing steep gradients in dilute solutions at low or high pH values.

The specific goal of the present theoretical treatment is to develop a mathematical description of an M-component IEF system capable of predicting the component concentrations, pH, conductance, and related parameters at every point along the column axis as a function of the components' mobilities and proton dissociation constants. Graphical data from the computer simulations are presented for several two-component systems to illustrate the treatment and the results.

Recently a great deal of interest has focused upon the modification of pH gradients established by commercially available carrier ampholytes [9,10] and the generation of natural pH gradients by use of well-defined ampholytes or buffers [11–14]. It is generally appreciated that the amounts of the individual ampholytes and their electrochemical characteristics must uniquely determine the shape of the pH gradient, the sample capacity, and the resolution. It should also be appreciated that the empirical approaches presently employed suffer from the absence of a model which demonstrates the contribution of individual ampholytes to gradient formation. Significant progress could not be reasonably expected without the development of a theoretical treatment to accompany this empirical thrust.

In fact, our accumulated library of simulations, comprising numerous mixtures of compounds with actual and arbitrarily assigned electrochemical properties, has proved most useful in the selection of components utilized in experimental IEF. The experimental studies, which include a verification of the model, confirm a most surprising outcome of the simulations, namely, that very low voltages are sufficient for full deployment of pH gradients. In contrast, at the voltages normally used in IEF with commercial carrier ampholytes, steep step-gradients are obtained, such as are more readily associated with isotachophoresis than with IEF. These detailed results will be presented in a separate paper [15].

2. Basic equations of steady state isoelectric focusing

The model describing a one-dimensional IEF system is based upon the following assumptions:

(1) The system is at steady state and the net flux of any species is zero, i.e., there is a balance between the mass transport by electromigration and diffusion.

(2) The concentrations of component subspecies are related by equations of chemical equilibria, i.e., chemical reactions (proton associations and dissociations) are rapid relative to the transport processes.

(3) The system is one-dimensional and the effects of electro-osmosis and radial and longitudinal temperature gradients are omitted.

(4) The condition of electroneutrality prevails on the physical scale of the system under consideration.

In the computations to date it was also assumed that only the two apparent dissociation constants (pK's) nearest the isoelectric point (pI) of an ampholyte influence its behavior in the steady state, i.e., only simple ampholytes are considered. This assumption is not essential and the model can readily be extended to include more complicated ampholytes or non-amphoteric buffers, where necessary.

2.1. General equations

Flux, F_i, (mole/m^{2}s) of ith species can be expressed as

$$-F_i = w_i z_i n_i \frac{d\phi}{dx} + \frac{RT}{e} w_i \frac{dn_i}{dx}, \qquad (1)$$

where n_i is concentration (mole/m^3), z_i is valence (dimensionless), ϕ is local electric potential (V), R is universal gas constant (8.314 J/K mole), e is Faraday constant (96 500 C/mole), T is temperature (K), C is Coulomb, and x is the distance along the column axis (m). The symbol w_i represents a mobility coefficient (m^{2}s^{-1}V^{-1}) related to conventional electrophoretic mobility (μ_i) by $\mu_i = w_i z_i$ and to the diffusion constant (D_i) by $D_i = (RT/e)w_i$. Scaling was introduced in the computer implemented model to reduce the round-off error in integration.

Conservation of mass (conservation of ith species) implies that

$$dF_i/dx + R_i = 0, \qquad (2)$$

where R_i is the rate of production of ith species per unit volume (mole/m^{3}s). The Poisson equation describes the relation between the potential and the net charge

$$\epsilon \mathrm{d}^2\phi/\mathrm{d}x^2 = -e\sum_{i=1}^{N} z_i n_i \qquad (3)$$

where ϵ is dielectric constant of water ($80 \times 8.85 \times 10^{-12}$ C/Vm), and N is number of species.

We assume that in the system under consideration there is no net production of charge, i.e., conservation of charge is expressed by the equation

$$\sum_{i=1}^{N} e z_i R_i = 0\,. \qquad (4)$$

From the assumption that there is no net flux of any ampholyte the conservation of jth ampholyte can be written in the following form

$$\sum_{i=m_{j1}}^{i=m_{j2}} R_i = 0 \qquad (5)$$

where $R_{m_{j1}}, R_{m_{j1+1}} \ldots, R_{m_{j2}}$ denote rates of production of species with subscripts $m_{j1}, m_{j1+1}, \ldots m_{j2}$, which result from the dissociation of the jth ampholyte.

2.2. Principles of model construction

2.2.1. The conservation of charge equation

Multiplying (2) by the charge ez_i, adding these equations for all species and taking (4) into account gives

$$\frac{\mathrm{d}}{\mathrm{d}x}\sum_{i=1}^{N} e z_i F_i = 0\,. \qquad (6)$$

Eq. (6) can be integrated once, giving

$$\sum_{i=1}^{N} e z_i F_i = -J\,, \qquad (7)$$

where J is current density.

Using (1) in the above we get

$$e\left(\sum_{i=1}^{N} z_i^2 w_i n_i\right)\frac{\mathrm{d}\phi}{\mathrm{d}x} + RT\sum_{i=1}^{N} z_i w_i \frac{\mathrm{d}n_i}{\mathrm{d}x} = -J\,, \qquad (8)$$

which represents the generalized form of Ohm's law for an electrolyte in steady state.

2.2.2. Ampholyte conservation

A procedure similar to the one used above can be applied here. The equations (2) for component subspecies of jth ampholyte are added, and taking into account (5) we obtain

$$\frac{\mathrm{d}}{\mathrm{d}x}\sum_{i=m_{j1}}^{i=m_{j2}} F_i = 0\,. \qquad (9)$$

Eq. (9) can be integrated, which gives the flux

$$\sum_{i=m_{j1}}^{i=m_{j2}} F_i = c_j, \quad j = 1, 2, \ldots, M \qquad (10)$$

of a jth ampholyte. The flux of any ampholyte is zero ($c_j = 0$) since we assume steady state. Making use of (1) in (10) with $c_j = 0$ results in the following M equations

$$e\left(\sum_{i=m_{j1}}^{i=m_{j2}} z_i w_i n_i\right)\frac{\mathrm{d}\phi}{\mathrm{d}x} + RT\sum_{i=m_{j1}}^{i=m_{j2}} w_i \frac{\mathrm{d}n_i}{\mathrm{d}x} = 0\,, \qquad (11)$$

$$j = 1, 2, \ldots, M$$

Eq. (8) and eqs. (11) constitute a system of $M + 1$ differential equations with $N + 1$ unknowns ($n_1, n_2, \ldots, n_N$). For model completeness, some other relations are needed since $N > M$.

2.2.3. Chemical equilibria

The concentrations of component subspecies are related by the dissociation constants. Consider, for example, a simple ampholyte A_j which dissociates into single valence ions A_j^+ and A_j^-. In such a case $N = 3M + 2$, taking into account the 3 species of each ampholyte and the hydrogen and hydroxyl ions.

We use the following notation:

$$[\mathrm{H}^+] = n_1, \qquad [\mathrm{OH}^-] = n_2\,,$$

$$[\mathrm{A}_j] = n_{m_{j1}} = n_{3j}, \quad [\mathrm{A}_j^+] = n_{m_{j1+1}} = n_{3j+1}\,,$$

$$[\mathrm{A}_j^-] = n_{m_{j2}} = n_{3j+2}, \quad j = 1, 2, \ldots, M\,. \qquad (12)$$

The chemical reactions are characterized by the dissociation constants, which are defined in terms of concentrations

$$K_{j1} = n_1 n_{3j}/n_{3j+1}, \qquad K_{j2} = n_1 n_{3j+2}/n_{3j}\,,$$

$$K_w = n_1 n_2\,. \qquad (13)$$

For such a system of simple ampholytes the valences are

$$z_{3j} = 0, \qquad z_{3j+1} = 1, \qquad z_{3j+2} = -1. \tag{14}$$

The total ampholyte concentration (n_{tj}) can be expressed in the form

$$n_{tj} = n_{3j} + n_{3j+1} + n_{3j+2}. \tag{15}$$

2.3. *Electroneutrality condition*

We assume that the length of the x axis is of the order of centimeters and, thus, the rate of change of the electric field with x is small compared to eM_i/ϵ, which is of the order of 10^{16} for a 0.1 M solution. Thus, we can replace the Poisson equation by an algebraic one representing the electroneutrality approximation in the form

$$\sum_{i=1}^{N} z_i n_i = 0. \tag{16}$$

Making use of (14) and (16) we obtain

$$n_1 - n_2 + \sum_{j=1}^{M} (n_{3j+1} - n_{3j+2}) = 0. \tag{17}$$

The model of IEF consists of eq. (8) representing the charge conservation, M equations expressing the ampholytes conservation, (11), $2M + 1$ equations describing chemical reactions, (13), and one equation representing the electroneutrality condition, (17). Thus we have $3M + 3$ equations describing fully the system with M simple ampholytes which has $3M + 3$ unknowns.

3. Construction of the model for computer implementation

3.1. *Mathematical model of IEF with simple ampholytes*

We shall denote the conductivity as

$$\sigma = e \sum_{i=1}^{N} z_i^2 w_i n_i \tag{18}$$

and write the charge conservation equation in the form

$$\sigma \frac{d\phi}{dx} + RT \sum_{i=1}^{N} z_i w_i \frac{dn_i}{dx} = -J. \tag{19}$$

Eqs. (11) for simple ampholytes have the form

$$e(w_{3j+1} n_{3j+1} - w_{3j+2} n_{3j+2}) d\phi/dx + RT\left(w_{3j} \frac{dn_{3j}}{dx} + w_{3j+1} \frac{dn_{3j+1}}{dx} + w_{3j+2} \frac{dn_{3j+2}}{dx}\right) = 0, \quad j = 1, 2, \ldots, M. \tag{20}$$

In order to represent eqs. (20) in a more convenient matrix form we introduce the column vectors $\boldsymbol{b}$ and $\boldsymbol{y}$, and the matrix A with entries defined as follows

$$b_j = e(n_{3j+1} w_{3j+1} - n_{3j+2} w_{3j+2}), \tag{21}$$

$$y_k = n_k, \tag{22}$$

$$a_{jk} = \begin{cases} 0, & k < 3j \text{ or } k > 3j + 2 \\ \sigma w_k, & 3j \leqslant k \leqslant 3j + 2 \end{cases} \qquad j = 1, 2, \ldots, M, \quad k = 1, 2, \ldots, N. \tag{23}$$

The notation (20)–(23) yields the ampholyte conservation equations in the form

$$\boldsymbol{b} \frac{d\phi}{dx} + RT\mathsf{A} \frac{d}{dx} \boldsymbol{y} = 0. \tag{24}$$

Taking (14) into account and introducing the N-dimensional row vector $\boldsymbol{c}$ with the entries defined by the following relations

$$c_k = \begin{cases} 0 & k = 3j, j = 1, 2, \ldots, M \\ (-1)^{k+1} w_k & \text{for all other } k\text{'s} \end{cases}, \qquad k = 1, 2, \ldots, 3M + 2 \tag{25}$$

and using the vector $\boldsymbol{y}$ we can write (19) in the form

$$\sigma \frac{d\phi}{dx} + RT\boldsymbol{c} \frac{d}{dx} \boldsymbol{y} = -J. \tag{26}$$

Multiplying (24) by the scalar σ and using (26) to eliminate $\sigma\, d\phi/dx$ yields

$$(-\boldsymbol{bc} + \sigma\mathsf{A}) \frac{d}{dx} \boldsymbol{y} = -\frac{J}{RT} \boldsymbol{b}. \tag{27}$$

The product of column vector $\boldsymbol{b}$ and row vector $\boldsymbol{c}$ forms a rectangular matrix with entries defined as follows

$$(-bc)_{jk}=\begin{cases}0 & k=3l,\ l=1,2,\ldots,M,\\ (-1)^j b_j w_k & \text{in all other cases},\end{cases}$$
$$j=1,2,\ldots,M,\quad k=1,2,\ldots,3M+2\,. \tag{28}$$

The equations (27) with rectangular $M\times(3M+2)$ matrix $(-bc+\mathbf{A})$ are basic differential equations with $3M+2$ unknowns. The algebraic relations (13) and (17) can be easily represented in the form

$$n_1=\left[\frac{K_w+\Sigma_{i=1}^M K_{i2}n_{3i}}{1+\Sigma_{i=1}^M n_{3i}/K_{i1}}\right]^{1/2},\quad n_2=K_w/n_1\,,$$
$$n_{3j+1}=\frac{1}{K_{j1}}\,n_1 n_{3j},\qquad n_{3j+2}=K_{j2}n_{3j}/n_1\,. \tag{29}$$

The model of steady state IEF with simple ampholytes which involves $3M+2$ unknowns is thus composed of M differential equations (27) and $2M+2$ algebraic equations (29).

3.2. Computer implementation

The model has to be transformed to a form suitable for computer implementation. There is readily available simulation software designed for models written in the form of a system of first order differential equations, and so the relations (29) were used to generate additional $2M+2$ differential equations of the form

$$\mathbf{D}\frac{\mathrm{d}}{\mathrm{d}x}y=0\,, \tag{30}$$

where the entries of the matrix $\mathbf{D}$ are functions of the unknowns n_i. The matrix $\mathbf{D}$ is rectangular with dimensions $(2M+2)\times(3M+2)$.

In order to simplify the expressions for the entries of matrix $\mathbf{D}$ which appears to be sparse, we introduce the following definitions

$$Q=\tfrac{1}{2}\left[\left(K_w+\sum_{i=1}^M K_{i2}n_{3i}\right)\left(1+\sum_{j=1}^M n_{3j}/K_{j1}\right)^3\right]^{-1/2},$$
$$B_j=Q(K_{j2}-K_w/K_{j1})\,,$$
$$P_{ij}=Q(K_{j2}/K_{i1}-K_{i2}/K_{j1})\,,$$
$$G_j=B_j+\sum_{i=1}^M P_{ij}n_{3i}\,. \tag{31}$$

Using the function G_j defined above we can represent the entries of matrix $\mathbf{D}$ in compact form. The first two rows of the matrix are defined by the formulas:

$$d_{1k}=\begin{cases}1 & k=1\,,\\ -G_j & k=3j\,,\\ 0 & \text{all other } k\text{'s}\,,\end{cases}$$
$$d_{2k}=\begin{cases}K_w/n_1^2 & k=1\,,\\ 1 & k=2\,,\\ 0 & \text{all other } k\text{'s}\,,\end{cases} \tag{32}$$
$$k=1,2,\ldots,3M+2\,.$$

The remaining entries of the matrix $\mathbf{D}$ are defined as follows for the odd rows ($i=2j+1$; $j=1,2,\ldots,M$)

$$d_{ik}=\begin{cases}-n_{3j}/K_{j1} & k=1\,,\\ -n_1/K_{j1} & k=3j\,,\\ 1 & k=3j+1\,,\\ 0 & \text{all other } k\text{'s}\,,\end{cases} \tag{33a}$$

for even rows ($i=2j+2$; $j=1,2,\ldots,M$)

$$d_{ik}=\begin{cases}K_{j2}n_{3j}/n_1^2 & k=1\,,\\ -K_{j2}/n_1 & k=3j\,,\\ 1 & k=3j+2\\ 0 & \text{all other } k\text{'s}\,.\end{cases} \tag{33b}$$

The M differential equations (27) and $2M+2$ differential equations (30) constitute the complete model of IEF in steady state with $3M+2$ unknowns. These equations can be written together in the form

$$\begin{array}{r}M\left\{\vphantom{\begin{array}{c}a\\a\end{array}}\right.\\ 2M+2\left\{\vphantom{\begin{array}{c}a\\a\\a\end{array}}\right.\end{array}\underbrace{\left[\begin{array}{c}-bc+\sigma\mathbf{A}\\ \\ \hline \\ \mathbf{D}\end{array}\right]}_{3M+2}\frac{\mathrm{d}}{\mathrm{d}x}y=-\frac{J}{RT}\left[\begin{array}{c}b\\ \hline 0\\ \vdots\\ 0\end{array}\right]\begin{array}{l}\left.\vphantom{\begin{array}{c}a\end{array}}\right\}M\\ \left.\vphantom{\begin{array}{c}a\\a\\a\end{array}}\right\}2M+2\end{array} \tag{34}$$

The total amount of each ampholyte in the column is given by

$$A_j=S\int_0^L n_{tj}(n_1(0),n_2(0),\ldots,n_{3M+2}(0),x)\,\mathrm{d}x\,, \tag{35}$$

where L is the column length and S is the cross-sectional area of the column. For actual simulations, initial boundary concentrations need to be assigned. In practice, these were usually computed by assigning the desired concentrations at the center of the column axis and then integrating backward and forward.

4. Computer simulations

Computer simulations have been implemented using DARE P simulation software [16] with the Runge-Kutta-Merson method chosen for integration. A variety of systems were studied at the level of one, two and three ampholytes. The simulations are set up by entering specific values for the applied current, the initial (boundary) concentrations of the ampholytes, and the electrochemical characteristics for each ampholyte which define its electrophoretic behavior, i.e., its proton dissociation constants (pK) and mobility coefficients (w). Different mobility coefficients can be entered for the negative, neutral and positive species of each ampholyte, as they may differ by as much as 5%. To represent a given experimental setup, the cross-sectional area and length of the column also have to be specified.

In some cases the above parameters have been given values which reflect real compounds, while in other instances values representing purely hypothetical compounds have been used. Simulations with hypothetical compounds, i.e., ampholytes with arbitrarily assigned parameters, have permitted us to study the effects of a systematic variation of one parameter at a time. Two such studies will be presented here, as they are of particular interest for the experimentalist: the effects of varying the spread between the pI values (ΔpI) and the spread between the pK values (ΔpK) of constituent ampholytes in two-component systems.

4.1. Effects of varying ΔpI at constant ΔpK

The following computations illustrate the effects of varying the spread between the isoelectric points (ΔpI) of ampholytes in binary mixtures. Three such mixtures were compared, with ΔpI of 0.5, 1 and 2 pH units, symmetrically distributed around pH 6.5. Table 1 summarizes the input parameters: As can be seen, all compounds were assumed to have the same mobility coefficient ($cm^2/V\ s$) and the model was exercised for an assumed column length of 1 cm.

The pH profiles for the above three systems are shown in fig. 1. As expected, the pH profiles span the range between the pI values of the constituent ampholytes in each instance.

Fig. 2 shows the profiles along the column length for the total ampholyte concentrations corresponding to the above pH profiles. As can be seen, these profiles are nearly superimposable. To achieve this, it was necessary to exercise the models at varying current densities, as reported in table 1. While these profiles are superimposable, significant differences are seen when the concentrations of the neutral species alone are plotted, as shown in fig. 3. In all systems, the more acidic components focus at the anode as zwitterions, the more basic ones focusing at the cathode. The system with a ΔpI of 2 has the lowest concentration of

Table 1
Effect of varying ΔpI at constant ΔpK: input parameters

System #	Component #	ΔpI	pK_1	pK_2	Mobility coef. × 10^4	Current (mA/cm^2)
A	1	0.5	5.25	7.25	3.0	0.046
	2		5.75	7.75	3.0	
B	3	1.0	5.0	7.0	3.0	0.0288
	4		6.0	8.0	3.0	
C	5	2.0	4.5	6.5	3.0	0.023
	6		6.5	8.5	3.0	

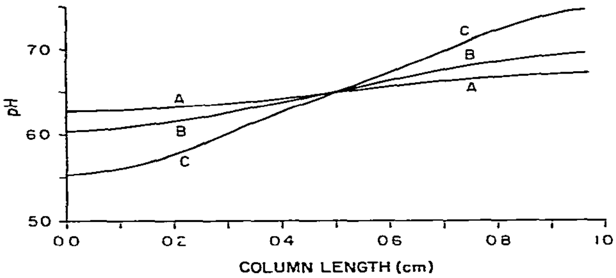

Fig. 1. Computed pH profiles for IEF of three two-component systems with ΔpI of 0.5, 1, and 2 pH units and ΔpK of 2. For other input parameters see table 1.

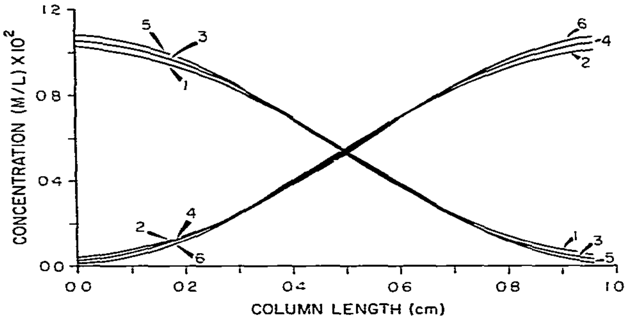

Fig. 2. Computed profiles of total ampholyte concentration corresponding to the three pH profiles presented in fig. 1.

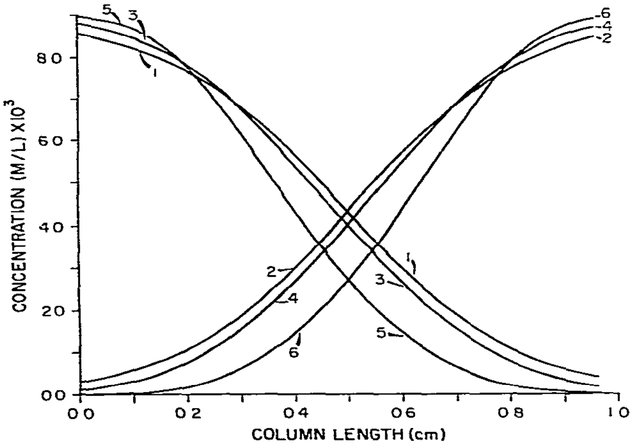

Fig. 3. Profiles of the concentration of neutral ampholyte species corresponding to figs. 1 and 2.

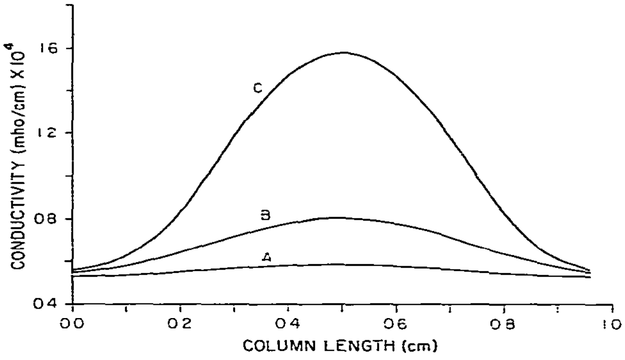

Fig. 4. Conductivity profiles corresponding to figs. 1–3.

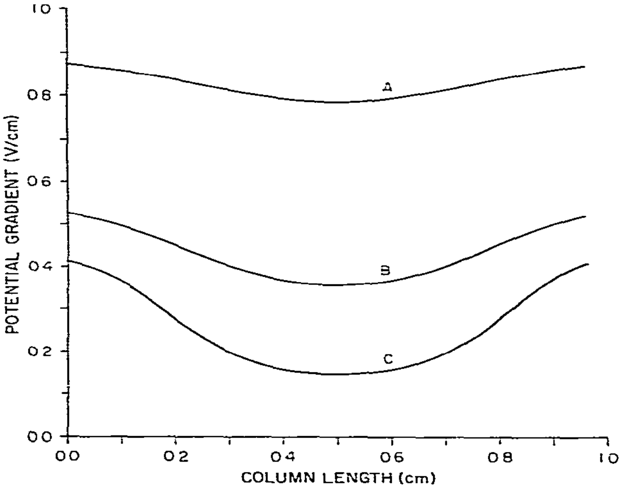

Fig. 5. Potential gradient profiles corresponding to figs. 1–4.

the neutral species. Thus, by difference, this system will have the highest concentration of charged species and, therefore, the highest conductivities (fig. 4) and the lowest potential gradients (fig. 5).

4.2. Effects of varying ΔpK at constant ΔpI

In the context of IEF, the difference between the pK values of the two dissociable groups of a biprotic ampholyte is of prime importance. A compound with a small ΔpK is considered to be a good ampholyte. Our simulations have compared three binary mixtures

Table 2
Effects of varying ΔpK at constant ΔpI: input parameters

System #	Component #	ΔpK	pK_1	pK_2	Mobility Coef. × 10^4	Current (mA/cm^2)
A	1	1	5.5	6.5	3.0	0.0288
	2		6.5	7.5	3.0	
B	3	2	5.0	7.0	3.0	0.0288
	4		6.0	8.0	3.0	
C	5	4	4.0	8.0	3.0	0.0288
	6		5.0	9.0	3.0	

with ΔpK's of 1, 2 and 4, while maintaining a ΔpI of 1. Table 2 summarizes the input parameters and it will be noticed that the system B is identical to system B of table 1. As in the foregoing set, all mobility coefficients were assumed to be identical and the column was kept at 1 cm length.

The pH profiles for the above three systems are shown in fig. 6. The pH gradients are nearly superimposable, as all three systems had the same ΔpI of 1. The concentration profiles for total ampholytes are also nearly superimposable, as shown in fig. 7. It will

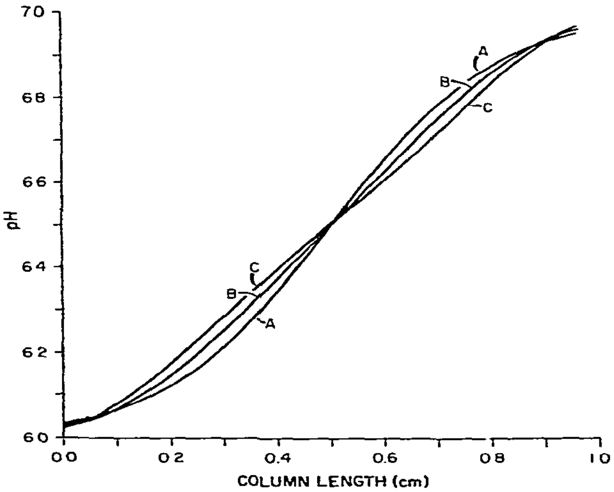

Fig. 6. pH profiles for IEF of three two-components systems with ΔpK of 1, 2, and 4 and ΔpI of 1 pH unit. For other input parameters see table 2.

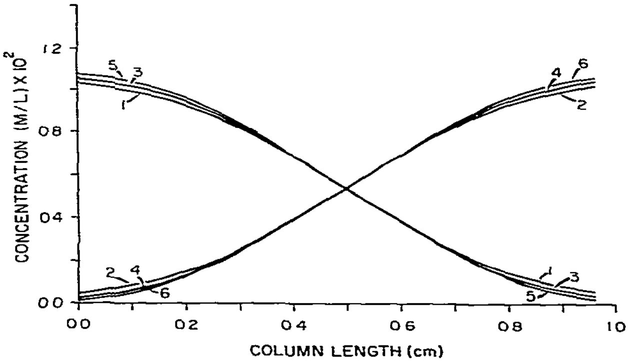

Fig. 7. Profiles of total ampholyte concentration corresponding to the three pH profiles presented in fig. 6.

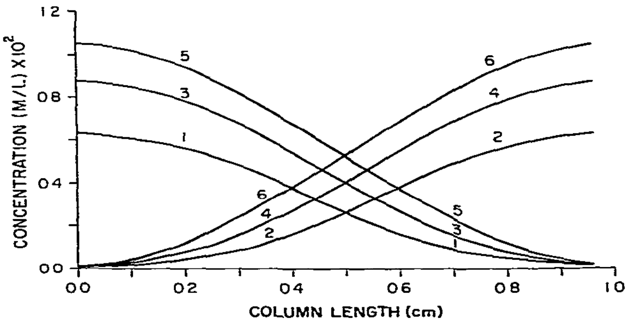

Fig. 8. Profiles of the concentration of neutral ampholyte species corresponding to figs. 6 and 7.

be noted that with this series, the applied current density in all three systems was identical. As before, significant differences are seen in the plots of the neutral species, shown in fig. 8. Highest concentration of the neutral species is seen for the system with the widest spread between the pK values of components. Thus, this system has also the lowest conductivity (fig. 9) and the highest voltage gradient (fig. 10).

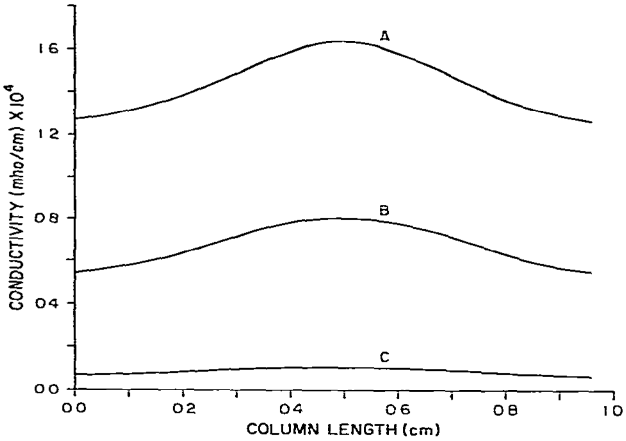

Fig. 9. Conductivity profiles corresponding to figs. 6–8.

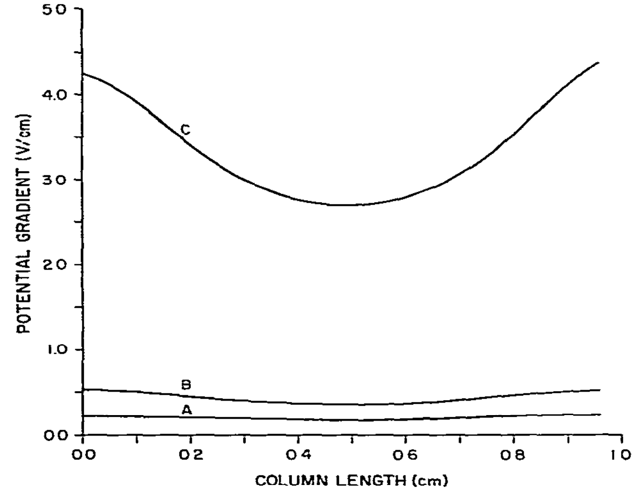

Fig. 10. Potential gradient profiles corresponding to figs. 6–9.

4.3. Effects of other variables

Other simulations, not reported in detail here, have clarified the effects of varying other parameters:

a) Current density: an increase in applied current density will cause a sharpening of all profiles, i.e., a doubling of the current will result in the deployment of identical profiles over exactly half the column length.

b) Column length: as a corollary of (a) if one doubles the column length one needs to reduce the current density by half to obtain identical profiles. The longer column will, however, contain twice the amount of ampholyte.

c) Concentration: if the initiating concentrations are reduced by half, the conductivity is reduced comparably. Thus, half the current needs to be applied to produce comparable concentration profiles and the total ampholyte contents of the column will be reduced proportionally.

d) Mobility: if the mobility coefficient is reduced by half, the net effect is similar to that obtained in reducing the concentration. The conductivity will be halved and the applied current needs to be reduced in half. Total ampholyte content of the column will, however, not change.

5. Conclusions

The aim of this paper is to present a theory of the structure of natural pH, concentration, conductivity, and potential gradients at the steady state of IEF using electrochemically well defined ampholytes. The mathematical model developed allows, for the first time, simulation of the IEF process using input parameters characterizing real or hypothetical ampholytes. Simulations have clarified the effects of varying the column dimensions, applied current, and the electrochemical parameters of components in binary mixtures.

In the computer implementation only the two apparent dissociation constants (pK's) nearest the isoelectric point of the ampholytes were considered. For ampholytes with more than two pK's the present model constitutes a good approximation, provided the neglected pK's differ from those considered by more than one unit. For the same reason, the model can be used for the simulation of focusing of non-amphoteric monovalent buffers or mixtures of such buffers and ampholytes. This requires that the buffers be assigned a second "convenience" pK, removed by several units from the pH range under consideration.

Particularly useful were simulations with hypothetical ampholytes, i.e., ampholytes with arbitrarily assigned electrochemical parameters. Some of the results can be summarized as follows:

A fully developed pH profile will always extend between the pI's of constituent ampholytes. To obtain a smooth pH gradient, very low current density has to be applied: at current densities usually applied in conventional IEF with commercial carrier ampholytes (e.g., Ampholine) steep and virtually useless step-gradients will be obtained, confined by "conductivity gaps" at either side of the step-gradient. This is the most striking and unexpected result of our simulations, which readily explains the many difficulties previously encountered by experimentalists in trying to develop Ampholine-free buffer systems.

Nearly identical pH and total concentration profiles can be obtained with compounds of varying ΔpK at the same current density. The larger the ΔpK, the higher the potential gradient, but lower the buffering capacity of the system.

Extensive simulations have been carried out also with parameters representative of actual compounds and these will be reported in a subsequent paper [15]. These simulations were of great value in the design of experimental systems for actual IEF of proteins. The resulting experimental work has validated, at least qualitatively, the present model [15].

Simulation studies with 3 component systems are currently under way, as well as simulations with non-interactive protein samples, i.e., proteins present in trace quantities and not affecting the pH gradient. Simulations with M components are also possible, but would require further improvement in the computer algorithms to increase their efficiency. For this purpose, advantage may be taken of the D-matrix sparseness. It is also possible to reduce the size of the problem by eliminating some variables, $(2M+2)$ in number, using algebraic equations and solving somewhat more complicated (dense matrices) differential equations for M unknowns only (basic concentrations). The concentrations of $2M+2$ remaining species would be computed for output purposes using again the algebraic relations.

These studies refer only to IEF at the steady state. Of equal importance is the elucidation of the transient states in IEF, and some progress in this direction has been made.

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References

[1] H. Svensson, Acta Chem. Scand. 15 (1961) 325.
[2] J.R. Cann, Biophys. Chem. 11 (1980) 249.
[3] J.R. Cann and K.J. Gardiner, Biophys. Chem. 10 (1979) 203.
[4] J.R. Cann and D.I. Stimpson, Biophys. Chem. 7 (1977) 103.
[5] S.K. Park, D.J. Cox, D.I. Stimpson and J.R. Cann, Biophys. Chem. 7 (1978) 367.
[6] G.H. Weiss, N. Catsimpoolas and D. Rodbard, Arch. Biochem. Biophys. 163 (1974) 106.
[7] O. Vesterberg, Acta Chem. Scand. 23 (1969) 2653.
[8] M. Almgren, Chemica Scripta 1 (1971) 69.
[9] M.L. Caspers, Y. Posey and R.K. Brown, Anal. Biochem. 79 (1977) 166.
[10] U. Stenman and R. Grasbeck, Biochem. Biophys. Acta 286 (1972) 243.
[11] M.L. Caspers and A. Chrambach, Anal. Biochem. 81 (1977) 28.
[12] A. Chrambach and N.Y. Nguyen, in: Electrofocusing and isotachophoresis, eds. B.J. Radola and D. Graesslin (W. de Gruyter, Berlin, 1977) p. 51.
[13] N.Y. Nguyen and A. Chrambach, Anal. Biochem. 74 (1976) 145.
[14] N.Y. Nguyen and A. Chrambach, Anal. Biochem. 79 (1977) 462.
[15] M. Bier, R.A. Mosher, T.T. Allgyer, E. Skeel and O.A. Palusinski, submitted to J. Chromatography.
[16] G.A. Korn and J.C. Wait, Digital continuous system simulation (Prentice-Hall, Englewood Cliffs, N.J. 1978).