[Short Communication]

Distribution of Carrier Ampholytes in Isoelectric Focusing*

— A Mathematical Approach —

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Basic equations of isoelectric focusing were discussed by Svensson,^{1,2)} but general solution giving distribution of carrier ampholytes, pH and conductance has not been obtained.

A new mathematical approach to describe distribution of carrier ampholytes in the steady state of isoelectric focusing and examples of the numerical solution are given here.

Neglecting the conductance by hydrogen and hydroxyl ions, general equation of mass balance of the i -th component of carrier ampholytes in the steady state of isoelectric focusing and the condition of electric neutrality are

$$\frac{1000 IU_i}{F} \cdot \frac{\partial}{\partial x} \left(\frac{C_i^+ - C_i^-}{\sum_{j=1}^n U_j (C_j^+ + C_j^-)} \right) - D_i \frac{\partial^2 C_i}{\partial x^2} = - \frac{\partial C_i}{\partial t} = 0, \quad i=1 \text{ to } n \quad \dots\dots\dots (1)$$

$$\sum_{j=1}^n (C_j^+ - C_j^-) = 0 \quad \dots\dots\dots (2)$$

where,

C_i^+ , C_i^- and C_i : cationic, anionic and total molar concentration of the i -th component, respectively,

U_i : absolute value of ionic mobility of monovalent cation and anion of the i -th component (assumed to be equal),

D_i : diffusion coefficient of cation, anion and zwitterion of the i -th component (assumed to be equal),

I : current density,

x : distance along the direction of current,

t : time,

F : Faraday's constant (965,00 C/mole),

n : number of components.

Integrating equation (1) (Eq. 1) with respect to x and dividing by U_i , we get

$$\frac{1000I}{F} \cdot \frac{C_i^+ - C_i^-}{\sum_{j=1}^n U_j (C_j^+ + C_j^-)} - \frac{D_i}{U_i} \cdot \frac{dC_i}{dx} = A_i = 0, \quad i=1 \text{ to } n \quad \dots\dots\dots (3)$$

where A_i 's are integration constants.

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Integration constants, A_i 's, are zero, because there is no net flow of carrier ampholytes under steady state of isoelectric focusing. The expression, $\frac{F}{1000} \sum_{j=1}^n U_j (C_j^+ + C_j^-)$ in eq. 1 and eq. 3 is specific conductance, κ , of the medium, so that eq. 3 corresponds to Svensson's equation (11) of the reference¹⁾ summed for cation, anion and zwitterion of the i -th component.

When Eq. 3 for all the components are summed up, the first term vanishes because of Eq. 2 (electric neutrality), and we get

$$\sum_{j=1}^n \frac{D_j}{U_j} \cdot \frac{dC_j}{dx} = 0.$$

Integration of the above equation gives

$$\sum_{j=1}^n \frac{D_j}{U_j} C_j = B \quad \dots\dots\dots (4)$$

Where B is an integration constant.

If current density is high, pure zone of the isoelectric i -th component may exist. Then, $C_j = 0$ for $j \neq i$, and concentration of the i -th component is given by

$$C_i = B \frac{U_i}{D_i}.$$

In a boundary zone between two isoelectric zones of pure component 1 and 2, concentrations of the two components satisfy Eq. 2 and Eq. 3 adapted for two component system. Remembering that $A_1 = A_2 = 0$, these equations are

$$\frac{1000I}{F} \cdot \frac{C_i^+ - C_i^-}{\sum_{j=1}^2 U_j (C_j^+ + C_j^-)} - \frac{D_i}{U_i} \cdot \frac{dC_i}{dx} = 0, \quad i=1 \text{ and } 2 \quad \dots\dots\dots (5)$$

$$\sum_{j=1}^2 (C_j^+ - C_j^-) = 0. \quad \dots\dots\dots (6)$$

Introducing cationic and anionic dissociation constants of the i -th component, K_{i1} and K_{i2} , respectively, we get from Eq. 5 and Eq. 6

$$\frac{1000I}{F} \cdot \frac{C_i \frac{[H^+]^2 - K_{i1}K_{i2}}{[H^+]^2 + [H^+]K_{i1} + K_{i1}K_{i2}}}{\sum_{j=1}^2 C_j \frac{U_j ([H^+]^2 + K_{1j}K_{2j})}{[H^+]^2 + [H^+]K_{1j} + K_{1j}K_{2j}}} - \frac{D_i}{U_i} \cdot \frac{dC_i}{dx} = 0, \quad i=1 \text{ and } 2 \quad \dots\dots\dots (7)$$

$$\sum_{j=1}^2 C_j \frac{[H^+]^2 - K_{1j}K_{2j}}{[H^+]^2 + [H^+]K_{1j} + K_{1j}K_{2j}} = 0. \quad \dots\dots\dots (8)$$

where,

$[H^+]$: molar concentration of hydrogen ion

K_{i1} : cationic dissociation constant ($= [H^+]C_i^{\pm}/C_i^+$) of the i -th component

K_{i2} : anionic dissociation constant ($= [H^+]C_i^{\pm}/C_i^-$) of the i -th component

$C^{\pm}$: zwitterionic molar concentration of the i -th component, and $C_i = C_i^+ + C_i^- + C_i^{\pm}$

If numerical values of the two components at any position, say $x = x_0$, and those of the physical constants and parameters appearing in Eq. 7 and Eq. 8 are given, $C_1(x)$ and $C_2(x)$ can be calculated by the following numerical integration procedure.

$[H^+]$ at $x = x_0$ is calculated first by introducing $C_1(x_0)$ and $C_2(x_0)$ into Eq. 8. Introducing the value of $[H^+]$ thus obtained into Eq. 7, we can get the values of $\frac{dC_1}{dx}$ and $\frac{dC_2}{dx}$ at $x = x_0$. Taking sufficiently small Δx , $C_1(x_0 + \Delta x)$ and $C_2(x_0 + \Delta x)$ can be calculated as

$$C_i(x_0 + \Delta x) = C_i(x_0) + \Delta x \left(\frac{dC_i}{dx} \right)_{x=x_0}, \quad i=1 \text{ and } 2.$$

Repeating the same procedure, concentration change along x of the two components can be obtained.

Table 1. Physical constants of carrier ampholytes, initial concentrations and parameters used for numerical integration of Fig. 1 and 2.

Carrier ampholyte, i	1	2	3
pK ₁	5	5	5.2
pK ₂	6.5	6.6	6.5
pI	5.75	5.8	5.85
D (10 ⁻⁶ cm ² /sec)	3	4	2
U (10 ⁻⁴ cm ² /v·sec)	1.5	2	1
Initial value of C (mol)			
Fig. 1, A	.03	.03	0
Fig. 1, B	.03	0	.03
Fig. 1, C	0	.03	.03
Fig. 2	.005	.05	.005
Current density (A/cm ²)	.02		
Δx (cm)	$\pm .001$

Fig. 1. Concentration (C in moles/l) of carrier ampholytes, pH and specific conductance (κ in mho/cm) in two component system.

See Table 1 for initial conditions and values of physical constants and parameters.

Calculations and graphic representations were done by YHP 9825 A personal computer and 9872 A plotter.

pH change has been obtained in the calculation procedure. Specific conductance is easily calculated from

$C_1(x)$ and $C_2(x)$.

Three examples of the numerical integration for arbitrarily chosen and probable values of the concentrations at $x=0$ and the physical constants (Table 1) are shown in Fig. 1.

If current density is low, two components with the nearest isoelectric points may exist at the isoelectric region of one component. The three components are named as 1, 2 and 3. If concentrations of the three components at any position and numerical values of the physical constants are given, $C_i(x)$ ($i=1, 2$ and 3), pH and specific conductance along x can be calculated by numerical integration of the following equation by the similar way as shown for two component system.

$$\frac{1000I}{F} \cdot \frac{\sum_{j=1}^3 C_j \frac{[H^+]^2 - K_{1j}K_{2j}}{U_j([H^+]^2 + [H^+]K_{1j} + K_{1j}K_{2j})}}{\frac{[H^+]^2 - K_{1i}K_{2i}}{[H^+]^2 + [H^+]K_{1i} + K_{1i}K_{2i}}} - \frac{D_i}{U_i} \cdot \frac{dC_i}{dx} = 0, \quad i=1, 2 \text{ and } 3 \quad \dots\dots\dots(9)$$

$$\sum_{j=1}^3 C_j \frac{[H^+]^2 - K_{1j}K_{2j}}{[H^+]^2 + [H^+]K_{1j} + K_{1j}K_{2j}} = 0 \quad \dots\dots\dots(10)$$

An example of the numerical integration of Eq. 9 and Eq. 10 for the components and initial conditions shown in Table 1 is given in Fig. 2.

Experimental proof of the results is left to be performed.

Fig. 2. Concentration (C in moles/l) of carrier ampholytes, pH and specific conductance (κ in mho/cm) in three component system.

See Table 1 for initial conditions and values of physical constants and parameters. Calculations and graphic representations were done by YHP 9825 A personal computer and 9872 A plotter.

References

1) Svensson, H. : Acta Chem. Scand., **15** : 425, 1961.
2) Svensson, H. : Ibid., **16** : 456, 1962.