THE THEORY OF ELECTROPHORESIS-CONVECTION

by

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I.

The fractionation of proteins in solution by an electrophoretic adaptation of the principles of the Clusius column has been described by us in a series of articles[1]. Fractionation occurs in a narrow vertical convection channel between two semi-permeable membranes connecting an upper and a lower reservoir. Separation depends upon the superposition of differential horizontal electrophoretic transport of the mobile components on vertical convective transport of the solution as a whole. The vertical convective transport arises from the horizontal density gradient produced by the electrophoretic migration of the proteins across the channel.

In the present article we shall develop a theory of transport in the electrophoresis-convection channel which is valid under certain limiting and ideal conditions. Since these conditions are only approximately realized in practice, exact quantitative agreement with the theory is not to be expected. However, since it yields times of transport and separation factors of the correct magnitude, the theory is useful as a guide in designing equipment and in planning fractionations.

In the development of the theory, it is assumed that there is no thermodynamic interaction between the mobile components, that is that the solution is ideal with respect to them and that they obey the ideal diffusion laws. It is further assumed that the flow in the channel is laminar and that the following relations are satisfied,

$$\frac{\mu_{\max} E a}{D_{\min}} << 1$$

$$\frac{h}{a} << 1$$

$$h = \left(\frac{2 \eta l D_{\max}}{a_{\min} \varrho_0 g C_0}\right)^{1/4}$$

where a is the channel width, l its length, E the electric field strength, C_0 the total protein concentration, η and ϱ_0 the viscosity and density of the solvent, and g the acceleration of gravity. $\mu_{\max}$ is the absolute magnitude of the greatest of the mobilities of the components and $D_{\min}$ and $D_{\max}$ are the smallest and largest values of the diffusion constants of the components. $a_{\min}$ is the least value of $(\partial\varrho/\partial C_K)_{T,p}$ of the components.

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The second of the stated conditions is generally fulfilled under practical operating conditions. The first is not always fulfilled. Never the less, in spite of failure to fulfil the stated conditions exactly and in spite of perturbing influences affecting operation, the theory provides a semi-quantitative description of transport in the column.

II.

We wish to investigate the transport of a system of ions at concentrations $C_1, \dots C_\nu$, in a conducting solvent by convection, diffusion and electrical migration in a vertical rectangular channel of width a and length l, connecting two reservoirs of volume V. The channel walls are supposed impermeable to the ν ion species in question (proteins) but permeable to certain ion species contained in the solvent (buffer ions). The concentrations of the permeable ions are assumed to be maintained at constant values by buffer reservoirs exterior to the channel walls. Their function is to carry a horizontal electric current density i through the channel walls, maintaining a horizontal electric field strength E in the interior of the channel equal to i/σ, where σ is the specific conductance of the solution.

The general equations of transport are

$$\varrho \frac{d\vec{u}}{dt} = -\varrho g \vec{1}_z - \nabla p + \eta \nabla^2 \vec{u} + \frac{\eta}{3} \nabla \nabla \cdot \vec{u}$$

$$\frac{d\varrho}{dt} = \varrho \nabla \cdot \vec{u} \tag{1}$$

$$\frac{\partial C_K}{\partial t} = \nabla \cdot \left\{ D_K \nabla C_K - (\mu_K \vec{E} + \vec{u})\, C_K \right\};\ K = 1, \dots \nu$$

where $\vec{u}$ is the convective velocity of the solution, ϱ the density, η the viscosity, D_K the diffusion constant and μ_K the electrophoretic mobility of ion constituent, K and g is the acceleration of gravity acting in the negative vertical direction, denoted by the unit vector $\vec{1}_z$. Eqs. (1) are to be solved subject to the boundary conditions that the convective velocity $\vec{u}$ vanishes at the channel walls and the horizontal components of the ionic current densities $C_K(\mu_K \vec{E} + \vec{u}) - D_K \nabla C_K$ also vanish at the walls. We now introduce a coordinate system x', y', z', with origin midway between the walls at the base of the channel, z'-axis in the direction of the vertical and x'-axis in the horizontal direction perpendicular to the parallel channel walls. Since in channels of finite breadth, we will neglect end effects, we consider the channel to be of infinite breadth in the y' direction. We now introduce reduced variables,

$$\begin{gathered} x = x'/a \\ -{}^1\!/_2 \leqslant x \leqslant +{}^1\!/_2 \\ z = z'/l \\ 0 \leqslant z \leqslant 1 \end{gathered} \tag{2}$$

The pressure may be eliminated from the z' and x' components of the first of Eqs. (1)

by differentiating the x'-component with respect to z' and z'-component with respect to x' and subtracting. When this is done, the reduced variables, Eq. (2), introduced into Eqs. (1), and terms of order a^2/l^2 are neglected, one obtains, in the quasi-stationary case $d\vec{u}/dt = 0$; $\partial C_K/\partial t = 0$),

$$\begin{gathered}
\frac{\partial^2 v}{\partial X^2} - \sum_{K=1}^{\nu} \frac{a_K\, \varrho_0\, g\, a^3}{\eta} \frac{\partial C_K}{\partial X} = 0 \\
a_K = \frac{1}{\varrho_0} \left(\frac{\partial \varrho}{\partial C_K}\right)_{T,p,C_j} \quad ; \; v\left(\pm {}^1/_2, Z\right) = 0 \\
\frac{\partial}{\partial X}\left(\frac{\partial C_K}{\partial X} - \frac{a\,\mu_K\, E}{D_K}\, C_K\right) - \frac{a^2 v}{l D_K} \frac{\partial C_K}{\partial Z} = 0 \;;\; K = 1, \ldots. \nu \\
\frac{\partial C_K}{\partial X} - \frac{a \mu_K E C_K}{D_K} = 0 \;;\; x = \pm {}^1/_2
\end{gathered} \tag{3}$$

where E is the magnitude of the electric field in the x-direction and v is the z-component of convective velocity. The horizontal component of convective velocity as well as terms involving vertical diffusion are neglected since they are of order a^2/l^2, relative to terms retained. It will be assumed the solution is sufficiently dilute to allow us to consider the coefficients a_k as constants, that is, that

$$\varrho = \varrho_0 \left[1 + \sum_{K=1}^{\nu} a_K\, C_K\right] \tag{4}$$

For aqueous solutions of proteins, the a_k are of the order of $2.5 \cdot 10^{-3}$, if the C_k are expressed in grams per 100 ml.

Eqs. (3) are non-linear, but become linear in the first order, when v and the C_k are expanded in powers of the field strength E, in the form,

$$\begin{gathered}
v = \frac{\varrho_0\, g\, a^3}{\eta}\, \mu^{(1)}\, \lambda(z) \left(\sum_K \frac{a_K\, C_K^0}{D_K}\right) F(x)\, E + O(E^2) \\
C_K = C_K^0 \lambda_K(z) + \frac{\mu_K\, a\, E\, C_K^0}{D_K}\, \varphi_K(x, Z) + O\left(E^2\right) \\
\mu^{(1)} = \sum_{K=1}^{\nu} g_K\, \mu_K \\
g_K(z) = \left[a_K\, C_K^0\, \lambda_K(z)/D_K\right] \Big/ \sum_l a_l\, C_l^0\, \lambda_l/D_l \\
\lambda(z) = \left(\sum_K a_K\, C_K^0\, \lambda_K/D_K\right) \Big/ \sum_K a_K\, C_K^0/D_K
\end{gathered} \tag{5}$$

where $O(E^2)$ means terms depending on the second and higher powers of the field strength, and C_K^0 is the initial uniform concentration of ion constituent K in the channel and in the reservoir at top and bottom. The quantity $\mu^{(1)}$ is the mean value or first moment of the mobility with respect to the distribution function g_K, which reduces in zero order to the weight fraction of constituent K at level z, if all constituents possess the same value of a_K and D_K. Differentiation of the first Eqs. (4) with respect to x and substitution of the power series, Eq. (5), into the resulting equation yields,

$$\begin{aligned}
&\frac{d^4F}{dx^4} + 4\beta_0^4 F = 0 \\
&F(\pm {}^1/_2) = 0\,;\ \left(\frac{d^3F}{dx^3}\right)_{x=\pm {}^1/_2} = 1 \\
&F(x) = \frac{1}{\beta^3}\,\frac{\sin\beta/2 \sinh\beta x - \sinh\beta/2 \sin\beta x}{\sin\beta/2 \cosh\beta/2 + \sinh\beta/2 \cos\beta/2} \\
&\beta = (1+i)\,\beta_0 \\
&\beta_0^4 = -\frac{1}{2}\,\frac{a^4}{h^4}\,\frac{d\lambda}{dz} \\
&h = \left[\frac{2\eta\, l}{\varrho_0\, g \sum_K a_K C_K^0/D_K}\right]^{1/4}
\end{aligned} \tag{6}$$

with due regard to all boundary conditions on v and the current densities of the ion constituents K. Use of the power series, Eqs. (5), in the remaining Eqs. (4), yields after integration of the equation for each C_K from $-\,{}^1\!/_2$ to x,

$$\begin{aligned}
&\frac{\partial x}{\partial \varphi_K} = \lambda_K(z) - \frac{\mu^{(1)}}{\mu_K}\,\lambda\,\frac{d\lambda_K}{d\lambda}\,G(x) \\
&G(x) = 4\beta_0^4 \int_{-1/2}^{x} F(x)\,dx \\
&= 1 - \frac{\sin\beta/2 \cosh\beta x + \sinh\beta/2 \cos\beta x}{\sin\beta/2 \cosh\beta/2 + \sinh\beta/2 \cos\beta/2}
\end{aligned} \tag{7}$$

The total current of any component K flowing in the negative z-direction through a section of the channel of breadth b is given by

$$\begin{aligned}
&J_K = -\,a\,b \int_{-1/2}^{+1/2} C_K\, v\, dx \\
&0 = \int_{-1/2}^{+1/2} \varrho\, v\, dx
\end{aligned} \tag{8}$$

The second of Eqs. (8) expresses the condition that net fluid flow in the vertical direction in the channel is zero. With the aid of Eqs. (5), (7), and (8), we find after partial integration

$$J_K = -\frac{a\,b\,l\,\mu_K\,\mu^{(1)}\,C^0_K\lambda\,E^2}{D_K\,d\lambda/dz}\int\limits_{-1/2}^{+1/2} G(x)\frac{\partial\varphi_K}{\partial x}\,dx + O(E^3) \tag{9}$$

Evaluation of the integral in Eq. (9) leads to the result

$$\begin{gathered} J_K = -\frac{a\,b\,l\,\mu^{(1)}\,\mu_K\,C^0_K\,\lambda\,E^2}{D_K\,d\lambda/dz}\left\{\lambda_K(z)\;\frac{\mu^{(1)}}{\mu_K}\lambda\,\frac{d\lambda_K}{d\lambda}\right. \\ \left.-\frac{2h}{a}\,\frac{1}{(-d\lambda/dz)^{1/4}}\left[K_0\lambda_K - \frac{5}{4}\,\frac{\mu^{(1)}}{\mu_K}K_1\lambda\,\frac{d\lambda_K}{d\lambda}\right]\right\} \\ K_0 = \frac{\sinh^2\beta_0/2\,\cos^2\beta_0/2 + \cosh^2\beta_0/2\,\sin^2\beta_0/2}{\sinh\beta_0/2\,\cosh\beta_0/2 + \sin\beta_0/2\,\cos\beta_0/2} \\ K_1 = K_0 - \frac{2\,\beta_0}{5}\,\frac{\sinh\beta_0\,\sin\beta_0}{[\sinh\beta_0 + \sin\beta_0]^2} \end{gathered} \tag{10}$$

When both sides of Eq. (10) are summed over all ion constituents, the initial terms independent of h/a vanish and we have,

$$\begin{gathered} j = \frac{2\,h\,b\,l\,\mu^{(1)^2}\lambda^2 E^2}{(-d\lambda/dz)^{5/4}}\left[\frac{5}{4}K_1 - K_0\right]\sum_K \frac{a_K\,C^0_K}{D_K} \\ j = \sum_{K=1}^{\nu} a_K\,J_K \end{gathered} \tag{11}$$

where j/a is the total mass current of all ion constituents down a section of channel of breadth b if all a_k are equal. The functions K_1 and K_0 rapidly approach unity as $1 + o(e^{-\beta_0})$, and can be approximated by unity if

$$-\frac{d\lambda}{dZ} > \left(\frac{h}{a}\right)^4 \tag{12}$$

Under representative conditions h/a is in general small, say of the order of 0.1 or less. Therefore except in an initial stage in which $(d\lambda/dz)$ is less than 10^{-4}, K_1 and K_0 may be set equal to unity and Eq· (11) becomes,

$$\begin{gathered} j = \frac{h\,b\,l\,\mu^{(1)^2}\lambda^2 E^2}{2\,(-d\lambda/dZ)^{4/5}}\sum_{K=1}^{\nu} a_K\,C^0_K/D_K \\ \frac{d\lambda}{dZ} = -\left(\frac{\mu^{(1)}}{\mu^{(1)}_0}\right)^{8/5}\frac{\lambda^{8/5}}{L} \\ \frac{1}{L} = \left[\frac{h\,b\,l\,\mu_0^{(1)^2}E^2}{2\,j}\sum_{K=1}^{\nu}\frac{a_K\,C^0_K}{D_K}\right]^{4/5} \end{gathered} \tag{13}$$

$$\mu_0^{(1)} = \sum_K g_K^0 \mu_K \quad ; \quad g_K^0 = \frac{a_K\, C_K^0 / D_K}{\sum_l a_l\, C_l^0 / D_l}$$

Using Eq. (13) in Eq. (10), we obtain

$$\lambda \frac{d\lambda_K}{d\lambda} - \frac{\mu_K}{\mu^{(1)}} \lambda_K = - \frac{D_K J_K}{2j} \left(\sum \frac{a_l C_l^0}{D_l} \right) \left(- \frac{d\lambda}{dZ} \right)^{1/4} \frac{h}{a} + O \left(\frac{h}{a} \right)^2 \tag{14}$$

For small h/a, we may therefore write

$$\begin{aligned} \frac{d \log g_K}{d \log \lambda} &= (\mu_K - \mu^{(1)}/\mu^{(1)}) \\ g_K &= \lambda_K\, g_K^0/\lambda \end{aligned} \tag{15}$$

Since in the quasi-stationary state, the currents J_k and therefore j must be independent of z, Eqs. (13) and (15) describe the manner in which the total ion concentration and the composition vary as a function of reduced height z in the convection channel. In the special case in which all components have the same diffusion constant and the same density coefficient a_K, g_K is the fraction of component K at level z and λ is the ratio of total protein concentration at level z to initial total protein concentration.

Eqs. (13) and (15) can be reduced to the form

$$\begin{aligned} g_K &= \varkappa^{\mu_K/\mu_0^{(1)}}\, g_K^0/\lambda \\ \lambda &= \sum_{K=1}^{\nu} \varkappa^{\mu_K/\mu_0^{(1)}}\, g_K^0 \\ \frac{d \log \varkappa}{dz} &= - \frac{1}{L} \left[\sum_{K=1}^{\nu} \frac{g_K^0\, \mu_K}{\mu_1^{(0)}}\, \varkappa^{\mu_K/\mu_0^{(1)}} \right]^{3/5} \end{aligned} \tag{16}$$

The solution of the ordinary differential equation for the parameter $\varkappa$, the last of Eqs. (16), may be carried out either with the specification of $\varkappa$ at $z = 0$ or $z = 1$ and the parameter L or with the specification of at both points $z = 0$ and $z = 1$, in which case L is determined. The first case corresponds to the specification of total protein concentration and composition at one end of the column together with the currents. The second case corresponds to specifying total protein concentration at both ends of the column and the composition at one end. The solutions thus obtained are stationary solutions.

In the quasi-stationary state with the channel connecting an upper and a lower reservoir of finite volumes V_1 and V_0 respectively, we assume the stationary solutions to be valid in the channel with boundary conditions changing slowly with time as the upper reservoir is impoverished and the lower reservoir is enriched in the mobile components. We shall assume the uniform concentrations in the top reservoir are equal to the first order terms $\lambda_K^{(1)} C_K^0$ at $z = 1$, the top of the channel and that the total protein concentration in the bottom reservoir is equal to the channel concentration at $z = 0$. In the multicomponent case, it is not possible to avoid composition gradients in the bottom reservoir, which would be determined by vertical diffusion, neglected in the present theory. The postulated boundary conditions are somewhat idealized but plausible. A more accurate specification would require an analysis of diffusive and convective transport in the reservoirs themselves. Conservation of mass then gives,

$$\begin{aligned} C_K^0 \frac{d\lambda_K^{(1)}}{dt} &= -\frac{J_K}{V} \\ \gamma(1) + \frac{V_0}{V_1}\gamma(0) &= 2 \\ \gamma &= \sum_{K=1}^{\nu} X_K^0 \lambda_K(Z) \\ X_K^0 &= C_K^0 \Big/ \sum_{l=1}^{\nu} C_l^0 \end{aligned} \tag{17}$$

Eqs. (13) and (17) then lead to the result

$$\begin{aligned} \frac{d\omega(\varkappa_1)}{d\tau} &= -L^{5/4} \\ \tau &= t/\Theta \\ 1/\Theta &= h\,b\,l\,\mu_0^{(1)^2} E^2/2\,V_1 D_0^{(1)} \\ 1/D_0^{(1(} &= \sum_{K=1}^{\nu} a_K X_K^0/D_K \\ \omega(\varkappa) &= \left(\sum_{K=1}^{\nu} a_K C_K^0 \varkappa^{\mu_K/\mu_0^{(1)}}\right) \Big/ \sum_{K=1}^{\nu} a_K C_K^0 \\ \gamma(\varkappa_1) + \frac{V_0}{V_1}\gamma(\varkappa_0) &= 2 \\ \gamma(\varkappa) &= \sum_{K=1}^{\nu} X_K^0 \varkappa^{\mu_K/\mu_0^{(1)}} = C(z)/C^0 \end{aligned} \tag{18}$$

The solution of Eqs. (16) and (18) determines $\varkappa$ as a function of reduced height z in the channel and the reduced time τ. The integrals of Eq. (16) may be written in the form,

$$\begin{aligned} Z/L &= \psi(\varkappa) - \psi(\varkappa_0) \\ \psi(\varkappa) &= \int_{\varkappa}^{1} \frac{d\varkappa}{\varkappa\left[\sum_{K=1}^{\nu} g_K^0 \frac{\mu_K}{\mu_0^{(1)}} \varkappa^{\mu_K/\mu_0^{(1)}}\right]^{3/5}} \\ L &= \frac{1}{\psi(\varkappa_1) - \psi(\varkappa_0)} \end{aligned} \tag{19}$$

Substitution of L from Eq. (19) into Eq. (18) yields,

$$\begin{gathered}\frac{d\varkappa_1}{d\tau} = -\frac{1}{\omega'(\varkappa)\left[\psi(\varkappa_1) - \psi(\varkappa_0)\right]^{5/4}} \\ \sum_{K=1}^{\nu} X_K^0 \left[\varkappa_1^{\mu_K/\mu_0^{(1)}} + \frac{V_0}{V_1} \varkappa_0^{\mu_K/\mu_0^{(1)}} \right] = 2 \\ \omega' = d\omega/d\varkappa \end{gathered} \tag{20}$$

where the second of Eqs. (20) determines $\varkappa_0$ as a function of $\varkappa_1$. Eq. (19) determines $\varkappa$ as a function of z and $\varkappa_1$, in the form

$$\psi(\varkappa) - \psi(\varkappa_0) = Z\left[\psi(\varkappa_1) - \psi(\varkappa_0)\right] \tag{21}$$

and Eq. (20) determines $\varkappa_1$ as a function of time τ with the initial condition $\varkappa_1(0) = 1$.

$$\begin{gathered} I(\varkappa_1) = \tau \\ I(\varkappa_1) = \int_{\varkappa_1}^{1} \left[\psi(\varkappa) - \psi(\varkappa)\right]^{5/4} \frac{d\omega}{d\varkappa}\, d\varkappa \\ \sum_{K=1}^{\nu} X_K^0 \left[\varkappa^{\mu_K/\mu_0^{(1)}} + \frac{V_0}{V_1} \varkappa_0^{\mu_K/\mu_0^{(1)}} \right] = 2 \end{gathered} \tag{22}$$

where the last of Eqs. (22) determines the value of S_0 as a function of S to be used in the evaluation of the integral I $(\varkappa_1)$. When $\varkappa$ is given the total protein concentration at any level z is given by $\gamma(\varkappa)$, that is

$$\begin{gathered} \gamma = \sum_{K=1}^{\nu} X_K^0\, \varkappa^{\mu_K/\mu_0^{(1)}} \\ \frac{C(z)}{C^0} = \frac{\sum_{K=1}^{\nu} C_K(z)}{\sum_{K=1}^{\nu} C_K^0} = \gamma \end{gathered} \tag{23}$$

The local compositions at any level z are given by

$$\begin{gathered} X_K = \frac{C_K(z)}{C(z)} = \frac{X_K^0\, \varkappa^{\mu_K/\mu_0^{(1)}}}{\gamma} \\ f_K(z) = \frac{X_K}{1 - X_K} \cdot \frac{1 - X_K^0}{X_K^0} \\ = \left(\sum_{\substack{l \neq K \\ = 1}}^{\nu} X_l^0\, \varkappa^{\mu_K/\mu_0^{(1)}} \right) \Big/ \sum_{\substack{l \neq K \\ = 1}}^{\nu} X_l^0\, \varkappa^{\mu_K/\mu_0^{(1)}} \end{gathered} \tag{24}$$

where f_K is the separation factor for component K at level z. Thus $f_K(1)$ is the separation factor in the top reservoir and $f_K(0)$ that in the bottom. Since $\varkappa$ is determined as a function of γ by Eq. (23), the local compositions X_K and separation factors f_K may be regarded as functions of γ alone and thus are determined by Eqs. (23) and (24) as functions of total protein concentration at the given level and time.

III.

In this section we will consider applications of the general theory to transport in solutions containing one and two mobile components. We shall first treat the transport of one component as a function of time and position in the column. For one component, λ, $\varkappa$, ω, and γ become identical and equal to the ratio of concentration $C(z, \tau)$ to initial concentration C^0. Also $\mu_0^{(1)}$ becomes equal to μ, the mobility of the single component. The function $\psi(\varkappa)$ of Eq. (19) therefore becomes,

$$\psi(\varkappa) = \int_{\varkappa}^{1} \varkappa^{-8/5} d\varkappa = 5/3 \; [\varkappa^{-3/5} - 1] \qquad (25)$$

$$\varkappa_1 + \varkappa_0 = 2$$

with $V_0 = V_1$. Eqs. (19) and (21) then give $\varkappa$ as a function of z in the form,

$$\varkappa(Z) = \frac{\varkappa_0}{\left(1 + 3/5 \; \varkappa_0^{-3/5} \dfrac{Z}{L}\right)^{5/3}}$$

$$L = 3/5 \; \frac{\varkappa_0^{3/5} \varkappa_1^{3/5}}{\varkappa_0^{3/5} - \varkappa_1^{3/5}} \qquad (26)$$

$$\varkappa_1 = 2 - \varkappa_0$$

To determine $\varkappa_1$ as a function of τ, we employ Eq. (22), which reduces to,

$$\tau = I(\varkappa_1)$$

$$I(\varkappa_1) = \left(\frac{5}{3}\right)^{5/4} \int_0^{1-\varkappa} \left\{\frac{1}{(1-y)^{3/5}} - \frac{1}{(1+y)^{3/5}}\right\}^{5/4} dy \qquad (27)$$

$$\tau = t/\Theta \quad ; \quad 1/\Theta = \frac{h\,b\,l\,\mu^2 E^2}{2\,VD}$$

The exhaust time t_e for the top reservoir is given by

$$t_e = I(0)\,\Theta \qquad (28)$$

The integral I equal to t/Θ is presented as a function of the fraction $1 - \varkappa_1$ of the mobile component transported out of the top reservoir in Table I. The characteristic time Θ is given by Eqs. (6) and (27) to be,

$$\Theta = \left(\frac{8\,\alpha\,\varrho_0\,g\,C_0}{\eta}\right)^{1/4} \frac{D^{3/4}\,V}{3600\,b\,l^{5/4}\,\mu^2\,E^2}\ \text{hours}$$

In water at 10°C with $\alpha = 2.5 \cdot 10^{-3},\ \overline{D} = 10^6 D,\ \overline{u} = 10^5\,\mu$ (29)

$$\Theta = \frac{546\,\overline{D}^{3/4}\,C_0^{1/4}\,V}{b\,l^{5/4}\,\overline{u}^2\,E^2}\ \text{hours}$$

where C_0 is expressed in grams/100 ml, E in volts/cm, and all lengths in cm. The value of the density coefficient α of $2.5 \cdot 10^{-3}$ is representative of the plasma proteins. The magnitude of Θ in a representative case, $C_0 = 1$, $\overline{D} = 1$, $V = 50$, $l = 16$, $b = 5$, $\overline{u} = 5$, $E = 1$, is 7.1 hours. The exhaust time from Table I is therefore 39.5 hours, the time for 90% transport 11.1 hours and the time for 20% transport 0.2 hours. Because of the non-ideal behavior of the apparatus, these estimates although of the correct magnitude are only approximately realized in practice.

TABLE I

TIME OF TRANSPORT AS A FUNCTION OF FRACTION OF MOBILE COMPONENT TRANSPORTED

t/Θ	$1 - \varkappa_1$	t/Θ	$1 - \varkappa_1$
0.006	0.10	0.631	0.70
0.029	0.20	0.972	0.80
0.074	0.30	1.57	0.90
0.145	0.40	2.13	0.95
0.253	0.50	3.21	0.99
0.407	0.60	5.57	1.00

We now turn to the system of two mobile components. In this case we obtain for $\psi(\varkappa)$

$$\psi(\varkappa) = {g_1^0}^{-3/5} \left(\frac{\mu_0^{(1)}}{\mu_1}\right)^{8/5} B_{nr}\left(\varkappa^{\mu^1/\mu_0^{(1)}}\right)$$

$$B_{nr}(X) = \int_1^X X^{-3/4}\left[1 + nr\,X^{n-1}\right]^{-3/5} d\,X$$

$$n = \mu_r/\mu_1 \qquad r = \frac{g_2^0}{g_1^0} = \frac{\alpha_2\,D_1 X_2^0}{\alpha_1\,D_2 X_1^0} \tag{30}$$

$$\mu_0^{(1)} = g_1^0\,\mu_1 + g_2^0\,\mu_2$$

$$\omega(\varkappa) = \left(\alpha_1\,X_1^0\,\varkappa^{\mu_1/\mu_0^{(1)}} + \alpha_2\,X_2^0\,\varkappa^{\mu_2/\mu_0^{(1)}}\right) / \left(\alpha_1\,X_1^0 + \alpha_2\,X_2^0\right)$$

$$\gamma(\varkappa) = X_1^0\,\varkappa^{\mu_1/\mu_0^{(1)}} + X_2^0\,\varkappa^{\mu_2/\mu_0^{(1)}}$$

For the dependence of $\varkappa_1$ on time of transport, we obtain,

$$\tau = \frac{a_1 X_1^0}{a_1 X_1^0 + a_2 X_2^0}\left(\frac{\mu_0^{(1)}}{\mu_1}\right)^2 g_1^{0\,-3/5}\left\{A_{nrs}^{(1)}\left(\varkappa_1^{\mu_1/\mu_0^{(1)}}\right) + (n+1)\frac{a_2 S}{a_1} A_{nrs}^{(2)}\left(\varkappa_1^{\mu_1/\mu_0^{(1)}}\right)\right\}$$

$$S = X_2^0/X_1^0$$

$$A_{nrs}^{(1)}(X) = \int_X^1 \left\{B_{nr}(X) - B_{nr}(X_0)\right\}^{5/4} dX \tag{31}$$

$$A_{nrs}^{(2)}(X) = \int_X^1 X^n\left\{B_{nr}(X) - B_{nr}(X_0)\right\}^{5/4} dX$$

$$\begin{cases} X_0 = X_0(X, S) \\ X_0 + S X_0^{n+1} = 2 - X - S X^{n+1} \end{cases}$$

Since the integrals $A_{nrs}^{(1)}$ and $A_{nrs}^{(2)}$ depend on three parameters, they have not been calculated and tabulated.

When the mobilities of the two components are of the same sign an upper bound to the time of transport to a fraction γ_1 of total solute in the upper reservoir may be obtained by use of the formula,

$$\tau = I(\gamma_1)$$

$$1/\Theta = \frac{h\,b\,l\,\mu_1^2 E^2}{2 V_1 D_0^{(1)}} \tag{32}$$

$$\frac{1}{D_0^{(1)}} = \frac{a_1 X_1^0}{D_1} + \frac{a_2 X_2^0}{D_2}$$

where I(γ_1) is given by Eq. (22) and Θ is calculated with the mobility μ_1 of the slower component. A lower bound is obtained by calculating Θ with the mobility μ_2 of the faster component. For practical purposes, these estimates usually suffice.

When the mobilities are of opposite sign, transport from the top reservoir ceases when $\mu^{(1)}$ vanishes, that is when,

$$\frac{a_1 X_1^0 \mu_1}{D_1}\varkappa_1^{\mu_1/\mu_0^{(1)}} + \frac{a_2 X_2^0 \mu_2}{D}\varkappa_1^{\mu_2/\mu_0^{(1)}} = 0$$

$$\varkappa_1 = \left[-\frac{a_2 X_2^0 \mu_2 D_1}{a_1 X_1^0 \mu_1 D_2}\right]^{\frac{\mu_0^{(1)}}{\mu_1 - \mu_2}} \tag{33}$$

due to cessation of convection at the top of the channel. The fraction γ_1 of protein remaining in the top reservoir at the end of transport is given by,

$$\gamma_1 = X_1^0\left[-\frac{a_2 X_2^0 \mu_2 D_1}{a_1 X_1^0 \mu_1 D_2}\right]^{\frac{\mu_1}{\mu_1 - \mu_2}} + X_2^0\left[-\frac{a_2 X_2^0 \mu_2 D_1}{a_1 X_1^0 \mu_1 D_2}\right]^{\frac{\mu_2}{\mu_1 - \mu_2}} \tag{34}$$

If the two components have equal diffusion constants and equal density coefficients, the state of zero transport is reached when the top reservoir becomes isoelectric.

For a given value of γ_1, the fraction of total solute remaining in the top reservoir at the time of sampling, Eq. (24) gives the separation factor for component 2, $f_2^{(1)}$ as

$$\frac{f_2^{(1)}}{[1 + X_2^0(f_2^{(1)} - 1)]^{\mu_2/\mu_1 - 1}} = \gamma_1^{(\mu_2/\mu_1 - 1)} \tag{35}$$

$$f_2^{(1)} = \gamma_1^{(\mu_2/\mu_2 - 1)} \quad ; \quad X_2^0 \leqslant 1$$

Eq. (35) may be used to estimate separation factors even without an estimate of the time required to attain a specified value of γ_1.

The bottom separation factor can be calculated by conservation of mass when $f_2^{(1)}$ is known. $f_2^{(1)}$ is presented as a function of X_2^0, $1 - \mu_2/\mu_1$, and γ in Tables II.

TABLE II

SEPARATION FACTORS, $f_1^{(2)}$, IN TOP RESERVOIR FOR THE TWO COMPONENT SYSTEM

γ_1 = RESIDUAL FRACTION OF TOTAL PROTEIN IN TOP

x_2^0 = INITIAL WEIGHT FRACTION OF COMPONENT 2

$\beta = 1 - \mu_2/\mu_1$

$x_2^0 = 0.1$					$x_2^0 = 0.4$				
γ_1	β 0.1	0.4	0.7	0.9	γ_1	β 0.1	0.4	0.7	0.9
0.1	1.26	2.50	4.16	5.64	0.1	1.25	2.15	3.21	3.92
0.2	1.17	1.85	2.77	3.48	0.2	1.17	1.72	2.30	2.68
0.3	1.13	1.58	2.15	2.59	0.3	1.12	1.50	1.88	2.12
0.4	1.10	1.42	1.80	2.08	0.4	1.09	1.37	1.62	1.78
0.5	1.07	1.30	1.56	1.75	0.5	1.07	1.27	1.45	1.56
0.6	1.05	1.22	1.39	1.51	0.6	1.05	1.19	1.32	1.39
0.7	1.04	1.15	1.26	1.34	0.7	1.04	1.13	1.21	1.26
0.8	1.02	1.09	1.16	1.20	0.8	1.02	1.08	1.13	1.16
0.9	1.01	1.04	1.07	1.09	0.9	1.01	1.04	1.06	1.07

$x_2^0 = 0.7$					$x_2^0 = 0.9$				
γ_1	β 0.1	0.4	0.7	0.9	γ_1	β 0.1	0.4	0.7	0.9
0.1	1.24	2.02	2.83	3.31	0.1	1.24	1.96	2.65	3.08
0.2	1.16	1.64	2.09	2.35	0.2	1.16	1.60	1.98	2.20
0.3	1.12	1.45	1.74	1.90	0.3	1.12	1.42	1.67	1.81
0.4	1.09	1.33	1.53	1.64	0.4	1.09	1.31	1.48	1.57
0.5	1.07	1.24	1.38	1.46	0.5	1.07	1.23	1.34	1.41
0.6	1.05	1.17	1.27	1.32	0.6	1.05	1.16	1.25	1.29
0.7	1.03	1.12	1.18	1.21	0.7	1.03	1.11	1.17	1.19
0.8	1.02	1.07	1.11	1.13	0.8	1.02	1.07	1.10	1.12
0.9	1.01	1.03	1.05	1.06	0.9	1.01	1.03	1.05	1.05

All of our equations apply to hypothetical continuous distributions in mobility if the sums over components are replaced by integrals over mobility. In such cases the function g_K of Equation (15) is replaced by $g(\mu)$ where $g(\mu)d\mu$ means the fraction of mobile solute, weighted with the factor a/D having mobilities between μ and $\mu + d\mu$. Eq. (15) then becomes,

$$\frac{d \log g}{d \log \lambda} = \frac{\mu - \mu^{(1)}}{\mu^{(1)}}$$

$$\mu^{(1)} = \int_{-\infty}^{\infty} \mu\, g(\mu)\, d\mu \tag{36}$$

$$\lambda = \int_{-\infty}^{\infty} \frac{C(\mu)}{C^0(\mu)}\, g^0(\mu)\, d\mu$$

$$\gamma = C/C_0 \qquad C = \int_{-\infty}^{\infty} C(\mu)\, d\mu$$

where $C(\mu)d\mu$ is the concentration of solute with mobilities between μ and $\mu + d\mu$, and C is the total solute concentration as in the discrete distribution. If we define mobility moments in the following manner

$$\Delta_n = \int_{-\infty}^{\infty} (\mu - \mu^{(1)})^n\, g(\mu)\, d\mu \tag{37}$$

we find from Eqs. (13) and (36),

$$\frac{d\, \Delta_n}{d \log \lambda} = \frac{\Delta_{n+1} - n\, \Delta_2\, \Delta_{n-1}}{\mu^{(1)}}$$

$$\frac{d\, \mu^{(1)}}{d \log \lambda} = \frac{\Delta_2}{\mu^{(1)}} \tag{38}$$

$$\frac{d\lambda}{d z} = -\left(\frac{\mu^{(1)}}{\mu_0^{(1)}}\right)^{3/5} \frac{\lambda^{3/5}}{L}$$

$$\frac{1}{L} = \left[\frac{h\, b\, l\, \mu_0^{(1)2}\, E^2}{2 j} \int_{-\infty}^{\infty} \frac{a}{D}\, C(\mu)\, d\mu\right]^{4/5}$$

The Gaussian distribution is of special interest since all moments Δ_n remain unchanged during transport, and the distribution remains Gaussian at all levels in the channel with a second moment Δ_2 equal Δ_2^0, that of the original distribution. By the second of Eqs. (38), we find that the average mobility $\mu^{(1)}$ is given by,

$$\mu^{(1)2} = \mu_0^{(1)2} + 2\, \Delta_2^0 \log \lambda \tag{39}$$

where $\mu_0^{(1)}$ is the initial value of the average mobility. A stationary state of no transport is achieved when $\mu_1^{(1)}$ in the top reservoir vanishes, corresponding to a final value λ_1 given by

$$\lambda_1 = e^{-\mu_0^{(1)2}/2\Delta_2^0} \qquad \mu_1^{(1)} = 0 \tag{40}$$

When a and D are identical for all components, λ_1 reduces to γ_1, the residual fraction of solute in the top reservoir. The time required to achieve a given value of γ_1 is rather complicated to calculate, although the formulas may be set up by the methods we have presented. An upper bound to the time required to achieve a given transport $1 - \gamma_1$ from the top reservoir is however easily obtained,

$$\frac{t(\gamma_1)}{\Theta} = \left[1 - \frac{1\,\Delta_2^0}{\mu_0^{(1)2}} \log \gamma_1\right] I(\gamma_1) \qquad \Theta = \left(\frac{8\,a\,\varrho_0\,h\,C_0}{\eta}\right)^{1/4} \frac{D^{3/4}\,V}{3600\,b\,l^{5/4}\,\mu_0^{(1)2}\,E^2} \tag{41}$$

if a and D are the same for all components. The integral I (γ_1) is given again by Table I.

SUMMARY

The theory of transport in the electrophoresis-channel is developed in the quasi-stationary approximation on the basis of the equations of hydrodynamics and the equations of forced diffusion of ion constituents under the influence of an applied electric field. Times of transport of proteins through the channel and separation factors for representative protein mixtures are estimated by means of the theory.

RÉSUMÉ

Nous avons développé la théorie du transport dans le canal à électrophorèse, en approximation quasi-stationnaire, en nous basant sur les équations de l'hydrodynamique et celles de la diffusion forcée de constituents ioniques sous l'influence d'un champ électrique appliqué. Nous avons évalué, à l'aide de cette théorie, les vitesses de transport de protéines à travers le canal et les facteurs de séparation de mélanges charactéristiques de protéines.

ZUSAMMENFASSUNG

Die Theorie der Überführung im Elektrophoresekanal wurde, in quasi-stationärer Annäherung, auf Grund der hydrodynamischen Gleichungen und der Gleichungen, welche für die Diffusion ionisierter Bestandteile unter dem Einfluss eines elektrischen Feldes gelten, entwickelt. Überführungszeiten von Eiweisstoffen durch den Kanal und Trennungsfaktoren charakteristischer Proteingemische wurden mit Hilfe dieser Theorie ermittelt.

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