

THEORY OF PAPER ELECTROPHORESIS. I.

EQUATION OF CONTINUITY
IN PAPER ELECTROPHORESIS

J. VACÍK

*Department of Physical Chemistry,
Charles University, Prague 2*

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A continuity equation for paper electrophoresis was derived. It involves the influences of longitudinal diffusion, slow attainment of equilibrium, distribution function, osmotic and sucking flows of electrolyte, dissociation of transferred substance and unequal distribution of potential gradient.

The paper electrophoresis is to a certain extent a combination of free (classical) electrophoresis and paper chromatography¹. Its common feature with free electrophoresis is the migration of ions in the electric field (differences in mobility among various components in a mixture enabling separation in free electrophoresis). The common feature of paper electrophoresis and chromatography is the paper as supporting medium and the interaction between it and the analyzed substance (differences in interaction of various components of a mixture with the paper enabling separation in paper chromatography). The shape of the zone or front in paper electrophoresis is determined by the same effects as its shape in classical electrophoresis, or as the shape of the spot in paper chromatography. The object of the present work is to derive a continuity equation in a general form that includes as many effects as possible.

Mathematical

We shall deal with electrophoresis under the following assumptions: 1. The problem is linear, the motion proceeds along the x axis; 2. the dissolved components are mutually independent (*i.e.* neither their mobilities nor their sorption properties depend on one another), therefore we shall not distinguish them by indexes; 3. the influence of activity coefficients can be neglected; 4. the diffusion coefficients are independent of concentration; 5. the system is isothermal and isobaric. The continuity equation has then the general form

$$\partial c / \partial t = -\partial j / \partial x - q_M, \quad (1)$$

where the first term on the right side characterizes the transport of a component,

the second one its interaction with the paper, *i.e.* the rate of increase of its concentration c as a result of the interaction, j denotes mass flow of the dissolved component in a given place parallel to the x axis, and t time.

The mass flow of the component is given by the familiar equation

$$j = -D \frac{\partial c}{\partial x} + cEU + cv, \quad (2)$$

where D means the diffusion coefficient of the component, U its velocity in a unit electric field, E intensity of the electric field and v velocity of motion of the solution in a given point. The quantity U is taken as positive regardless of the charge of the corresponding ion; this means that the direction of the x axis is the same as the direction of migration.

The rate of change of concentration in the mobile phase, q_M , due to interaction with the stationary phase must be equal in absolute value to the rate q_S of change of concentration C of the substance bound to the stationary phase:

$$\frac{\partial C}{\partial t} = p_0 q_S = -p_0 q_M, \quad (3)$$

where p_0 denotes free pore volume (porosity).

The equilibrium between the analyzed substance in the mobile phase (in supporting electrolyte) and in the stationary phase (adsorbed on paper) is not always attained immediately but rather slowly depending on the sorption kinetics. It is often assumed² that the rate of attainment of equilibrium is proportional to the difference between the equilibrium and actual concentrations: $q_M = (H/p_0)(f^*(c) - C)$. Here H denotes the coefficient of transfer of the separated substance from the solution to the solid phase, $f^*(c)$ separation function equal to the amount of substance per 1 g of stationary phase in equilibrium with its solution of concentration c , and C (as above) amount of substance adsorbed per 1 g of stationary phase at a given instant (*i.e.* nonequilibrium one). On introducing the separation function³ $f(c) = f^*(c) + p_0 c$ equal to the amount of substance in the mobile and stationary phases per 1 g of support, we can write

$$q_M = (H/p_0)(f(c) - C - p_0 c). \quad (4)$$

On introducing Eqs (2) and (4) into (1) we obtain for the equilibrium in the mobile phase

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} + U \left(c \frac{\partial E}{\partial x} + E \frac{\partial c}{\partial x} \right) - v \frac{\partial c}{\partial x} - c \frac{\partial v}{\partial x} - \frac{H}{p_0} (f(c) - C - p_0 c). \quad (5)$$

For the equilibrium in the stationary phase we obtain from Eqs (3) and (4)

$$\frac{\partial C}{\partial t} = H(f(c) - C - p_0 c). \quad (6)$$

The rate of flow of the mobile phase, v , can be expressed as the sum of three components, $V + v(x) + v(c)$, where V is the constant velocity component, $v(x)$ is that one which depends on distance, and $v(c)$ that which depends on concentration of the substance to be separated. From Eqs (5) and (6) we then obtain the final equation for the distribution of concentration of the given component in the mobile phase at any instant:

$$\begin{aligned} & \frac{1}{H} \frac{\partial^2 c}{\partial t^2} + \left[\frac{1}{H} \frac{\partial}{\partial x} (v(x) + EU) + \frac{1}{p_0} \frac{df(c)}{dc} \right] \frac{\partial c}{\partial t} - D \frac{\partial^2 c}{\partial x^2} + \\ & + \left[V + v(x) + v(c) + EU + c \frac{dv(c)}{dc} + \frac{U}{H} \frac{\partial E}{\partial t} \right] \frac{\partial c}{\partial x} - \frac{D}{H} \frac{\partial^3 c}{\partial x^2 \partial t} + \\ & + \frac{1}{H} \left[V + v(x) + v(c) + EU + c \frac{dv(c)}{dc} \right] \frac{\partial^2 c}{\partial x \partial t} + \frac{1}{H} \left(2 \frac{dv(c)}{dc} + c \frac{d^2 v(c)}{dc^2} \right) \frac{\partial c}{\partial t} \frac{\partial c}{\partial x} + \\ & + c \frac{dv(x)}{dx} + \frac{U}{H} c \frac{\partial^2 E}{\partial x \partial t} + cU \frac{\partial E}{\partial x} = 0. \end{aligned} \quad (7)$$

The potential gradient is given in general by the distribution of all kinds of ions in the paper and is a complicated function of distance and time. In many cases, however, the concentration of all ions can be regarded as independent of time and distance except for the concentration of ions to be separated. The potential gradient is then a function of the latter quantity and Eq. (7) takes the form

$$\begin{aligned} & \frac{1}{H} \frac{\partial^2 c}{\partial t^2} + \left(\frac{1}{H} \frac{dv(x)}{dx} + \frac{1}{p_0} \frac{df(c)}{dc} \right) \frac{\partial c}{\partial t} - D \frac{\partial^2 c}{\partial x^2} + \\ & + \left[V + v(x) + v(c) + EU + c \left(\frac{dv(c)}{dc} + U \frac{\partial E}{\partial c} \right) \right] \frac{\partial c}{\partial x} - \frac{D}{H} \frac{\partial^3 c}{\partial x^2 \partial t} + \\ & + \frac{1}{H} \left[V + v(x) + v(c) + EU + c \left(\frac{dv(c)}{dc} + U \frac{\partial E}{\partial c} \right) \right] \frac{\partial^2 c}{\partial x \partial t} + \\ & + \frac{1}{H} \left[2 \left(U \frac{\partial E}{\partial c} + \frac{dv(c)}{dc} \right) + c \left(\frac{d^2 v(c)}{dc^2} + U \frac{\partial^2 E}{\partial c^2} \right) \right] \frac{\partial c}{\partial t} \frac{\partial c}{\partial x} + c \frac{dv(x)}{dx} = 0. \end{aligned} \quad (8)$$

It is convenient for later calculations to include the migration of ions into the velocity component $v(c)$:

$$\begin{aligned}
& \frac{1}{H} \frac{\partial^2 c}{\partial t^2} + \left(\frac{1}{H} \frac{dv(x)}{dx} + \frac{1}{p_0} \frac{df(c)}{dc} \right) \frac{\partial c}{\partial t} - D \frac{\partial^2 c}{\partial x^2} + \left(V + v(x) + (c) + \right. \\
& \left. + c \frac{dv(c)}{dc} \right) \frac{\partial c}{\partial x} - D \frac{\partial^3 c}{\partial x^2 \partial t} + \frac{1}{H} \left(V + v(x) + v(c) + c \frac{dv(c)}{dc} \right) \frac{\partial^2 c}{\partial x \partial t} + \\
& + \frac{1}{H} \left(2 \frac{dv(c)}{dc} + c \frac{d^2 v(c)}{dc^2} \right) \frac{\partial c}{\partial t} \frac{\partial c}{\partial x} + c \frac{dv(x)}{dx} = 0. \quad (9)
\end{aligned}$$

DISCUSSION

Of chromatographic factors influencing the concentration profile of the zone (front) in paper electrophoresis, we shall consider longitudinal diffusion, slow attainment of equilibrium and the separation function; of electrophoretic factors, flows of electrolyte (osmotic and sucking), dissociation of the transferred compound and unequal distribution of potential gradient.

Electroosmotic flow. Its velocity, v_{os} , is proportional to the product of the intensity of the electric field and of the electrokinetic potential. The latter increases with decreasing concentration of the base electrolyte, c_e . (The concentration c of the separated substance is negligible against c_e .) In the concentration range usual in paper electrophoresis the dependence of the electrokinetic potential on concentration can be considered as linear. The potential gradient E is, as a first approximation, inversely proportional to the concentration of the base electrolyte. As a result,

$$v_{os} = k_1 + k_2/c_e, \quad (10)$$

where k_1 and k_2 are constants depending on the base electrolyte-stationary phase system used. Chromatographic paper causes with respect to other supports a relatively small electroosmotic flow towards the cathode (naturally the sort of paper plays a role^{4,5}). It follows that with constant concentration of the base electrolyte the electroosmotic flow is also constant and can be included in the term V . The latter can represent also the liquid flow in a countercurrent or vertical arrangement if no evaporation of the liquid from the paper occurs.

Sucking flow. In the case of an equal evaporation of the liquid from the whole paper area⁶⁻⁹, a flow of electrolyte towards the center of the paper strip is caused by capillary forces. It can be expressed as

$$v_s = \frac{1}{2} aL - ax; \quad 0 \leq x \leq L, \quad (11)$$

where L denotes total length of the paper and a ratio of the amount of water eva-

porated in a second to the total amount contained in the same paper area. Since the sucking flow is a function of distance, it can be included in the term $v(x)$. In more accurate calculations it should be taken into account that the rate of evaporation is proportional to the square of potential gradient^{8,10}:

$$a = k_3 E^2. \quad (12)$$

The term $v(c)$ represents the concentration-dependent velocity component, mainly migration of ions in the electric field, expressed as EU in the basic equations given above. When the electrophoretic process proceeds in a porous medium filled with an electrolyte, the porous structure has to be taken into account. If no interaction with the stationary phase takes place, the velocity of ions can be expressed as¹¹

$$v(c) = \alpha EU/\gamma, \quad (13)$$

where γ is the tortuosity factor and α dissociation grade, which is equal to one in the case of a strong electrolyte resulting in a simplification^{1,12}. The dissociation grade α , potential gradient E and velocity of ions U are generally functions of concentration of the substance to be separated.

The dependence of the quantity U both on concentration of the analyzed substance^{13,14} and on concentration of the supporting electrolyte is, however, only little pronounced and can be neglected in most cases. The dependence of the potential gradient on concentration of the analyzed substance is strongly influenced by the supporting electrolyte concentration (or more properly by its conductivity). If the latter is independent of time and coordinate, the current can be expressed as $i = E(\kappa_e + \kappa)$, where κ_e and κ denote the electrical conductivities of the supporting electrolyte and of the substance to be separated. Since κ is proportional to concentration c , we can express the potential gradient in electrophoresis with constant current as $E = K_a/(K_b + \alpha c)$, where K_a and K_b are constants. Introducing this into Eq. (13) and using the common relation among dissociation grade α , dissociation constant K_A and concentration c , we can express the term $v(c) + c dv(c)/dc$ in Eq. (9) as

$$v(c) + c \frac{dv(c)}{dc} = \frac{K_a K_b K_A U}{\gamma [K_b - \frac{1}{2} K_A + \frac{1}{2} (K_A^2 + 4 K_A c)^{1/2}]^2 (K_A^2 + 4 K_A c)^{1/2}}. \quad (14)$$

For $\alpha = 1$ Eq. (14) simplifies to

$$v(c) + c dv(c)/dc = K_a K_b U / \gamma (K_b + c)^{1/2} \quad (15)$$

and for $\partial E / \partial c = 0$ it takes the form

$$v(c) + c dv(c)/dc = EK_A U / \gamma (K_A^2 + 4 K_A c)^{1/2}. \quad (16)$$

Influence of the concentration of base electrolyte. In the above calculations, we considered the dependence of the potential gradient on concentration of the analyzed substance, *i.e.* the potential change in the place where this substance is present. The change of potential gradient in electrophoresis is caused also by changing concentration of the base electrolyte, c_e , due to evaporation of a volatile component. The change of the c_e value can be expressed by

$$\frac{\partial c_e}{\partial t} + c_e \frac{\partial v_e}{\partial x} + v_e \frac{\partial c_e}{\partial x} - D \frac{\partial^2 c_e}{\partial x^2} = 0, \quad (17)$$

where v_e means convective flow of electrolyte due to the combined effects of electroosmosis, evaporation of the solvent and its replenishment by sucking. By combining Eqs (10)–(12) and (16), deriving with respect to c_e and rearranging we obtain

$$\frac{\partial c_e}{\partial t} - \left(\frac{k_3 L}{c_e^2} + k_2 \right) \frac{\partial c_e}{\partial x} - D \frac{\partial^2 c_e}{\partial x^2} = 0. \quad (18)$$

The solution of this equation gives the concentration distribution of the base electrolyte as a function of time and distance, whence the dependence of the potential gradient on place and time can be derived. The concentration profile of the zone (front) cannot be calculated in this case from the simplified Eq. (9), but from (7).

The changes of base electrolyte concentration in electrophoresis are relatively small¹⁰, hence direct proportionality between concentration and specific conductance of the electrolyte can be assumed. Even if the base electrolyte is a buffer, or more often a mixture of a buffer and a neutral salt, the mentioned dependence is linear since the concentration ratio of dissociated and undissociated components in a buffer is practically constant¹⁵.

In constant current electrophoresis the concentration c_e can only increase, hence the potential gradient must diminish. In electrophoresis with constant applied voltage the current varies, the sign of this variation being different in different points of the paper surface; accordingly the potential gradient in certain places becomes larger, in other smaller.

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