[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF WISCONSIN]

Weak Electrolyte Moving Boundary Systems Analogous to the Electrophoresis of a Single Protein[1]

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Moving boundary systems obtained in the electrophoresis of proteins are compared with various types of weak electrolyte moving boundary systems. The latter are suitable analogs for interpreting certain phenomena encountered with proteins. A Kohlrausch regulating function applicable to monovalent weak electrolytes is derived, and the number of boundaries permitted in a weak electrolyte moving boundary system is discussed. A theory which deals with boundary displacements, compositions of phases, boundary sharpening factors and the number of boundaries is derived for a weak electrolyte system analogous to that of a protein above its isoelectric point in a buffer of the uncharged-acid type. The results of moving boundary experiments with sodium aspartate in sodium acetate buffers are compared with theoretical predictions.

Introduction

The moving boundary theory for strong electrolytes[3] predicts some of the effects encountered in the electrophoresis of proteins. These include the existence of ϵ- and δ-boundaries, the difference in velocity of ascending and descending protein boundaries, and the variation in apparent analysis with total protein concentration.[4-6] However, proteins are not strong electrolytes, and in solution they are in acid–base equilibrium with the buffer. Simple weak acids and bases are useful as models for proteins since their constituent mobilities are pH dependent.[7] Furthermore, when weak electrolyte constituents are caused to migrate by the passage of current, acid–base reactions occur which are similar to those in protein systems. An analog of a protein above its isoelectric point is the weak acid HS and its anion S^-, since the mobility of the S constituent increases as the pH is raised. Similarly, a base C and its cationic form CH^+ is an analog of a protein below its isoelectric point.

Electrophoresis experiments may be carried out in buffers of the uncharged-acid and the uncharged-base types. These buffer types are represented symbolically by HR, AR and B, BHX, respectively, where A is the cation of a strong base and X is the anion of a strong acid. The two protein analogs and these two types of buffers lead to eight distinct moving boundary systems since there are ascending and descending cases for each combination.

TYPE I, descending
HR, AR, HS, AS (α) ← HR, AR (β)::HR, AR (γ)

TYPE I, ascending
HR, AR (γ) ← HR, AR, HS, AS (β)::HR, AR, HS, AS (α)

TYPE II, descending
B, BHX, HS, BHS (α) ← B, BHX (β)::B, BHX (γ)

TYPE II, ascending
B, BHX (γ) ← B, BHX, HS, BHS (β)::B, BHX, HS, BHS (α)

TYPE III, descending
HR, AR (γ)::HR, AR (β) → HR, AR, C, CHR (α)

TYPE III, ascending
HR, AR, C, CHR (α)::HR, AR, C, CHR (β) → HR, AR (γ)

TYPE IV, descending
B, BHX (γ)::B, BHX (β) → B, BHX, C, CHX (α)

TYPE IV, ascending
B, BHX, C, CHX (α)::B, BHX, C, CHX (β) → B, BHX (γ)

The notation used in these moving boundary systems is that introduced by Longsworth.[4,8]

The Moving Boundary Equation and the Kohlrausch Regulating Function for Monovalent Weak Electrolytes.—Svensson[9] and Alberty[10] have independently derived the moving boundary equation for weak electrolyte systems, which may be written

$$\bar{u}_J{}^\alpha \bar{c}_J{}^\alpha/\kappa^\alpha - \bar{u}_J{}^\beta \bar{c}_J{}^\beta/\kappa^\beta = v^{\alpha\beta}(\bar{c}_J{}^\alpha - \bar{c}_J{}^\beta) \qquad (1)$$

This equation applies to any constituent J in the phases α and β which are separated by a boundary produced by the passage of current. The quantity $v^{\alpha\beta}$ is the volume in milliliters swept out by the boundary during the passage of one coulomb of electricity. The specific conductances of the two phases are κ^α and κ^β. The quantities $\bar{u}_J$ and $\bar{c}_J$ are the constituent mobility and constituent concentration (moles liter^{-1}), respectively, for the J constituent, and they are given by expressions

$$\bar{u}_J = \frac{\sum_{i=1}^{n} u_i c_i}{\sum_{i=1}^{n} c_i} \qquad (2)$$

and

$$\bar{c}_J = \sum_{i=1}^{n} c_i \qquad (3)$$

The subscript i in equations 2 and 3 denotes one of the n species in solution which comprise the J constituent; a given constituent is comprised by a species which cannot undergo dissociation and all forms which are in equilibrium with that species.

The Kohlrausch[11] regulating function ω for strong electrolytes has been redefined by Dole[3] in terms of relative ion mobilities as

$$\omega = \sum_{i=1}^{n} (c_i/r_i) \qquad (4)$$

Provided that relative ion mobilities are constant, the function ω has the same value in successive

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(2) Shell Fellow 1952–1953.

(3) V. P. Dole, THIS JOURNAL, **67**, 1119 (1945).

(4) L. G. Longsworth, *J. Phys. Colloid Chem.*, **51**, 171 (1947).

(5) R. A. Alberty, *J. Chem. Educ.*, **25**, 619 (1948).

(6) J. R. Cann, THIS JOURNAL, **71**, 907 (1949).

(7) J. C. Nichol, *ibid.*, **72**, 2367 (1950).

(8) L. G. Longsworth, *ibid.*, **67**, 1109 (1945).

(9) H. Svensson, *Acta Chem. Scand.*, **2**, 841 (1948).

(10) R. A. Alberty, THIS JOURNAL, **72**, 2361 (1950).

(11) F. Kohlrausch, *Ann. Physik*, **62**, 209 (1897).

phases that are separated by moving boundaries. It is possible to derive a similar function which is applicable to systems containing monovalent weak acids and bases. If the mobility of the uncharged form is zero, then it may be seen from equations 2 and 3 that $\bar{u}_J\bar{c}_J = u_jc_j$, where j designates the singly charged form of the constituent. It is now possible to rewrite the moving boundary equation 1 in the following way

$$u_j^\alpha c_j^\alpha/\kappa^\alpha - u_j^\beta c_j^\beta/\kappa^\beta = v^{\alpha\beta}(\bar{c}_J^\alpha - \bar{c}_J^\beta) \qquad (5)$$

The relative mobility r_j of any ion j is defined as u_j/u_m where u_m is the mobility of some particular ion m, and similarly the relative conductance σ is defined as κ/u_m. With the use of these definitions and the assumption that relative ion mobilities are constant, equation 5 may be rewritten as

$$c_j^\alpha/\sigma^\alpha - c_j^\beta/\sigma^\beta = v^{\alpha\beta}(\bar{c}_J^\alpha/r_j - \bar{c}_J^\beta/r_j) \qquad (6)$$

The summation of equations of the form 6 for all constituents is

$$\sum_j c_j^\alpha/\sigma^\alpha - \sum_j c_j^\beta/\sigma^\beta = v^{\alpha\beta}\left[\sum_J(\bar{c}_J^\alpha/r_j) - \sum_J(\bar{c}_J^\beta/r_j)\right] \qquad (7)$$

The electroneutrality condition states that $\sum_j c_j^\alpha = \sum_j c_j^\beta = 0$. Therefore, if $v^{\alpha\beta} \neq 0$

$$\sum_J(\bar{c}_J^\alpha/r_j) = \sum_J(\bar{c}_J^\beta/r_j) \qquad (8)$$

The function $\sum_J(\bar{c}_J/r_j)$ is designated as $\bar{\omega}$ and will be referred to as the Kohlrausch regulating function for monovalent weak electrolytes. For successive phases which are separated by moving boundaries, the function $\bar{\omega}$ has the same value in each phase. For the case of a stationary boundary (that is, $v^{\alpha\beta} = 0$), equation 6 shows that all ions are diluted by the same factor across the boundary.

In the expression for the Kohlrausch regulating function, concentrations and relative mobilities always appear as ratios, and it is not necessary to follow the sign convention described in connection with equation 1.[10] In the following calculations, the convention that all concentrations, mobilities and boundary displacements are positive quantities will be used. The only modification required in using this convention is that the term $(\bar{c}_J^\alpha - \bar{c}_J^\beta)$ in equations 1 and 5 must be changed to $(\bar{c}_J^\beta - \bar{c}_J^\alpha)$ if the boundary moves in the direction opposite to that of the constituent J.

The Number of Boundaries in a Weak Electrolyte Moving Boundary System.—For strong electrolytes Dole[3] has proved that a maximum of $n - 1$ boundaries can form in a system containing n different ionic species. One of these boundaries must be stationary if relative ion mobilities are constant, leaving a maximum of $n - 2$ moving boundaries. By reasoning similar to that used by Dole, the maximum number of boundaries permitted in a weak electrolyte system may be derived.[12]

The total number of constituents contained in a weak electrolyte system is designated as N. Of these the concentrations of $N - 2$ constituents in each phase are independently variable. It is convenient to regard the constituent concentrations of hydrogen and hydroxyl, which are present in any aqueous solution, as the only concentrations which are not independently variable. Another way of expressing this number of degrees of freedom is in terms of the number S of species, including water molecules, which are present. There are less than S degrees of freedom in determining the composition of a phase since the definition of a concentration scale imposes one restriction on the number of independently variable concentration values, the electroneutrality condition imposes another, and E additional restrictions are imposed if there are E independent expressions for chemical equilibria among the S species. The number of degrees of freedom $N - 2$ may also be expressed as $S - E - 2$. In the case of the α solution of the Type I system, made up by adding HR, HS and AOH to water, there are five constituents (R, S, A, H and OH) and eight species (R^-, HR, S^-, HS, A^+, H^+, OH^- and H_2O).

If B is the number of boundaries which form, there are $B + 1$ phases, of which $B - 1$ appear after the current has been turned on. The number of unknowns which must be evaluated in order to describe the system completely is $(B - 1)(N - 2) + B$, where $(B - 1)(N - 2)$ is the number of unknown concentrations required to give the compositions of the new phases and B is the number of boundary displacements. For each boundary, $N - 2$ independent moving boundary equations can be written, and there are therefore a total of $B(N - 2)$ relationships that are available. In order that the values of the unknowns may be unique and determinate, the number of unknowns must be equal to the number of available relationships. Therefore

$$(B - 1)(N - 2) + B = B(N - 2)$$
$$B = N - 2$$

It should be stressed that $N - 2$ is the maximum number of boundaries permitted, but in certain experiments all of them may not exist or they may not be detected. For any particular system, a complete description can be obtained by solving $(N - 2)^2$ relationships for the unknown quantities in terms of the compositions of the two original phases.

In view of the fact that in each of the weak electrolyte moving boundary systems formulated above there are five constituents present, three boundaries are permitted. The absence of one of the boundaries implies that each initial system has some special property that prevents the formation of the second moving boundary. In the analogous protein systems which are encountered experimentally, it is probable that there are actually three boundaries in many cases, but the concentration gradients across one of the moving boundaries are too small to be detected by the usual experimental methods.

Weak Electrolyte Systems Analogous to Those Containing a Protein Above Its Isoelectric Point in an Uncharged-acid Type Buffer

A theory will be derived for the moving boundary systems of Type I which form when current is passed through initial boundaries between the solutions HR, AR, HS, AS (α) and HR, AR (γ). The

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[12] O. J. Plescia and R. A. Alberty, presented at the Chicago Meeting of the American Chemical Society, September, 1950.

compositions of the initial phases may not be arbitrarily chosen if only two boundaries are formed by the passage of current. The special properties that lead to the formation of one less than the maximum number of boundaries will be discussed.

In order to simplify the problem of describing these systems, the following assumptions are made: (1) the relative mobilities of the ions are constant throughout the system, (2) the mobilities of uncharged weak acid molecules are zero, and (3) the relative values of ionization constants for the weak acids expressed in terms of concentrations are constant throughout the system. An important corollary of assumption (1) is that ionic conductances are additive.[8] The additivity of ionic conductances is expressed by $\kappa = (F/1000) \sum_i u_i c_i$, where F is the faraday. The conductance due to hydrogen and hydroxyl ions will be ignored. A corollary of assumption 2 and the stoichiometry of reactions of the type $R^- + HS = HR + S^-$ which occur in the moving boundaries is that the total concentration of weak acid at any level in the cell is constant.

In the descending system, the migration of S^- ions tends to leave a region adjacent to the initial boundary position in which there are no S^- ions. However, in the adjustment of equilibrium, R^- ions react with HS molecules to form additional S^- ions in this region. If, as is the case for systems discussed in this paper, the continuous migration of S^- ions and the adjustment of equilibrium results in the complete disappearance of the S constituent from this region, designated as the β-phase, the moving boundary equation for the S constituent simply reduces to

$$\bar{u}_S^\alpha = v^{\alpha\beta}\kappa^\alpha \tag{9}$$

In the ascending system, the migration of S^- ions leads to the formation of the β-phase, which contains HS molecules as a result of the reaction of S^- ions with HR molecules. The moving boundary equation for S at the $\beta\gamma$-boundary is

$$\bar{u}_S^\beta = v^{\beta\gamma}\kappa^\beta \tag{10}$$

In each system there will be a stationary boundary, the $\beta\gamma$-boundary in the descending system and the $\alpha\beta$-boundary in the ascending system, since (1) there will be gradients in concentration near the initial boundary positions if the choice of concentrations for the two initial phases is arbitrary and (2) the positions of these gradients will not change during the passage of current if relative ion mobilities are constant.

Compositions of Intermediate Phases.—The concentrations of HR and AR in the β-phase of the descending system may be calculated from the equality of Kohlrausch regulating functions of the α- and β-phases (equation 11), the electroneutrality condition, and the equation for conservation of weak acid at any level of the cell (equation 12).

$$\frac{c_A^\beta}{r_A} + \frac{c_R^\beta + c_{HR}^\beta}{r_R} = \frac{c_A^\alpha}{r_A} + \frac{c_R^\alpha + c_{HR}^\alpha}{r_R} + \frac{c_S^\alpha + c_{HS}^\alpha}{r_S} \tag{11}$$

$$c_{HR}^\beta = c_{HR}^\alpha + c_{HS}^\alpha \tag{12}$$

Thus, the concentration of AR in the β-phase is

$$c_R^\beta = c_R^\alpha + \frac{r_R(r_A + r_S)}{r_S(r_A + r_R)}\, c_S^\alpha + \frac{r_A(r_R - r_S)}{r_S(r_A + r_R)}\, c_{HS}^\alpha \tag{13}$$

The four unknown concentrations in the β-phase of the ascending system may be calculated from the dilution ratio across the stationary $\alpha\beta$-boundary (equation 14), the equality of Kohlrausch regulating functions (equation 15), conservation of weak acid (equation 16), and the mass action relationship (equation 17)

$$c_R^\beta/c_S^\beta = c_R^\alpha/c_S^\alpha \tag{14}$$

$$\frac{c_R^\beta + c_S^\beta}{r_A} + \frac{c_R^\beta + c_{HR}^\beta}{r_R} + \frac{c_S^\beta + c_{HS}^\beta}{r_S} = \frac{c_R^\gamma}{r_A} + \frac{c_R^\gamma + c_{HR}^\gamma}{r_R} \tag{15}$$

$$c_{HR}^\beta + c_{HS}^\beta = c_{HR}^\gamma \tag{16}$$

$$K_{HR}c_{HR}^\beta/c_R^\beta = K_{HS}c_{HS}^\beta/c_S^\beta \tag{17}$$

The solution of equations 14, 15, 16 and 17 leads to

$$c_{HR}^\beta = \frac{c_{HR}^\gamma}{1 + \dfrac{K_{HR}}{K_{HS}}\dfrac{c_S^\alpha}{c_R^\alpha}} \tag{18}$$

$$c_{HS}^\beta = \frac{c_{HR}^\gamma}{1 + \dfrac{K_{HS}}{K_{HR}}\dfrac{c_R^\alpha}{c_S^\alpha}} \tag{19}$$

$$c_R^\beta = \frac{c_R^\gamma - c_{HS}^\beta\dfrac{r_A(r_R - r_S)}{r_S(r_A + r_R)}}{1 + \dfrac{c_S^\alpha}{c_R^\alpha}\dfrac{r_R(r_A + r_S)}{r_S(r_A + r_R)}} \tag{20}$$

$$c_S^\beta = \frac{c_R^\gamma - c_{HS}^\beta\dfrac{r_A(r_R - r_S)}{r_S(r_A + r_R)}}{\dfrac{c_R^\alpha}{c_S^\alpha} + \dfrac{r_R(r_A + r_S)}{r_S(r_A + r_R)}} \tag{21}$$

Sharpening Factors at the Moving Boundaries.—A moving boundary in a weak electrolyte system will have sharp gradients in concentration if the constituent disappearing across the boundary has a greater velocity in the phase behind the boundary than in the phase ahead of the boundary. Differences in field strength and *p*H across a boundary produce the conductivity effect[13] and the *p*H effect,[4] which influence the sharpness of the boundary. Although these effects may be discussed separately, it is the net effect which determines the change in velocity of a constituent across the boundary.

If the velocity of the constituent is greater behind the boundary than ahead of it, the boundary will eventually reach a steady state in which the tendency to diffuse is balanced by the tendency of the material in the region behind the boundary to overtake that in the region ahead of the boundary. For the descending $\alpha\beta$-boundary, the condition may be expressed as

$$\bar{u}_S^\beta E^\beta > \bar{u}_S^\alpha E^\alpha \tag{22}$$

where $\bar{u}_S^\beta$ is the mobility that the S constituent would have at the *p*H of the β-phase

$$\bar{u}_S^\beta = u_S^\beta\left(1 + \frac{c_{HS}^\beta}{c_S^\beta}\right)^{-1} = u_S\left(1 + \frac{K_{HR}}{K_{HS}}\frac{c_{HR}^\beta}{c_R^\beta}\right)^{-1} \tag{23}$$

Inequality 22 may be combined with equations 12 and 13 to obtain the following condition for the movement of the $\alpha\beta$-boundary in a steady state

$$\frac{K_{HR}}{K_{HS}} < 1 + \left(1 + \frac{c_S^\alpha}{c_{HS}^\alpha}\right)\left(1 - \frac{r_R}{r_S}\right)\left(\frac{r_A + r_S}{r_A + r_R}\right) \tag{24}$$

For any two acids, the only variable which determines whether the boundary moves in a steady

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[13] L. G. Longsworth and D. A. MacInnes, THIS JOURNAL, **62**, 705 (1940).

state is the *p*H of the α-phase. The boundary should move in a steady state under the following conditions

(1) $K_{HS} > K_{HR}$ and $r_S \geq r_R$

(2) $K_{HS} > K_{HR}$ and $r_S < r_R$

if c_S^α/c_{HS}^α is sufficiently small

(3) $K_{HS} < K_{HR}$ and $r_S > r_R$

if c_S^α/c_{HS}^α is sufficiently large. In a similar way the following inequality is obtained for the steady state condition in the ascending system

$$\frac{r_R}{r_S} - 1 > \left\{\frac{r_A + r_R}{r_A + r_S}\left(1 - \frac{K_{HR}}{K_{HS}}\right) + 1 - \frac{r_R}{r_S}\right\}\frac{c_R^\alpha c_{HR}^\gamma}{c_S^\alpha c_R^\gamma} + \left\{1 - \frac{K_{HR}}{K_{HS}}\frac{r_R}{r_S}\right\}\frac{c_{HR}^\gamma}{c_R^\gamma} + \left\{\frac{K_{HS}}{K_{HR}}\left(1 - \frac{r_R}{r_S}\right)\right\}\frac{c_R^\alpha}{c_S^\alpha} \quad (25)$$

It is of interest to consider the factors that determine whether the conductivity and *p*H effects are favorable for a sharp moving boundary. A boundary $\zeta\eta$ moving toward the ζ-phase will be sharpened by the conductivity effect if $E^\eta/E^\zeta > 1$ where E is the field strength. If ion mobilities are equal on both sides of the boundary, which is the case if the ionic strengths are approximately equal, then the ratio of field strengths may be written in terms of relative conductances.

$$E^\eta/E^\zeta = \sigma^\zeta/\sigma^\eta \quad (26)$$

Since the mobility of the anion constituent of a weak acid increases with the *p*H, the constituent mobility will be greater in the η-phase than in the ζ-phase if $c_H^\zeta/c_H^\eta > 1$, and the $\zeta\eta$-boundary will be sharpened by the *p*H effect if the constituent disappears across the boundary.

For the descending $\alpha\beta$-boundary, equations 12 and 13, which give the composition of the β-phase, may be used to obtain the ratios of field strengths and hydrogen ion concentrations across the boundary.

$$\frac{E^\beta}{E^\alpha} = \frac{(r_A + r_R)c_R^\alpha + (r_A + r_S)c_S^\alpha}{(r_A + r_R)c_R^\alpha + \frac{r_R}{r_S}(r_A + r_S)c_S^\alpha + \frac{r_A}{r_S}(r_R - r_S)c_{HS}^\alpha} \quad (27)$$

$$\frac{c_H^\alpha}{c_H^\beta} = \frac{1 + \frac{K_{HS}}{K_{HR}}\frac{c_{HS}^\alpha}{c_{HR}^\alpha}\left\{\frac{r_R(r_A + r_S)}{r_S(r_A + r_R)} + \frac{c_{HS}^\alpha}{c_S^\alpha}\frac{r_A(r_R - r_S)}{r_R(r_A + r_R)}\right\}}{1 + \frac{c_{HS}^\alpha}{c_{HR}^\alpha}} \quad (28)$$

For the $\beta\gamma$-boundary in the ascending limb, the corresponding relations are

$$\frac{E^\beta}{E^\gamma} = \frac{1 + \frac{K_{HS}}{K_{HR}}\frac{c_R^\alpha}{c_S^\alpha}}{1 + \frac{K_{HS}}{K_{HR}}\frac{c_R^\alpha}{c_S^\alpha} - \frac{r_A(r_R - r_S)}{r_S(r_A + r_R)}\frac{c_{HR}^\gamma}{c_R^\gamma}} \cdot \frac{\frac{c_R^\alpha}{c_S^\alpha}\frac{(r_A + r_R)}{(r_A + r_S)} + \frac{r_R}{r_S}}{\frac{c_R^\alpha}{c_S^\alpha}\frac{(r_A + r_R)}{(r_A + r_S)} + 1} \quad (29)$$

$$\frac{c_H^\gamma}{c_H^\beta} = \frac{1 + \frac{K_{HR}}{K_{HS}}\frac{c_S^\alpha}{c_R^\alpha}\left\{1 - \frac{r_A(r_R - r_S)}{r_S(r_A + r_R)}\frac{c_{HR}^\gamma}{c_R^\gamma}\right\}}{1 + \frac{r_R(r_A + r_S)}{r_S(r_A + r_R)}\frac{c_S^\alpha}{c_R^\alpha}} \quad (30)$$

TABLE I

SUMMARY OF CONDUCTIVITY AND *p*H EFFECTS IN SYSTEMS OF TYPE I

(A sharpening effect is denoted by +, a spreading effect by −, and no effect by 0.)

Conditions	Descending Conductivity	Descending *p*H	Ascending Conductivity	Ascending *p*H
$K_{HS} \geqslant K_{HR}$, $r_S \leqslant r_R$	− or 0	+	+ or 0	−
$K_{HS} \leqslant K_{HR}$, $r_S \geqslant r_R$	+ or 0	−	− or 0	+
$K_{HS} \gg K_{HR}$, $r_S > r_R$	+	+	−	−
$K_{HS} \ll K_{HR}$, $r_S < r_R$	−	−	+	+

In Table I the directions of the conductivity and *p*H effects under various conditions are listed. As a general rule, the mobility of a protein is less than that of the buffer ion of the same sign. Avoiding the extreme case in which $K_{HS} << K_{HR}$, the case most representative of protein-like experiments is taken to be that in which $K_{HS} \geq K_{HR}$ and $r_R > r_S$. From the table, it is seen that the directions of the conductivity and *p*H effects are opposed in each moving boundary system. Furthermore, if one of the effects is favorable in either the descending or ascending system, it is unfavorable in the other system. These conclusions were reached by Longsworth[4] from a consideration of the theory for strong electrolytes by assuming the concentrations of undissociated species to be constant throughout the system. Longsworth showed that in the case of ovalbumin in an acetate buffer there is a higher degree of enantiography between the ascending and descending patterns when the protein is above its isoelectric point than when the protein is below its isoelectric point.

Conditions under which Only One Moving Boundary Is Obtained.—Consider any two solutions ζ and η which contain the same constituents and which are separated by the $\zeta\eta$-boundary. The flow $\mathfrak{F}$ of any constituent J in one of the solutions as current is passed through the boundary is $\bar{u}_J\bar{c}_J/1000\kappa$ moles coulomb^{-1}, and so the difference in flow in the ζ- and η-phases is expressed by writing equation 1 as

$$\Delta\mathfrak{F} = \bar{u}_J^\zeta\bar{c}_J^\zeta/1000\kappa^\zeta - \bar{u}_J^\eta\bar{c}_J^\eta/1000\kappa^\eta \quad (31)$$

If each constituent J has only one ionic form j and if relative ion mobilities are constant, then equation (31) may be rewritten as

$$\Delta\mathfrak{F} = (c_j^\zeta/\sigma^\zeta - c_j^\eta/\sigma^\eta)r_j/1000 \quad (32)$$

If every ion is diluted by the same factor across the $\zeta\eta$-boundary, then $c_j^\zeta/\sigma^\zeta = c_j^\eta/\sigma^\eta$ and $\Delta\mathfrak{F} = 0$. If every ion is not diluted by the same factor across the $\zeta\eta$-boundary, then $c_j^\zeta/\sigma^\zeta \neq c_j^\eta/\sigma^\eta$ and $\Delta\mathfrak{F} \neq 0$. In the latter case, a moving boundary will sweep out a new phase between the ζ- and η-phases. Conversely, if the passage of current does not produce a new phase, the flow of each constituent must be the same in the two phases, as in the former case.

Therefore, it is clear that in the moving boundary systems discussed in the theory hydrogen ion is diluted across the stationary boundaries by the same factor as the other ions. If k_D is the ratio c_j^γ/c_j^β at the $\beta\gamma$-boundary in the descending system, the following is obtained from equation 12

$$c_{HR}^\gamma = k_D^2(c_{HR}^\alpha + c_{HS}^\alpha) \quad (33)$$

Similarly, if c_j^β/c_j^α at the ascending $\alpha\beta$-boundary is designated by k_A, the relation for the ascending system is

$$c_{HR}^\gamma = k_A^2(c_{HR}^\alpha + c_{HS}^\alpha) \quad (34)$$

These relations show that the compositions of the initial phases are not arbitrary if only two boundaries are to form in each system.

According to equations 33 and 34, $k_D = k_A$. Therefore it would be possible to derive the conditions involving the compositions of the initial phases under which only two boundaries would form in each limb. However, whether a particular system meets these conditions may be determined more simply. This is done by obtaining the compositions of the two intermediate phases in the manner described above and by examining the ratios of ion concentrations across the stationary boundaries. If at each boundary the ratios for all ions are not equal, then the conditions are not met. In this case it is to be expected that three boundaries will occur in each limb, and the simplified theory is inadequate for a complete prediction of the behavior of the system.

Moving Boundary Systems Formed by Aspartate in Acetate Buffers

A study has been made of moving boundary systems which form during the passage of current through an initial boundary separating a sodium acetate buffer from a sodium acetate buffer to which aspartic acid has been added. In the acetate buffering region, aspartic acid exists predominantly as the dipolar ion $^-O_2CCH(NH_3^+)CH_2CO_2H$ and the negative ion $^-O_2CCH(NH_3^+)CH_2CO_2^-$ which will be designated as HAsp and Asp^-, respectively. The two initial phases in the boundary system are indicated by HOAc, NaOAc (γ) and HOAc, NaOAc, HAsp, NaAsp (α). This system is similar to that discussed in the preceding section, in which A = Na, R = OAc, and S = Asp.

Number of Boundaries.—In a number of these aspartate–acetate moving boundary systems, the maximum allowable number of boundaries (three) were observed. Figure 1a shows the pattern for the ascending boundary system for experiment 8. In addition to the extremely diffuse $\beta\gamma$-boundary across which aspartate disappears there is a boundary moving toward the negative electrode across which no constituent disappears. Also shown in Fig. 1b is the pattern for the descending boundary system for experiment 11, in which convection apparently occurs as the second moving boundary is formed.

The theoretical description of the systems containing three boundaries would be considerably more complicated, and therefore it is of interest to consider to what extent the theory derived for systems containing only two boundaries is applicable. In equations 12 and 13 the only concentrations required are those of the α-phase in the descending system; therefore, the composition of the β-phase and the displacement of the $\alpha\beta$-boundary are independent of the existence of a boundary moving in the opposite direction. On the other hand, the relations 18 to 21 for the composition of the β-phase in the ascending limb involve the concentrations of the solution below the stationary boundary in addition to the top solution, but if there is a boundary moving with the current the composition of the new phase below the stationary boundary is not known. Therefore, the composition of the ascending β-solution and the displacement of the $\beta\gamma$-boundary in that limb which are calculated from the theory will not be exactly correct, although the errors in the calculated values should decrease as the concentration gradients across the second moving boundary decrease.

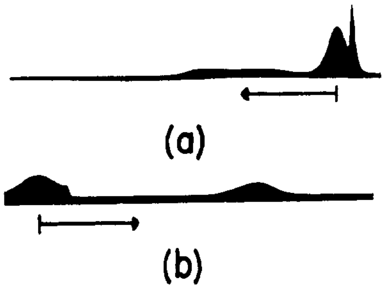

Fig. 1.—Schlieren patterns for systems showing two moving boundaries: a, experiment 8, ascending, after 50 min. at 12 ma.; b, experiment 11, descending, after 220 min. at 12 ma.

In the case of the descending system, an additional moving boundary would be expected if $c_H^\beta/\sigma^\beta - c_H^\gamma/\sigma^\gamma$ is not equal to zero (see equation 32). In nine experiments in which the absolute values of $c_H^\beta/\sigma^\beta - c_H^\gamma/\sigma^\gamma$ calculated from the compositions of the phases are greater than 0.10, three boundaries are observed in the descending channel. In five experiments in which the absolute values are less than 0.10, only two boundaries appear. The only exception is a three-boundary system in which the quantity is equal to +0.07. In other words, three boundaries usually appear when the difference in flow of hydrogen ions is numerically large, as expected.

In five ascending systems in which the absolute values of the quantity $c_H^\beta/\sigma^\beta - c_H^\alpha/\sigma^\alpha$ are greater than 0.94, there are three boundaries. In ten patterns in which the absolute values are less than 0.94, only two boundaries appear. It is interesting to observe this correlation for the ascending system, although the value of the term c_H^β/σ^β used in these calculations is only approximately correct, for the reason discussed above.

Boundary Displacements and Conductances of Intermediate Phases.—It is of interest to compare the values of these quantities determined experimentally with the values that are calculated with the theory. In order to obtain the latter, it is necessary to know the compositions of the various phases in the moving boundary systems. The concentrations of the various species in the initial phases were calculated from the ionization constants of aspartic and acetic acids and the known concentrations of the aspartate, acetate and sodium constituents, which are listed in Table II. The compositions of the intermediate phases were calculated by use of equations 12 and 13 and 18, 19, 20 and 21. The values of the ionization constants and relative mobilities used for these calculations are $K_{HAsp} = 11.7_5 \times 10^{-5}$, $K_{HOAc} = 2.00 \times 10^{-5}$, $r_{Na} = 1.000$, $r_{OAc} = 0.751$, and $r_{Asp} = 0.512$.

The theoretical values of the boundary displacements were obtained from equations 9 and 10, which were rewritten as follows in terms of relative mobilities and concentrations

TABLE II

STOICHIOMETRIC COMPOSITIONS OF INITIAL PHASES IN MOVING BOUNDARY SYSTEMS

(moles/l. at 0°)

Exp.	α-Phase c_{HAsp}	α-Phase c_{HOAc}	α-Phase c_{NaOH}	α-Phase pH	γ-Phase c_{HOAc}	γ-Phase c_{NaOH}	γ-Phase pH	No. of boundaries Desc.	No. of boundaries Asc.
1	0.0400	0.0600	0.0500	4.40	0.0600	0.0200	4.40	3	3
2	.0350	.0400	.0500	4.70	.0400	.0200	4.70	3	3
3	.0600	.4375	.0938	3.93	.4375	.0638	3.93	3	3
4	.0425	.2036	.0953	4.36	.2036	.0953	4.64	2	2
5	.0509	.3156	.0953	4.11	.2036	.0545	4.26	3	2
6	.0606	.2146	.0953	4.23	.1578	.0783	4.69	2	2
7	.0851	.1527	.0953	4.23	.1578	.0783	4.69	2	2
8	.0953	.1221	.0953	4.24	.1507	.0300	4.10	3	3
9	.0953	.1221	.0953	4.24	.1629	.0817	4.70	2	2
10	.0953	.1221	.0953	4.24	.1018	.0817	5.30	2	2
11	.0474	.2470	.0953	4.24	.1375	.0409	4.33	3	2
12	.0284	.2963	.0953	4.24	.1375	.0409	4.33	3	2
13	.1144	.0751	.0953	4.23	.1588	.0790	4.70	3	3
14	.1135	.2647	.0953	3.93	.1629	.0817	4.70	3	2
15	.0604	.4581	.0953	3.92	.1375	.0409	4.33	3	2

TABLE III

BOUNDARY DISPLACEMENTS[a] AND CONDUCTANCES[b] OF INTERMEDIATE PHASES OF ASPARTATE–ACETATE MOVING BOUNDARY SYSTEMS

Exp.	Descending $v^{\alpha\beta}$ (theor.)	Descending $v^{\alpha\beta}$ (exp.)	Descending Dev., %	Descending $10^3\,\kappa^\beta$ (theor.)	Descending $10^3\,\kappa^\beta$ (exp.)	Descending Dev., %	Ascending $v^{\beta\gamma}$ (theor.)	Ascending $v^{\beta\gamma}$ (exp.)	Ascending Dev., %	Ascending $10^3\,\kappa^\beta$ (theor.)	Ascending $10^3\,\kappa^\beta$ (exp.)	Ascending Dev., %
4	0.0243	0.0246	+1.2	4.00	3.87	−3.2	0.0285	0.0289	+1.4	3.12	3.16	+ 1.3
5	.0200	.0203	+1.5	4.07	4.10	+ .7	.0386	.0388	+ .5	1.78	1.93	+ 8.5
6	.0225	.0230	+2.2	4.16	3.90	−6.3	.0361	.0367	+1.4	2.47	2.55	+ 3.2
7	.0230	.0234	+1.7	4.41	4.11	−6.8	.0364	.0368	+1.1	2.27	2.42	+ 6.6
9	.0235	.0244	+3.8	4.48	4.28	−4.5	.0357	.0354	− .8	2.19	2.55	+16.5
10	.0235	.0240	+2.1	4.48	4.25	−5.1	.0440	.0441	+ .2	2.39	2.46	+ 2.9
11	.0223	.0226	+1.3	4.04	3.93	−2.7	.0544	.0554	+1.8	1.36	1.46	+ 7.4
12	.0219	.0221	+ .9	3.87	4.07	+5.2	.0542	.0567	+4.4	1.46	1.65	+13.0
15	.0164	.0149	−9.2	4.16	4.19	+ .7	.0544	.0556	+2.2	1.37	1.47	+ 7.3

[a] Ml. coulomb^{-1}. [b] Ohm^{-1} cm.$^{-1}$ at 0°.

Descending:

$$v^{\alpha\beta} = \frac{r_{\text{Asp}}(1 + c_{\text{HAsp}}^{\alpha}/c_{\text{Asp}}^{\alpha})^{-1}}{c_{\text{Asp}}^{\alpha}(r_{\text{Na}} + r_{\text{Asp}}) + c_{\text{OAc}}^{\alpha}(r_{\text{Na}} + r_{\text{OAc}})} \quad (35)$$

Ascending:

$$v^{\beta\gamma} = \frac{r_{\text{Asp}}(1 + c_{\text{HAsp}}^{\beta}/c_{\text{Asp}}^{\beta})^{-1}}{c_{\text{Asp}}^{\beta}(r_{\text{Na}} + r_{\text{Asp}}) + c_{\text{OAc}}^{\beta}(r_{\text{Na}} + r_{\text{OAc}})} \quad (36)$$

The results of these calculations and the experimentally determined displacements are given in Table III for a series of experiments, which do not include any experiments in which two moving boundaries were observed in the ascending limb. The uncertainty of the calculated values of the displacements was estimated by assuming an error of 5% in the ratio $K_{\text{HAsp}}/K_{\text{HOAc}}$ and an error of 2% in the relative mobilities of aspartate and acetate. As a consequence of these errors, the uncertainty in calculated values for $v^{\alpha\beta}$ and $v^{\beta\gamma}$ is approximately 3–5%. The experimental error is approximately 1%. Therefore, the difference between experimental and calculated values is generally less than would have been expected.

At the conclusion of these experiments, a sample of each intermediate phase was removed from the cell and its specific conductance was determined. The results of these determinations are shown in Table III. By use of the calculated concentration values and the relative mobilities of the ions, relative conductances of the solutions were calculated from $\sigma = 96.5\,\{c_{\text{Asp}}(r_{\text{Na}} + r_{\text{Asp}}) + c_{\text{OAc}}(r_{\text{Na}} + r_{\text{OAc}})\}$. The value of the relative conductance of each phase was multiplied by the absolute mobility of sodium[14] at that ionic strength in order to obtain a calculated value of the specific conductance. These calculated values are also given in Table III. Because of the uncertainties in quantities that must be used to calculate relative conductances, the calculated values are uncertain to the extent of about 2–3%. Furthermore, the assumption that relative ion mobilities are constant, which is required to calculate the specific conductances, leads to an estimated error in the latter values of 5%, which corresponds to the average error indicated in Table III. In some cases among the ascending phases, the difference is considerably larger which may in some cases result from the difficulty of obtaining the sample for a conductance measurement. Although in none of the ascending systems listed in Table III was more than one moving boundary actually observed, the predictions for the ascending systems are less accurate than those for the descending systems for the reason discussed in the preceding section.

Boundary Sharpness.—The theory predicts the conditions under which aspartate boundaries should move in a steady state. Inequalities 24 and 25 for this case give the following steady state conditions

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[14] E. A. Anderson, Doctoral Dissertation, University of Wisconsin, 1949.

Descending:

$$c_{\mathrm{HAsp}}{}^{\alpha}/c_{\mathrm{Asp}}{}^{\alpha} > 0.945 \qquad (37)$$

Ascending:

$$0.50 > 0.47\,\frac{c_{\mathrm{OAc}}{}^{\alpha}}{c_{\mathrm{Asp}}{}^{\alpha}}\,\frac{c_{\mathrm{HOAc}}{}^{\gamma}}{c_{\mathrm{OAc}}{}^{\gamma}} + 0.73\,\frac{c_{\mathrm{HOAc}}{}^{\gamma}}{c_{\mathrm{OAc}}{}^{\gamma}} - 2.92\,\frac{c_{\mathrm{OAc}}{}^{\alpha}}{c_{\mathrm{Asp}}{}^{\alpha}} \qquad (38)$$

It is often difficult to determine experimentally whether boundaries move in a steady state. In many cases the boundaries become more diffuse as long as they are observed. At present, there is no way of predicting how long a time would be required for a moving boundary to reach a steady state. For this reason, it cannot be stated from rather limited observation that the boundary does not reach a steady state, although a boundary which does move in a steady state is easily identified. In many cases, it is possible only to remark how the relative sharpness of boundaries is changed when certain experimental conditions are altered.

The sharpness of the descending $\alpha\beta$-boundary has been observed for different solutions in which the ratios $c_{\mathrm{HAsp}\alpha}/c_{\mathrm{Asp}\alpha}$, $c_{\mathrm{OAc}\alpha}/c_{\mathrm{Asp}\alpha}$ and $c_{\mathrm{HOAc}\gamma}/c_{\mathrm{OAc}\gamma}$ were varied systematically. In several series of experiments in which only one of these ratios was varied, the sharpness of the boundary appeared to be independent of the values of $c_{\mathrm{OAc}\alpha}/c_{\mathrm{Asp}\alpha}$ and $c_{\mathrm{HOAc}\gamma}/c_{\mathrm{OAc}\gamma}$ but increased as the value of $c_{\mathrm{HAsp}\alpha}/c_{\mathrm{Asp}\alpha}$ increased. These observations are in accordance with the predictions made with inequality 37 about the effects of these variables on the relative velocities of the aspartate constituent in the α- and β-phases. The results of tests of the steady state condition, however, are inconclusive.

Similar observations were made of the sharpness of the ascending $\beta\gamma$-boundary. The sharpness of the boundary increased as $c_{\mathrm{HOAc}\gamma}/c_{\mathrm{OAc}\gamma}$ decreased and increased as $c_{\mathrm{OAc}\alpha}/c_{\mathrm{Asp}\alpha}$ increased with $c_{\mathrm{HOAc}\gamma}/c_{\mathrm{OAc}\gamma}$ fixed at a value less than 6. On the other hand, the sharpness was independent of the value of $c_{\mathrm{HAsp}\alpha}/c_{\mathrm{Asp}\alpha}$. These observations are in accordance with predictions made with inequality (38). In experiments in which the experimental conditions satisfied inequality 38, the boundary appeared to move in a steady state in experiments 9 and 10, but it did not reach a steady state during the periods of observation in experiments 6, 7, 11, 12 and 13.

It is interesting that in nearly every experiment which has been performed the boundary across which aspartate disappears is asymmetrical. This cannot be the result of inhomogeneity of the aspartate constituent, although such a conclusion is often made about proteins that form asymmetrical boundaries. With the results obtained with aspartate systems in mind, it would not seem to be safe to state that a protein is heterogeneous simply because it forms asymmetrical peaks in electrophoresis experiments.

Experimental

Experiments were carried out at 1° with the use of an 11-ml. Tiselius cell in which boundaries were observed with a cylindrical lens schlieren optical system. The general techniques used in these experiments have been described previously.[7,15] The Tiselius cell which was used had mean cross-sectional areas of 0.7487 cm.^{2} and 0.7497 cm.^{2} for the two channels. These values were obtained from the weights of water and of mercury required to fill each channel and from the over-all length of the channels. Corrections (about 0.7%) for the larger cross-sectional areas within the end-plates were applied. During each experiment the current through the cell was maintained constant within 0.02% at about 12 ma. with a Rubicon regulating galvanometer.[16] Photographs of the schlieren patterns were made at recorded intervals of time on Eastman Panchromatic Tri X Type B plates. For accurate mobility determinations, boundary displacements were determined from measurements made with a comparator; in other cases, the displacements were determined from enlarged tracings of the patterns. Corrections (about 1%) were added to the observed displacements in order to correct for solvent displacement.[17] Conductances were measured at 0°.

The solutions which were used in the moving boundary experiments were prepared from reagent-grade chemicals. The solutions were prepared by dissolving recrystallized aspartic acid in water and adding aliquots of standardized acetic acid and sodium hydroxide.

The values of the ionization constants for aspartic (second acid dissociation) and acetic acids as determined from *p*H measurements on buffer solutions at 1° with the glass electrode are $11.7_6 \times 10^{-6}$ and 2.00×10^{-5}, respectively. Since the ionic strength was near 0.1 and no correction was made for activity coefficients, these values are apparent ionization constants at that ionic strength.

Mobilities of sodium, acetate and aspartate (Asp^-) ions were determined by use of the following moving boundary systems

$$\mathrm{LiCl}\ (\gamma)::\mathrm{LiCl}\ (\beta) \rightarrow \mathrm{NaCl}\ (\alpha)$$

$$\mathrm{NaOAc},\ p\mathrm{H}\ 8\ (\alpha) \leftarrow \mathrm{NaPropionate}\ (\beta)::\mathrm{NaPropionate}\ (\gamma)$$

$$\mathrm{NaAsp},\ p\mathrm{H}\ 7\ (\alpha) \leftarrow \mathrm{NaGlutamate}\ (\beta)::\mathrm{NaGlutamate}\ (\gamma)$$

The result for sodium ion at an ionic strength of 0.050 ($u_{\mathrm{Na}} = 23.2_3 \times 10^{-6}$ cm.^{2} volt^{-1} sec.$^{-1}$) is in excellent agreement with the previous determination by Anderson.[14] At an ionic strength of 0.10, Anderson reports a sodium mobility of $22.2_1 \times 10^{-5}$, and the mobilities of acetate and aspartate at this ionic strength relative to that sodium were found to be 0.751 and 0.512, respectively.

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