[Contribution from the Chemistry Department, University of Wisconsin]

Weak Electrolyte Moving Boundary Systems Analogous to the Electrophoresis of Two Proteins

By James C. Nichol, Edward B. Dismukes and Robert A. Alberty

Received December 6, 1957

A theoretical and experimental study is presented for low molecular weight weak electrolyte moving boundary systems analogous to those encountered in the electrophoresis of two proteins. The theory is applied to the system aspartate–glutamate in acetate buffer under conditions where the constituents exist in significant concentration only as the uncharged acids and univalent anions. The compositions of the various phases produced by the passage of a current are predicted. The expected apparent analyses for the aspartate–glutamate mixtures are calculated from the predicted changes in refractive index across the moving boundaries. The theory is tested using the experimental results of Longsworth along with data obtained in this Laboratory. It is shown that the true refractometric fraction of a "protein" component is not necessarily obtained from the apparent refractometric fraction by a linear extrapolation of the ratio of the "protein" concentration to buffer concentration to zero. The theoretical calculations also provide information on the sharpening and spreading effects which result from *p*H and conductivity gradients through the moving boundaries.

In giving a theoretical description of some of the effects encountered in protein electrophoresis, a number of workers[1–4] have made use of strong electrolyte moving boundary theory.[5,6] However, since both proteins and the buffers used are weak electrolytes, a complete theoretical treatment requires the application of the weak electrolyte moving boundary equation[7–9] to all the constituents present. Weak electrolyte systems analogous to those used in the determination of the transference numbers of strong electrolytes have been discussed by Svensson and co-workers[10] and by Alberty[9] and Nichol.[11] Dismukes and Alberty[12] have applied the weak electrolyte moving boundary equation to a system analogous to the electrophoresis of a single protein in a buffer.

In this paper we present moving boundary experiments with monovalent weak acids which correspond to the electrophoresis of mixtures of two proteins, where the "proteins" are at a *p*H above their isoelectric points in a buffer of the uncharged acid type. The theory developed is applied to the system aspartate–glutamate in acetate buffer over a range of *p*H in which the constituents exist in significant concentration only as the uncharged acids and univalent anions. This system was chosen because of earlier experiments by Longsworth[13] who pointed out that neither the descending nor ascending pattern permitted a linear extrapolation of apparent analysis *versus* the ratio of concentration of "protein" component to buffer salt to the true analysis. Refractive increment data[14] are available for use in predicting the refractive indices of solutions containing sodium acetate, aspartate, glutamate and the corresponding weak acids.

Of special interest from the point of view of electrophoretic analysis is the prediction of the magnitude of the individual concentration changes across each boundary in the above system, including those of constituents which do not disappear in the boundary. As a consequence of the superimposed refractive index gradients, the apparent refractometric analyses from the ascending and descending patterns are different, and the analyses also depend upon the ratio of the total protein concentration to the buffer concentration.[1–4,15,16] For the simple system discussed below quantitative predictions of these effects are made and confirmed by experiment.

In the preceding paper, Nichol and Gosting[17] have shown that for strong electrolyte systems analogous to those used for analyzing protein mixtures, the apparent fraction of a "protein" component does not in general approach the true refractometric fraction linearly as the ratio of total "protein" concentration to "buffer" concentration approaches zero. The theoretical calculations for the weak electrolyte aspartate–glutamate–acetate system discussed in this paper lead to the same conclusion. It is therefore evident that great care should be exercised in interpreting linear extrapolations of the apparent analyses to infinite dilution of the components being analyzed.

Theory

The descending and ascending moving boundary systems to be considered are illustrated in Fig. 1. These systems result when the initial boundaries are formed between a buffer (AR, HR; where AR is the salt of a weak acid HR and a strong base AOH) and a solution of two weak acids (HS and HT) in the buffer. The mobility of constituent T is greater than that of constituent S. In order to obtain a moving boundary system of this type, it is necessary to pick weak acids with ionization constants and anion mobilities such that the two protein-like constituents (R and S) will disappear in the moving boundaries. It should be noted that since there are six constituents (R, S, T, A, H and

---

[1] L. G. Longsworth, *J. Phys. and Colloid Chem.*, **51**, 171 (1947).

[2] S. H. Armstrong, Jr., M. J. E. Budka and K. C. Morrison, This Journal, **69**, 416 (1947).

[3] R. A. Alberty, *J. Chem. Educ.*, **25**, 619 (1948).

[4] J. R. Cann, This Journal, **71**, 907 (1949).

[5] V. P. Dole, *ibid.*, **67**, 1119 (1945).

[6] H. Svensson, *Arkiv Kemi, Mineral. Geol.*, **22A**, No. 10 (1946).

[7] H. Svensson, *Acta Chem. Scand.*, **2**, 841 (1948).

[8] R. A. Alberty and J. C. Nichol, This Journal, **70**, 2297 (1948).

[9] R. A. Alberty, *ibid.*, **72**, 2361 (1950).

[10] H. Svensson, A. Benjaminsson and I. Brattsten, *Acta Chem. Scand.*, **3**, 307 (1949); I. Brattsten and H. Svensson, *ibid.*, **3**, 359 (1949).

[11] J. C. Nichol, This Journal, **72**, 2367 (1950).

[12] E. B. Dismukes and R. A. Alberty, *ibid.*, **76**, 191 (1954).

[13] L. G. Longsworth, *Anal. Chem.*, **23**, 346 (1951).

[14] E. B. Dismukes and R. A. Alberty, This Journal, **75**, 809 (1953).

[15] H. Svensson, *Arkiv. Kemi, Mineral. Geol.*, **17A**, No. 14 (1943).

[16] G. E. Perlman and D. Kaufman, This Journal, **67**, 638 (1945).

[17] J. C. Nichol and L. J. Gosting, *ibid.*, **80**, 2601 (1958).

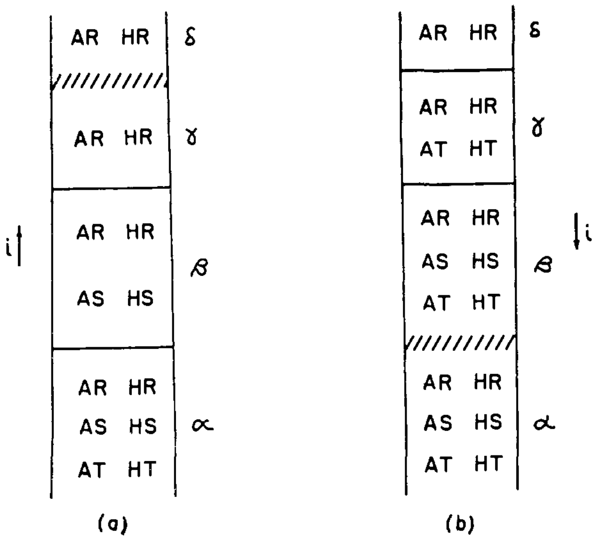

Fig. 1.—(a) Descending weak electrolyte moving boundary system analogous to the electrophoresis of two proteins S and T in a buffer, AR, HR. (b) Corresponding ascending system. The arrow indicates the direction of the current, and the boundary near the initial boundary position is indicated by / / / / / /.

OH), a maximum of four boundaries is permitted in each limb.[12] In the experiments reported only three boundaries are observed in each limb. Two of these are moving boundaries and the third is something like the concentration boundary which remains near the site of the initial boundary in strong electrolyte experiments but is different in that the concentrations of the salts and the different acids do not change across the boundary in the same ratio. The boundary which is not observed is of the type across which no constituent disappears and may be considered to be connected with the hydrogen and hydroxyl ions which have very low concentrations in the present experiments. The conditions under which such a boundary does not form in a system analogous to the electrophoresis of a single protein have been discussed earlier.[12]

In the following theoretical treatment use is made of the weak electrolyte moving boundary equation and of the Kohlrausch regulating function for monovalent weak electrolytes.[12] In deriving the equations it is assumed that the relative ion mobilities and relative values of the ionization constants for the weak acids are constant throughout the system, that the mobilities of uncharged weak acid molecules are zero, that the total concentration of weak acid at any level in the cell is constant and that the conductance due to H^+ and OH^- ions may be ignored.

Compositions of the End Phases.—In order to predict the compositions of the intermediate phases which are formed by the passage of current, it is necessary to know the compositions of the end phases as well as values for the mobilities of all ions and ionization constants of all weak acids. The composition of the δ solution (buffer) in terms of HR and AR is obtained directly from a knowledge of the number of moles of HR and AOH used to make up the solution. If the α solution is made up of A moles of HR, B moles of HS, C moles of HT and S moles of AOH per liter, the concentrations of the various weak acids and salts in this phase are given by

$$A = a + r,\ B = b + s,\ C = c + t,\ S = a + b + c \quad (1)$$

where $a = C_{\mathrm{AR}}$, $b = C_{\mathrm{AS}}$, $c = C_{\mathrm{AT}}$, $r = C_{\mathrm{HR}}$, $s = C_{\mathrm{HS}}$ and $t = C_{\mathrm{HT}}$. The equilibrium relations may be written as

$$K_{\mathrm{HR}}\frac{(A-a)}{a} = K_{\mathrm{HS}}\frac{(B-b)}{b} = K_{\mathrm{HT}}\frac{(C-c)}{c} \quad (2)$$

where the K's are apparent ionization constants at the ionic strength of the final solution. Since the ratios of these constants are assumed to be invariant throughout the system, the values of a and b may be obtained by simultaneous solution of the following two quadratic equations which are derived from (1) and (2)

$$(M-1)a^2 + \{A + S + M(C - S) + b(M-1)\}\, a + A(b - S) = 0 \quad (3)$$

$$(N-1)b^2 + \{B + S + N(C - S) + a(N-1)\}\, b + B(a - S) = 0 \quad (4)$$

where $M = K_{\mathrm{HT}}/K_{\mathrm{HR}}$ and $N = K_{\mathrm{HT}}/K_{\mathrm{HS}}$. Concentrations c, r, s and t may then be calculated from equation 1. In these equations and those which follow all concentrations are taken as positive quantities.

Compositions of Intermediate Phases in the Descending System.—The composition of the γ–phase may be calculated from the statement of the conservation of weak acid

$$C^{\gamma}_{\mathrm{HR}} = C^{\beta}_{\mathrm{HR}} + C^{\beta}_{\mathrm{HS}} = C^{\alpha}_{\mathrm{HR}} + C^{\alpha}_{\mathrm{HS}} + C^{\alpha}_{\mathrm{HT}} \quad (5)$$

and from the Kohlrausch regulating function for monovalent weak acids[12]

$$C^{\gamma}_{\mathrm{R}}\left(\frac{1}{r_{\mathrm{R}}} + \frac{1}{r_{\mathrm{A}}}\right) + \frac{C^{\gamma}_{\mathrm{HR}}}{r_{\mathrm{R}}} = \bar{\omega}^{\gamma} = \bar{\omega}^{\alpha} = \bar{\omega}^{\beta} \quad (6)$$

where

$$\bar{\omega}^{\alpha} = C^{\alpha}_{\mathrm{R}}\left(\frac{1}{r_{\mathrm{R}}} + \frac{1}{r_{\mathrm{A}}}\right) + C^{\alpha}_{\mathrm{S}}\left(\frac{1}{r_{\mathrm{S}}} + \frac{1}{r_{\mathrm{A}}}\right) + C^{\alpha}_{\mathrm{T}}\left(\frac{1}{r_{\mathrm{T}}} + \frac{1}{r_{\mathrm{A}}}\right) + \frac{C^{\alpha}_{\mathrm{HR}}}{r_{\mathrm{R}}} + \frac{C^{\alpha}_{\mathrm{HS}}}{r_{\mathrm{S}}} + \frac{C^{\alpha}_{\mathrm{HT}}}{r_{\mathrm{T}}} \quad (7)$$

The four unknown concentrations in the β-phase of the descending limb may be obtained by combining equation 5, equation 6, the mass action relationship (equation 8) and the weak electrolyte moving boundary equations[8,12] for the R and T constituents at the α,β-boundary (equations 9 and 10).[18]

$$K_{\mathrm{HR}}C^{\beta}_{\mathrm{HR}}/C^{\beta}_{\mathrm{R}} = K_{\mathrm{HS}}C^{\beta}_{\mathrm{HS}}/C^{\beta}_{\mathrm{S}} \quad (8)$$

$$\frac{C^{\alpha}_{\mathrm{R}}}{\sigma^{\alpha}} - \frac{1000C^{\beta}_{\mathrm{R}}/\mathfrak{F}}{C^{\beta}_{\mathrm{R}}(r_{\mathrm{A}} + r_{\mathrm{R}}) + C^{\beta}_{\mathrm{S}}(r_{\mathrm{A}} + r_{\mathrm{S}})} = \frac{v^{\alpha\beta}}{r_{\mathrm{R}}}(C^{\alpha}_{\mathrm{HR}} + C^{\alpha}_{\mathrm{R}} - C^{\beta}_{\mathrm{HR}} - C^{\beta}_{\mathrm{R}}) \quad (9)$$

$$r_{\mathrm{T}}C^{\alpha}_{\mathrm{T}}/(C^{\alpha}_{\mathrm{T}} + C^{\alpha}_{\mathrm{HT}}) = v^{\alpha\beta}\sigma^{\alpha} \quad (10)$$

where σ^{α} is the relative conductance, $(\mathfrak{F}/1000)\sum_{i=1}^{N} C_i r_i$, of the α-phase,[12] and $\mathfrak{F}$ is the faraday.

---

[18] In solving these equations, two simultaneous quadratic equations $f_1(x,y) = 0$ and $f_2(x,y) = 0$ involving the unknowns C^{β}_{R} and C^{β}_{S} are encountered. If the substitution $y = Vx$ is made and each term is divided by x, the two resulting equations may be combined to yield the cubic equation $g(V) = 0$ which may be solved for V by Newton's method.

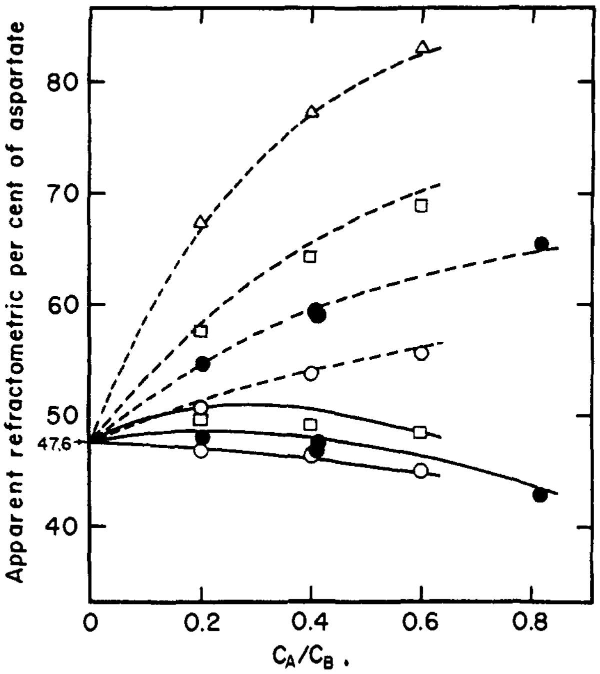

Fig. 2.—Plot of apparent refractometric per cent. aspartate *versus* C_A/C_B, the ratio of the sum of glutamate and aspartate constituent concentrations to the concentration of sodium acetate in the buffer. The curves have been computed theoretically; – – – –, ascending; ———, descending. The various symbols represent experimental points determined in the different buffers for which the theoretical curves were calculated: $C_{\mathrm{HOAc}}^{\delta}/C_{\mathrm{NaOAc}}^{\delta}$ = 2, ○; 1, ● (Longsworth's data, ref. 13); 0.5, □; 0, △.

Compositions of Intermediate Phases in the Ascending System.—In the β-phase of the ascending moving boundary system, the concentrations of the weak acids are calculated from the statement of conservation of weak acid

$$C_{\mathrm{HR}}^{\beta} + C_{\mathrm{HS}}^{\beta} + C_{\mathrm{HT}}^{\beta} = C_{\mathrm{HR}}^{\gamma} + C_{\mathrm{HT}}^{\gamma} = C_{\mathrm{HR}}^{\delta} \quad (11)$$

the mass action relationships

$$K_{\mathrm{HR}}C_{\mathrm{HR}}^{\beta}/C_{\mathrm{R}}^{\beta} = K_{\mathrm{HS}}C_{\mathrm{HS}}^{\beta}/C_{\mathrm{S}}^{\beta} = K_{\mathrm{HT}}C_{\mathrm{HT}}^{\beta}/C_{\mathrm{T}}^{\beta} \quad (12)$$

and the fact that all ions are diluted by a common factor across the α,β-boundary,[12] expressed in this case by

$$C_{\mathrm{R}}^{\beta}/C_{\mathrm{R}}^{\alpha} = C_{\mathrm{S}}^{\beta}/C_{\mathrm{S}}^{\alpha} = C_{\mathrm{T}}^{\beta}/C_{\mathrm{T}}^{\alpha} \quad (13)$$

The ion concentrations in the β-phase may then be obtained by considering the additional relations involving the Kohlrausch functions

$$\bar{\omega}^{\beta} = \bar{\omega}^{\gamma} = \bar{\omega}^{\delta} \quad (14)$$

The four unknown concentrations in the γ-phase of the ascending limb may be obtained by solving simultaneously equation 11, equation 14, the mass action relationship (equation 15) and the weak electrolyte moving boundary equations for the R and T constituents at the γ,δ-boundary (equations 16 and 17).[18]

$$K_{\mathrm{HR}}C_{\mathrm{HR}}^{\gamma}/C_{\mathrm{R}}^{\gamma} = K_{\mathrm{HT}}C_{\mathrm{HT}}^{\gamma}/C_{\mathrm{T}}^{\gamma} \quad (15)$$

$$\frac{C_{\mathrm{R}}^{\delta}}{\sigma^{\delta}} - \frac{1000C_{\mathrm{R}}^{\gamma}/\mathfrak{F}}{C_{\mathrm{R}}^{\gamma}(r_{\mathrm{A}} + r_{\mathrm{R}}) + C_{\mathrm{T}}^{\gamma}(r_{\mathrm{A}} + r_{\mathrm{T}})} = \frac{v^{\gamma\delta}}{r_{\mathrm{R}}}(C_{\mathrm{HR}}^{\delta} + C_{\mathrm{R}}^{\delta} - C_{\mathrm{HR}}^{\gamma} - C_{\mathrm{R}}^{\gamma}) \quad (16)$$

$$r_{\mathrm{T}}C_{\mathrm{T}}^{\gamma}/(C_{\mathrm{T}}^{\gamma} + C_{\mathrm{HT}}^{\gamma}) = (\mathfrak{F}/1000)v^{\gamma\delta}[C_{\mathrm{R}}^{\gamma}(r_{\mathrm{A}} + r_{\mathrm{R}}) + C_{\mathrm{T}}^{\gamma}(r_{\mathrm{A}} + r_{\mathrm{T}})] \quad (17)$$

Boundary Displacements.—Once the compositions of the various phases have been obtained, the relative conductances, σ, are readily calculable.[12] The boundary displacements, v, may then be obtained from $v = \bar{r}/\sigma$ where $\bar{r}$ is the relative constituent mobility of a constituent present on only one side of the boundary and σ is the relative conductance of the phase containing this constituent. It should be emphasized that $\bar{r}$ is not a constant throughout the system since it contains concentration terms varying from phase to phase.

Apparent Refractometric Fraction of "Protein" Components.—These are calculable from the phase compositions and refractive index increments, **k**, of the salts and weak acids. For example, the apparent refractometric fraction, F_{T}, of component T in the ascending channel is given by

$$F_{\mathrm{T(asc)}} = \frac{C_{\mathrm{AR}}^{\gamma}\mathbf{k}_{\mathrm{AR}} + C_{\mathrm{HR}}^{\gamma}\mathbf{k}_{\mathrm{HR}} + C_{\mathrm{AT}}^{\gamma}\mathbf{k}_{\mathrm{AT}} + C_{\mathrm{HT}}^{\gamma}\mathbf{k}_{\mathrm{HT}} - C_{\mathrm{AR}}^{\delta}\mathbf{k}_{\mathrm{AR}} - C_{\mathrm{HR}}^{\delta}\mathbf{k}_{\mathrm{HR}}}{C_{\mathrm{AR}}^{\beta}\mathbf{k}_{\mathrm{AR}} + C_{\mathrm{HR}}^{\beta}\mathbf{k}_{\mathrm{HR}} + C_{\mathrm{AS}}^{\beta}\mathbf{k}_{\mathrm{AS}} + C_{\mathrm{HS}}^{\beta}\mathbf{k}_{\mathrm{HS}} + C_{\mathrm{AT}}^{\beta}\mathbf{k}_{\mathrm{AT}} + C_{\mathrm{HT}}^{\beta}\mathbf{k}_{\mathrm{HT}} - C_{\mathrm{AR}}^{\delta}\mathbf{k}_{\mathrm{AR}} - C_{\mathrm{HR}}^{\delta}\mathbf{k}_{\mathrm{HR}}} \quad (18)$$

Experimental

Experiments were carried out with a Spinco Model H electrophoresis apparatus. An 11 ml. quartz Tiselius cell with reference paths was used. The cell had mean cross-sectional areas of 0.750 cm.² and 0.747 cm.² for the two channels, these values being obtained by determining the cross-sectional area at 5 mm. intervals with a Gaertner comparator. A current of 16 ma., constant to ±0.1%, was used.

Photographs of schlieren and interference fringe patterns were recorded simultaneously on Eastman Kodaline CTC plates. Boundary displacements were determined from the schlieren patterns by measuring the displacements of the maximum ordinates of the symmetrical peaks with a comparator. The refractometric analyses (recorded as apparent refractometric per cent. of aspartate in Fig. 2) were obtained from the Rayleigh fringe patterns. An initial fringe photograph of each channel was taken after the cell center section had been moved into line with top and bottom sections but before the current was turned on. Corrections determined from measurements of small deviations of the fringes from linearity (maximum 0.2 fringe) in these initial photographs were applied to the fringe displacements across the moving boundaries. At the start of the experiment, no compensation of the boundaries was carried out but in most experiments it was found necessary to back compensate at a later stage in the experiment in order to attain good resolution of the boundaries. The best results were obtained when the leading peak was kept stationary with respect to the cell. Too rapid compensation was found to result in changes in the total fringe displacement between the end solutions and in the refractometric analysis with time. In some experiments, notably for the descending boundaries, complete separation, as evidenced by a region of zero fringe displacement between the boundaries, was not obtained. In these cases the position of the minimum in the gradient curve between the boundaries was determined as described by Longsworth.[13] Solutions were prepared in volumetric flasks calibrated for 1°. Aspartic and glutamic acid were weighed directly, while the desired volumes of standard sodium hydroxide and acetic acid solutions were delivered from calibrated burets. The aspartic acid, supplied by the Eastman Kodak Company and the glutamic acid, supplied by the Pfanstiehl Company, were both twice recrystallized from water and dried at 110° for 36 hr.

Conductances were measured at 0° using a Shedlovsky type cell and a Jones bridge. Measurements of pH were made at 25° with a Beckman model G pH meter.

The values[12] of the relative mobilities for sodium, acetate and aspartate ions used were 22.2, 16.7 and 11.1. The value for glutamate ion, 9.8, was determined from a conductance measurement on sodium glutamate at 0°. The ionization constants were determined from pH measurements on buffer solutions at 1° with the glass electrode.

TABLE I

REFRACTOMETRIC ANALYSES OF EQUIMOLAR MIXTURES OF ASPARTIC AND GLUTAMIC ACIDS

Expt.	C_A/C_B	δ-Soln. C_{NaOH}	δ-Soln. C_{HOAc}	α-Soln. C_{NaOH}	α-Soln. C_{HOAc}	$C_{HAsp} + C_{HGlut}$	Refractometric % aspartate Asc. (Exp.)	Asc. (Theor.)	Desc. (Exp.)	Desc. (Theor.)
1	0.200	0.1000	0.3000	0.1000	0.3000	0.02000	50.7	51.38	46.8	47.10
2	.400	.1000	.3000	.1000	.3000	.04000	53.8	54.22	46.4	46.13
3	.600	.1000	.3000	.1000	.3000	.06000	55.6	56.17	45.0	44.71
4[a]	.204	.1965	.3930	.1965	.3930	.0401	54.7	54.64	48.0	48.58
5[a]	.407	.1990	.3980	.1990	.3980	.0811	59.1	59.61	47.6	47.86
6[a]	.405	.0996	.1992	.0996	.1992	.0403	59.3	59.58	46.8	47.87
7[a]	.809	.0999	.1998	.0999	.1998	.0808	65.5	64.80	42.7	43.68
8	.200	.1000	.1500	.1000	.1500	.02000	57.6	58.27	49.6	50.66
9	.400	.1000	.1500	.1000	.1500	.04000	64.3	65.58	49.2	50.50
10	.600	.1000	.1500	.1000	.1500	.06000	69.0	70.15	48.6	48.42
11	.200	.1000	.1000	.1200	.1000	.02000	67.4	66.59	..	...
12	.400	.1000	.1000	.1400	.1000	.04000	77.2	77.12	..	...
13	.600	.1000	.1000	.1600	.1000	.06000	83.1	82.62	..	...

[a] Calculated from Longsworth's data.[13]

TABLE II

CONDUCTANCE DATA AND BOUNDARY DISPLACEMENTS

Expt. no.[a]	Sp. cond., $\kappa \times 10^3$, 0°C. α-soln.	δ-soln.	Boundary displacement, $v \times 10^2$, ml./coul. Asc. $v\gamma\delta$ Obsd.	$v\gamma\delta$ Theor.	$v\beta\gamma$ Obsd.	$v\beta\gamma$ Theor.	Desc. $v\alpha\beta$ Obsd.	$v\alpha\beta$ Theor.	$v\beta\gamma$ Obsd.	$v\beta\gamma$ Theor.
1	3.595	3.682	2.28	2.24	1.48	1.50	2.20	2.14	1.33	1.39
2	3.522	3.682	2.31	2.27	1.47	1.51	2.09	2.06	1.27	1.31
3	3.490	3.682	2.36	2.29	1.44	1.50	1.97	1.93	1.24	1.11
8	3.626	3.736	2.81	2.83	2.34	2.32	2.67	2.65	2.02	2.02
9	3.538	3.736	2.94	2.94	2.36	2.42	2.63	2.60	1.87	1.86
10	3.461	3.736	3.07	3.03	2.46	2.49	2.49	2.59	1.76	1.73
11	4.297	3.753	3.15	3.11	..	..	..	..	..	..
12	4.835	3.753	3.29	3.25	..	..	..	..	..	..
13	5.370	3.753	3.35	3.37	..	..	..	..	..	..

[a] See Table I.

The values for acetic and aspartic acids have been given earlier[12] while the second acid dissociation constant for glutamic acid is 5.25×10^{-5} at 0.1 ionic strength.

Results

Measurements and theoretical predictions were made for both the ascending and descending systems which are represented by

Asc.: HOAc, NaOAc(δ) ←HOAc, NaOAc, HAsp, NaAsp(γ) ←HOAc, NaOAc, HGlut, NaGlut, HAsp, NaAsp(β)::HOAc, NaOAc, HGlut, NaGlut, HAsp, NaAsp(α)

Desc.: HOAc, NaOAc, HGlut, NaGlut, HAsp, NaAsp(α) ←HOAc, NaOAc, HGlut, NaGlut(β) ←HOAc NaOAc(γ)::HOAc, NaOAc(δ)

where, referring to Fig. 1, acetate = OAc^- = R, glutamate = $Glut^-$ = S, aspartate = Asp^- = T and sodium = Na^+ = A.

In Table I are given the compositions of the end solutions (α and δ) and the refractometric per cent. aspartate obtained from measurements on the fringe photographs of the moving boundary systems. Experiments 4–7 are those of Longsworth,[13] his weight concentrations having been converted to an equivalent per liter basis by means of approximate partial specific volumes.[19] Longsworth reported the apparent mole per cent. aspartate after correction for the unequal constituent refractive index increments of aspartate and glutamate, but the values given in Table I are the uncorrected refractometric per cent. of aspartate. The column C_A/C_B gives the ratio of the sum of glutamate and aspartate concentrations to the concentration of sodium acetate in the buffer. Included for comparison in Table I are the theoretical refractometric percentages of aspartate. In Table II are listed the conductance data for the end solutions and the observed and theoretical displacements of the two moving boundaries in each limb. For experiments 11, 12 and 13 (strong electrolyte case) no resolution occurred in the descending limb and hence no data are shown. Also, data are not shown for $v^{\beta\gamma}$ for the ascending system in 11, 12 and 13 because resolution into two peaks was attained only after back compensation.

To show more clearly the trends in the data and to compare the experimental results with the theory, the refractometric per cent. aspartate has been plotted against the ratio C_A/C_B in Fig. 2. The computed values are in agreement with the experimental results within the experimental errors and the uncertainty in the input data for the theoretical calculations. This agreement is taken to confirm the theoretical formulation for this particular type of weak electrolyte system. At much higher or lower *p*H values additional species would have to be considered in the calculation.

To establish precisely the limiting value of the refractometric per cent. aspartate as $C_A/C_B \rightarrow 0$, computations were carried out at values of this ratio extending considerably below those for which experimental data were obtained. Table III lists

---

(19) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, Chapters 7 and 16.

the calculated values of refractometric per cent. aspartate, in this range starting at the lowest experimental points. The strong electrolyte case is omitted, because the limiting behavior is discussed in the companion paper.[17]

TABLE III

THEORETICAL VALUES FOR THE APPARENT REFRACTOMETRIC PER CENT. OF ASPARTATE[a]

C_A/C_B	$C^{\delta}_{HOAc}/C^{\delta}_{NaOAc}$	Refractometric % aspartate Asc.	Refractometric % aspartate Desc.
0.025	1	48.56	47.81
.05	0.5	50.50	48.71
	1	49.52	48.02
	2	48.53	47.41
.10	0.5	53.31	49.62
	1	51.34	48.34
	2	49.56	47.36
.20	0.5	58.27	50.66
	1	54.57	48.58
	2	51.38	47.10

[a] $C^{\delta}_{NaOAc} = 0.1000$.

Discussion

Several significant effects are demonstrated by the theoretical calculations and confirmed by experiment for the aspartate–acetate–glutamate system. The non-linearity of the refractometric per cent. aspartate *versus* C_A/C_B plots observed by Longsworth[13] at pH 4.6 is evident, especially for the ascending system, over the whole pH range studied. It is clear that a linear extrapolation of the apparent refractometric per cent. to $C_A/C_B = 0$ using values in the experimental range would not give the true refractometric per cent. of aspartate. The difference may be particularly great if there is a maximum in the plot and experimental data for the extrapolation are available only on the high concentration side of the maximum. Furthermore, the theoretical calculations indicate that extrapolation of the apparent analyses obtained in a much lower concentration range will not necessarily yield the true refractometric per cent. of the component being measured. In the present example, the calculated values extrapolate to a limiting refractometric per cent. aspartate of 47.6% at $C_A/C_B = 0$ for both ascending and descending systems at all pH values used. However, the true refractometric per cent. aspartate, calculated from the concentrations and refractive index increments of the salts and acids in the original solutions, varies from 47.75 at pH 7.6–8.0 (the strong electrolyte case) to 48.58 at pH 4.40.

The theoretical calculations also provide information on the sharpening and spreading effects which result from pH and conductivity gradients through the moving boundaries. These effects were first discussed in detail by Longsworth,[1] and they are of interest in connection with the steady-state test for protein heterogeneity.[20,21] The necessary condition for a steady-state moving boundary is that the constituent which disappears in the boundary must have a higher constituent mobility behind the boundary than ahead of it.

The calculations for the descending system given in Table IV show that $\sigma^{\alpha} < \sigma^{\beta} < \sigma^{\gamma}$, so that the gradient in the electric field strength through the moving boundaries will tend to cause spreading. In the ascending system, on the other hand, $\sigma^{\beta} < \sigma^{\alpha} < \sigma^{\delta}$ (Table V) so that the gradient in the electric field strength will tend to cause sharpening.

In both of these systems the conductivity effect is partially counteracted by the pH effect. In the descending system $p\text{H}^{\alpha} < p\text{H}^{\beta} < p\text{H}^{\gamma}$, and in the ascending system $p\text{H}^{\beta} < p\text{H}^{\gamma} < p\text{H}^{\delta}$. The net effect of the change in conductivity and pH may be seen by comparing the velocity of a constituent in the phase adjoining the boundary in which it disappears with the velocity which the constituent would have on the other side of the boundary if an infinitely small amount diffused into that region. The mobilities in Tables IV and V for constituents not present in a phase were calculated using the equation

$$\bar{r}_x = \frac{r_x}{1 + \frac{K_{HR}C_{HR}}{K_{Hx}C_R}} \qquad (19)$$

where x is the constituent not present in the phase and R is a constituent which is present.

TABLE IV

THEORETICAL CALCULATIONS FOR THE DESCENDING SYSTEM

0.2 M HOAc, 0.02 M HGlut, 0.02 M HAsp, 0.1 N NaOH(α) ← HOAc, HGlut, NaOH(β) ← HOAc, NaOH(γ): : 0.2 M HOAc, 0.1 N NaOH(δ)

Concentrations

Phase	C_{HOAc}	C_{OAc}	C_{HGlut}	C_{Glut}	C_{HAsp}	C_{Asp}
δ	0.10	0.10	0	0	0	0
γ	.14	.11379	0	0	0	0
β	.13298	.09292	0.007022	0.012879	0	0
α	.12739	.07261	0.008012	0.011989	0.004599	0.015401

Derived quantities

Phase	σ	v	pH	$\bar{r}_{Glut}$	$\bar{r}_{Asp}$	Sharpening ratio
δ	375.4		4.70	.....	....	...
		0				
γ	427.2		4.61	(6.68)[a]	....	...
		0.01632				
β	388.6		4.54	6.34	(8.92)	0.959
		0.02380				
α	359.1		4.46	5.87	8.55	0.965

[a] The parentheses indicate that the constituent is not actually present in this phase.

TABLE V

THEORETICAL CALCULATIONS FOR THE ASCENDING SYSTEM

0.2 M HOAc, 0.1 N NaOH(δ) ← HOAc, HAsp, NaOH(γ) ← HOAc, HAsp, HGlut, NaOH(β): :0.2 M HOAc, 0.02 M HGlut, 0.02 M HAsp, 0.1 N NaOH (α)

Concentrations

Phase	C_{HOAc}	C_{OAc}	C_{HGlut}	C_{Glut}	C_{HAsp}	C_{Asp}
δ	0.10	0.10	0	0	0	0
γ	.09634	.07688	0	0	0.003656	0.017138
β	.09099	.06431	0.005723	0.010617	.003285	.013639
α	.12739	.07261	0.008012	0.011988	.004599	.015401

Derived quantities

Phase	σ	v	pH	$\bar{r}_{Glut}$	$\bar{r}_{Asp}$	Sharpening ratio
δ	375.4		4.70	.....	(9.48)	
		0.02662				1.029
γ	343.7		4.60	(6.63)[a]	9.15	
		0.02002				1.022
β	318.0		4.55	6.37	8.95	
		0				
α	359.1		4.46	5.87	8.55	

[a] The parentheses indicate that the constituent is not actually present in this phase.

---

(20) E. A. Anderson and R. A. Alberty, *J. Phys. and Colloid Chem.*, **52**, 1345 (1948).

(21) H. Hoch, *Biochem. J.*, **46**, 199 (1950).

The constituent velocity is proportional to $\bar{r}/\sigma$, and the ratio $(\bar{r}/\sigma)_{\text{behind}}/(\bar{r}/\sigma)_{\text{ahead}}$ will be referred to as the sharpening ratio. If this ratio is greater than one, the net effect is to sharpen the boundary; if the ratio is equal to one, there will be no sharpening or spreading effect, and if the ratio is less than one, the net effect is to spread the boundary. The calculated sharpening ratios for the aspartate and glutamate boundaries in both the ascending and descending channels are shown in the right hand columns of Tables IV and V. These calculations are in agreement with the fact that the ascending moving boundaries appear to move in a steady state and the descending boundaries continue to spread.

Since proteins are such complicated weak electrolytes, it is not possible to apply directly the equations derived here. However, these calculations and experiments indicate some of the problems in determining the true composition of a protein mixture by electrophoresis. If the components of a mixture can be isolated, it is always possible to obtain the true analysis of a mixture by an empirical method. This was done by Bock and Alberty[22] for mixtures of adenosine phosphates. Apparent analyses for mixtures of known composition were obtained by moving boundary experiments, and calibration curves showing the relationship between the actual composition and apparent analysis for a given set of conditions were constructed.

Acknowledgments.—The authors are indebted to Prof. Louis J. Gosting for helpful discussions and to Prof. Robert M. Bock for making available the Spinco Model H electrophoresis apparatus. The authors are also indebted to Prof. P. C. Hammer, Mrs. Hildegard Frazier and Mr. Werner Schanzenbach of the Numerical Analysis Laboratory of the University of Wisconsin for assistance with the numerical computations. This research was supported by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation and by a Grant-in-Aid from the du Pont Company.

(22) R. M. Bock and R. A. Alberty, *J. Biol. Chem.*, **193**, 435 (1951).

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NORTHWESTERN UNIVERSITY]

Semiempirical Potential Energy Functions. III. Generalization for Ionic Molecules and the Inclusion of London Forces[1]

BY ARTHUR A. FROST AND JOHN H. WOODSON

RECEIVED NOVEMBER 16, 1957

This semi-empirical potential energy function previously proposed for covalent diatomic molecules has been extended to apply to ionic molecules and to interaction of inert gas atoms. It is shown that this leads to a useful prediction of the dissociation energy and internuclear equilibrium distance of ionic molecules using as the only experimental information the known interaction of the corresponding inert gas atoms.

In previous papers[2,3] there has been proposed a semi-empirical function V for covalent diatomic molecules of the form

$$V = e^{-aR}\left(\frac{c}{R} - b\right) \qquad (1)$$

where R is the internuclear distance and a, b and c are parameters determined by fitting experimental data. c is interpreted as the product of the effective charges on the kernels of the two atoms.

This function was devised in order to provide a proper dependence of V on R as R goes to zero. The same idea is here extended to ionic molecules and to inert gas atom interaction.

Inert Gas Atom Interaction.—The usual empirical function for this case is the exp-six or Buckingham potential[4]

$$V = be^{-aR} - \frac{d}{R^6} \qquad (2)$$

where the first term is a repulsion due to nonbonded interaction of closed shells and the second term is a London attraction. It is obvious that as R goes to zero this V goes to minus infinity instead of positive infinity as it should reasonably do owing to internuclear coulomb repulsion. As a result various modified Buckingham potentials have been used. Buckingham and Corner[5] and Rice and Hirschfelder[6] have replaced the exp-six function at small distances by an arbitrary function that does behave more satisfactorily. This has been necessary in particular for virial coefficient calculations.

It is possible to remedy the negative infinity defect in a smooth manner by modifying the London term as follows: Replace $1/R^6$ by

$$\frac{1}{R^6}\left[1 - e^{-aR}\left(1 + aR + \frac{(aR)^2}{2!} + \frac{(aR)^3}{3!} + \frac{(aR)^4}{4!} + \frac{(aR)^5}{5!}\right)\right] \qquad (3)$$

which can also be expressed as

$$\frac{1}{R^6} - \frac{a^6}{5!}A_5(aR) \qquad (4)$$

where

$$A_5(\alpha) = \int_1^\infty e^{-\alpha x}x^5\,dx$$

---

[1] This research was supported by a grant from the National Science Foundation. This paper was presented at the September, 1957, meeting of the American Chemical Society.

(2) A. A. Frost and B. Musulin, *J. Chem. Phys.*, **22**, 1017 (1954). (Paper I.)

(3) P. S. K. Chen, M. Geller and A. A. Frost, *J. Phys. Chem.*, **61**, 828 (1957). (Paper II.)

(4) E. A. Mason and W. E. Rice, *J. Chem. Phys.*, **22**, 522, 843 (1954).

(5) R. A. Buckingham and J. Corner, *Proc. Roy. Soc.* (*London*), **A189**, 118 (1947).

(6) W. E. Rice and J. O. Hirschfelder, *J. Chem. Phys.*, **22**, 187 (1954).