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Series Solutions of the Dole Equations and their Implications for Electrophoretic Analysis

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Expressions are derived for the apparent refractometric fractions of components in solutions of strong electrolytes, as determined from moving boundary experiments; the derivations are based on Dole's moving boundary theory and they apply to electrophoresis experiments similar to those used for analyzing protein mixtures in buffer solutions. It is shown that the apparent fraction for a "protein" component does not in general approach the true refractometric fraction as the ratio of total "protein" concentration to "buffer" concentration approaches zero though in favorable cases the difference between the two values may be very small. Graphical comparisons of the apparent and actual fractions are presented for two representative systems containing four ionic species, and the rapidity with which the series solutions converge to the exact Dole theory is illustrated. Comparisons between predicted and observed apparent refractometric fractions are included for the two systems. The effects of the ionic mobilities and refractive increments on the difference between the actual and apparent fractions are discussed.

The moving boundary method as developed by Tiselius[1] and others has been used widely in the analysis of solutions of proteins and of other ionized macromolecules. A refractometric analysis is commonly involved, the quantities measured (such as Rayleigh fringe displacements or areas of schlieren patterns) being proportional to the differences in refractive index across the moving boundaries. In the analysis of a mixture of N proteins each protein i disappears in one of the boundaries, across which the refractive index difference is Δn_i. The apparent refractometric fraction of component i is therefore equal to $\Delta n_i / \sum_{l=1}^{N} \Delta n_l$. However, the true analysis in terms of weight fractions will differ from the apparent refractometric analysis because (1) the specific refractive index increments are not in general the same for different components and (2) across every moving boundary there exist small concentration differences of the components which do not disappear, in addition to the concentration change of the component which does disappear. As a result of these superimposed concentration differences the refractometric analyses obtained from the ascending and descending patterns are different and furthermore the analyses will vary with the ratio of the total protein concentration to the buffer concentration.[2-4]

In order to make quantitative corrections for the superimposed concentration differences occurring in the electrophoretic analysis of proteins and other weak electrolytes, a general theory is required corresponding to the theories of Dole[5] and Svensson[6] for strong electrolytes. Because of the complexity of weak electrolyte systems, no such general theory is at present available, although computations have been made for a few simple cases using moving boundary equations developed for univalent electrolytes.[7] However, calculations made on the assumption that the Dole theory can be applied approximately to protein systems support experimental observations that the predominant errors caused by the superimposed concentration differences tend to decrease as the ratio of total protein to buffer salt concentration decreases.[4,8-10] At-

(1) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

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

(3) G. E. Perlmann and D. Kaufman, THIS JOURNAL, **67**, 638 (1945).

(4) S. H. Armstrong, Jr., M. J. E. Budka and K. C. Morrison, *ibid.*, **69**, 416 (1947).

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

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

(7) J. C. Nichol, E. B. Dismukes and R. A. Alberty, THIS JOURNAL, **80**, 2610 (1958).

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

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

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

tempts have been made to eliminate these errors entirely by a linear extrapolation of the apparent composition to a value of zero for the ratio of the total protein concentration to buffer concentration[3,4] (appropriate corrections being made for any differences in specific refractive increments of the protein components). Sometimes this extrapolation has been performed using constant concentration either of the buffer[10] or of the total protein.[2] Cann[10] has cautioned that the extrapolated composition has not been shown to be the correct value. It will in fact be shown below that such extrapolation procedures do not in general lead to correct analyses of mixtures of strong electrolytes. The magnitude of the residual error depends on the nature of the system being studied. Available experimental evidence indicates that this error may be small for protein systems; however, since it was found to be large for experiments with chlorate and iodate as "protein" ions in a potassium chloride "buffer," data for that system are reported in the experimental part of this paper to illustrate the series expansions which are derived from the Dole theory. It is hoped that these series expansions will contribute to the general understanding of moving boundary electrophoresis which is needed to utilize fully the inherent precision of this analytical method.

In the following paper[7] it will be shown that corresponding errors also exist for the case of weak electrolytes.

Equations for Descending Boundaries

Specification of the System and Definition of Terms.—Suppose that in a vertical tube, Fig. 1a, a

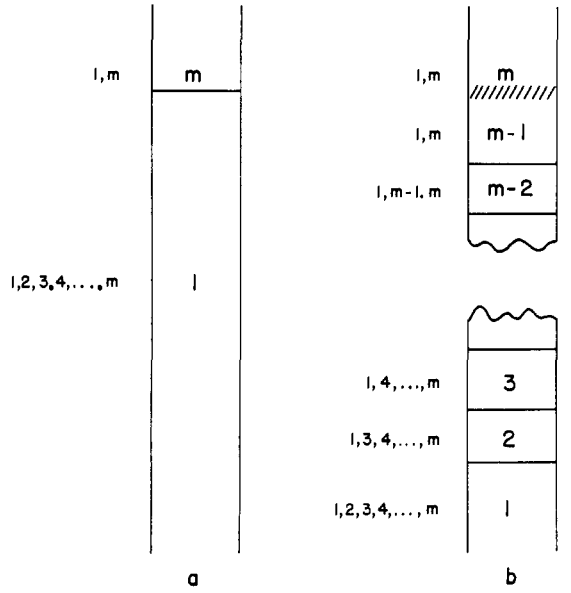

Fig. 1.—The descending limb of a moving boundary experiment with strong electrolytes: a, before current is passed; b, after current is passed. This experiment is analogous to the electrophoresis of a protein solution.

boundary is formed between solution m containing ionic species 1 and m and solution 1 containing ionic species 1, 2, 3, 4, . . . , m. The charges on all the species except m have the same sign. For the following derivations to be applicable it is not necessary that the concentrations of the two initial solutions satisfy the Donnan equilibrium, even though this equilibrium frequently exists between the two solutions used for protein electrophoresis experiments. On application of an electrical potential of appropriate sign the descending moving boundary system shown in Fig. 1b will develop provided there is no convection. It is assumed that ionic species 2, . . . , $m - 1$ all have different mobilities with the mobility decreasing as the number designating the ion increases. Ions 1 and m are considered to be present in relatively large amounts compared to the other ions so that they correspond to the two "buffer" ions while ions 2, . . . , $m - 1$ correspond to the "protein" ions. All the electrolytes are assumed to be completely ionized, but the ionization of the solvent is considered to be negligible. The numbers beside the channels indicate the ions present in the different phases, whereas the phases themselves are identified by the numbers within the channel.

Our procedure may be described as follows: Given the equivalent concentration, C_i, and the relative mobility,[5] r_i, of each ionic species i, together with the refractive increments per equivalent, $\mathbf{k}_i$, of each neutral salt consisting of ions i and m, we will derive an expression for the apparent refractometric fraction, F_j, of each "protein" salt containing ionic species j and m. This apparent fraction, F_j, is the value obtained from the usual refractometric measurements of the descending boundary system. The expression for F_j will be derived as a power series in ratios of the "protein" ion concentrations to the "buffer" ion concentrations; the limiting value of each F_j is then obtained by simply letting these ratios approach zero. Of importance to experimentalists is the inverse problem of determining the actual refractometric fractions of the "proteins" from measured values of their apparent refractometric fractions; the formulation of a practical procedure for performing such an inversion of these equations requires further study and will not be considered here.

To define F_j precisely, we first define the equivalent refraction of an electrically neutral salt by

$$\mathbf{k}_i = (\partial n/\partial |C_i|)_{C_{k\neq i}} \quad \begin{pmatrix} i \neq m \\ k \neq m \end{pmatrix} \qquad (1)$$

where n is the refractive index of the solution. Here C_i, the concentration of ionic species i, is given the same algebraic sign as that of the corresponding ion and is expressed as equivalents per unit volume of solution; $|C_i|$ denotes the absolute value of C_i. The subscript $k \neq i$ indicates that all ionic concentrations except C_i and C_m are held constant; it should be understood that the concentration of ionic species m is varied along with C_i during the differentiation indicated in equation 1 in such a way as to maintain electroneutrality. Although the equivalent refractions, $\mathbf{k}_i$, will be used throughout this article, it may be helpful to note that they are simply related to the specific refractions, $\mathbf{k}_i'$, per gram of component i by

$$\mathbf{k}_i = \mathbf{k}_i' (\hat{M}_i + \hat{M}_m) \qquad (2)$$

Here $\hat{M}_i$ and $\hat{M}_m$ are the weights per equivalent of ions i and m, respectively. Assuming that each $\mathbf{k}_i$ (or $\mathbf{k}_i'$) is independent of the concentration of every component over the concentration range encountered, we may now define F_j in terms of the refractive indices of phases $1, j-1, j$ and $m-1$ as

$$F_j = \frac{n_{j-1} - n_j}{n_1 - n_{m-1}} = \frac{\sum_{i=1}^{m-1} \mathbf{k}_i(C_{i,j-1} - C_{i,j})}{\sum_{i=1}^{m-1} \mathbf{k}_i(C_i - C_{i,m-1})} \quad (3)$$

The double subscript on the concentrations denotes first, the ionic species and second, the phase considered; when no second subscript is used the concentration is that in the starting solution, phase 1. Thus $C_{i,j}$ denotes the concentration of ionic species i in phase j while C_i denotes the concentration of ionic species i in phase 1. This notation will be used henceforth throughout this paper, except that for ascending boundaries C_i will denote the concentration of ionic species i in phase m.

Apparent Refractometric Fractions as Functions of Concentration Ratios in the Various Phases.—The actual refractometric fraction of each "protein" component j in the starting solution, phase 1 of Fig. 1, is denoted by

$$f_j = \frac{\mathbf{k}_j C_j}{\sum_{l=2}^{m-1} \mathbf{k}_l C_l} \quad (4)$$

Also, terms in equation 3 such as $C_{j,j}$, $C_{j,m-1}$, etc., which are zero for the system shown in Fig. 1b, are dropped. It is then possible to convert equation 3 to the form

$$F_j = \frac{f_j}{Q}\left[\frac{\mathbf{k}_1(C_{1,j-1} - C_{1,j})}{\mathbf{k}_j C_j} + \frac{C_{j,j-1}}{C_j} + \sum_{i=j+1}^{m-1} \frac{\mathbf{k}_i(C_{i,j-1} - C_{i,j})}{\mathbf{k}_j C_j}\right] \quad (5)$$

Here

$$Q = 1 + \left[\mathbf{k}_1(C_1 - C_{1,m-1}) \Big/ \sum_{i=2}^{m-1} \mathbf{k}_i C_i\right] = 1 - \sum_{i=2}^{m-1} \frac{\mathbf{k}_1 r_1 \delta_{im}}{\mathbf{k}_i r_i \delta_{1m}} f_i \quad (6)$$

in which the symbol δ denotes differences in relative mobilities

$$\delta_{ij} = r_i - r_j \quad (7)$$

All relative mobilities are assumed to be constant over the concentration range considered.[5] To derive the second expression for Q in equation 6, it is convenient to utilize the Kohlrausch regulating function,[5,11] which for any phase l is defined by

$$\omega_l = \sum_{i=1}^{m} \frac{C_{i,l}}{r_i} \quad (8)$$

Because this function has the same value across each moving boundary, it has the same value for phases 1 and $m-1$, *i.e.*, $\omega_1 = \omega_{m-1}$. From this fact and from the electroneutrality relation between the equivalent concentrations in any phase l

$$\sum_{i=1}^{m} C_{i,l} = 0 \quad (9)$$

it may be shown that

$$C_{1,m-1} = C_1\left[1 + \sum_{i=2}^{m-1} \frac{r_1 \delta_{im}}{r_i \delta_{1m}}\left(\frac{C_i}{C_1}\right)\right] \quad (10)$$

Substitution of this expression and equation 4 into the first definition of Q in equation 6 gives the desired second definition in terms of the actual refractometric fractions, f_i, in phase 1. It is to be noted that Q is independent of the ratio of total "protein" concentration to "buffer" concentration.

For subsequent use equation 5 is written in terms of concentration ratios

$$F_j = \frac{f_j}{Q}\left[\frac{\mathbf{k}_1 C_1}{\mathbf{k}_j C_j}\left(\frac{C_{1,j-1}}{C_1}\right)\left(1 - \frac{C_{1,j}}{C_{1,j-1}}\right) + \frac{C_{j,j-1}}{C_j} + \sum_{i=j+1}^{m-1} \frac{\mathbf{k}_i C_i}{\mathbf{k}_j C_j}\left(\frac{C_{i,j-1}}{C_i}\right)\left(1 - \frac{C_{i,j}}{C_{i,j-1}}\right)\right] \quad (11)$$

This expression is seen to involve no series approximations. In order to replace all concentrations in the intermediate phases $2, \ldots, m-1$ by the concentrations in phase 1, however, it is convenient to use series expansions. We will choose to express F_j as a series in

$$y = \frac{\sum_{i=2}^{m-1} \mathbf{k}_i C_i}{\mathbf{k}_1 C_1} \quad (12)$$

which is the ratio of total "protein" concentration to "buffer" concentration on the basis of refractive index.

Evaluation of Concentration Ratios in Equation 11 using Dole's Equation.—The concentration ratios in equation 11 are given by Dole's equation 31[5] in the form

$$\frac{C_{i,j}}{C_{i,j-1}} = \frac{x_j \delta_{ji}}{r_j(x_j - r_i)} \quad (13)$$

where the quantities x_j needed for this relation are the roots of Dole's equation 33, *i.e.*, of

$$\sum_{i=1}^{m-1} \frac{\delta_{im}}{(r_i - x_j)} C_i = 0 \quad (14)$$

These roots will be obtained as series expansions which converge rapidly provided the absolute concentrations of the "buffer" ions, $|C_1|$ and $|C_m|$, are considerably larger than those of the "protein" ions. To solve for a particular root, x_j, equation 14 is first written in the form

$$\frac{\delta_{1m}}{(r_1 - x_j)}\left[1 + \frac{(r_1 - x_j)}{\delta_{1m}} \sum_{\substack{k=2 \\ k \neq j}}^{m-1} \frac{\delta_{km}}{(r_k - x_j)}\left(\frac{C_k}{C_1}\right)\right] = -\frac{\delta_{jm}}{(r_j - x_j)}\left(\frac{C_j}{C_1}\right) \quad (15)$$

where the notation on the summation sign means that k is given all values from 2 to $m-1$ inclusive *except* the value j (which may have any value from 2 to $m-1$ inclusive). Rearrangement of equation 15 yields the expression in closed form

$$x_j = r_j + \frac{\delta_{jm}(r_1 - x_j)}{\delta_{1m}}\left[1 + \frac{(r_1 - x_j)}{\delta_{1m}} \sum_{\substack{k=2 \\ k \neq j}}^{m-1} \frac{\delta_{km}}{(r_k - x_j)}\left(\frac{C_k}{C_1}\right)\right]^{-1}\left(\frac{C_j}{C_1}\right) \quad (16)$$

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

When $C_j/C_1 << 1$ and $C_k/C_1 << 1$, the first approximate solution is seen to be

$$x_j = r_j + \ldots \quad (17)$$

The second approximation is

$$x_j = r_j + \frac{\delta_{1j}\,\delta_{jm}}{\delta_{1m}}\left(\frac{C_j}{C_1}\right) + \ldots \quad (18)$$

Substitution of the second approximation, equation 18, for x_j into the right side of equation 16, and expansion in the numerator of the term in brackets, provides the third approximation for x_j; substitution of this third approximation into the right side of equation 16 yields the fourth approximation

$$x_j = r_j + A' - A'' + A''' + \ldots \quad (19)$$

where

$$A' = \frac{\delta_{1j}\,\delta_{jm}}{\delta_{1m}}\frac{C_j}{C_1} \quad (19a)$$

$$A'' = \frac{\delta_{1j}\,\delta_{jm}}{(\delta_{1m})^2}\left[\delta_{jm}\left(\frac{C_j}{C_1}\right)^2 + \delta_{1j}\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{km}}{\delta_{kj}}\frac{C_jC_k}{C_1^2}\right] \quad (19b)$$

and

$$A''' = \frac{\delta_{1j}\,\delta_{jm}}{(\delta_{1m})^3}\left\{(\delta_{jm})^2\left(\frac{C_j}{C_1}\right)^3 + 3\delta_{1j}\,\delta_{jm}\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{km}}{\delta_{kj}}\frac{C_j^2C_k}{C_1^3} - (\delta_{1j})^2\,\delta_{jm}\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{km}}{(\delta_{kj})^2}\frac{C_j^2C_k}{C_1^3} + (\delta_{1j})^2\left[\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{km}}{\delta_{kj}}\frac{C_k}{C_1}\right]^2\frac{C_j}{C_1}\right\} \quad (19c)$$

It will be noted that the primes in equation 19 and in succeeding equations correspond to the powers to which the concentration ratios are raised; consequently A' is of order y (equation 12), A'' is of order y^2, etc. On substituting equation 19 into equation 13 we have

$$\frac{C_{i,j}}{C_{i,j-1}} = 1 - B' + B'' - B''' + \ldots \quad (20)$$

where

$$B' = \frac{r_i\delta_{1j}\delta_{jm}}{r_j\delta_{1m}\delta_{ji}}\frac{C_j}{C_1} \quad (20a)$$

$$B'' = \frac{r_i\delta_{1j}\delta_{jm}}{r_j(\delta_{1m})^2\delta_{ji}}\left[\frac{\delta_{1i}\delta_{jm}}{\delta_{ji}}\left(\frac{C_j}{C_1}\right)^2 + \delta_{1j}\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{km}}{\delta_{kj}}\frac{C_jC_k}{C_1^2}\right] \quad (20b)$$

and

$$B''' = \frac{r_i\delta_{1j}\delta_{jm}}{r_j(\delta_{1m})^3\delta_{ji}}\left\{\left(\frac{\delta_{1i}\delta_{jm}}{\delta_{ji}}\right)^2\left(\frac{C_j}{C_1}\right)^3 + 2\frac{\delta_{1i}\delta_{1j}\delta_{jm}}{\delta_{ji}}\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{km}}{\delta_{kj}}\frac{C_j^2C_k}{C_1^3} - \delta_{1j}\delta_{jm}\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{1k}\delta_{km}}{(\delta_{kj})^2}\frac{C_j^2C_k}{C_1^3} + (\delta_{1j})^2\left[\sum_{\substack{k=2\\k\neq j}}^{m-1}\frac{\delta_{km}}{\delta_{kj}}\frac{C_k}{C_1}\right]^2\frac{C_j}{C_1}\right\} \quad (20c)$$

The remaining ratios required are found by taking products of the above ratios, yielding

$$\frac{C_{i,j-1}}{C_i} = 1 - D' + D'' + \ldots \quad (21)$$

where

$$D' = \sum_{s=2}^{j-1}\frac{r_i\delta_{1s}\delta_{sm}}{r_s\delta_{1m}\delta_{si}}\frac{C_s}{C_1} \quad (21a)$$

and

$$D'' = \sum_{s=2}^{j-1}\left[\frac{r_i\delta_{1i}\delta_{1s}(\delta_{sm})^2}{r_s(\delta_{1m})^2(\delta_{si})^2}\left(\frac{C_s}{C_1}\right)^2 + \frac{r_i(\delta_{1s})^2\delta_{sm}}{r_s(\delta_{1m})^2\delta_{si}}\sum_{\substack{k=2\\k\neq s}}^{m-1}\frac{\delta_{km}}{\delta_{ks}}\frac{C_kC_s}{C_1^2}\right] + \sum_{s=3}^{j-1}\sum_{t=2}^{s-1}\frac{r_i^2\delta_{1s}\delta_{sm}\delta_{1i}\delta_{tm}}{r_sr_i(\delta_{1m})^2\delta_{si}\delta_{ti}}\frac{C_sC_t}{C_1^2} \quad (21b)$$

Final Series Expansions for the Apparent Refractometric Fractions.—Substitution of equations 20 and 21 into equation 11 yields the desired expression

$$F_{j(\text{desc})} = \frac{f_j}{Q}(1 - E_0 + E_1y + E_2y^2 + \ldots) \quad (22)$$

where

$$E_0 = \frac{\mathbf{k}_1r_1\delta_{jm}}{\mathbf{k}_jr_j\delta_{1m}} \quad (22a)$$

$$E_1 = \sum_{s=2}^{j-1}U_{j,s} + \sum_{s=j+1}^{m-1}V_{j,s} \quad (22b)$$

and

$$E_2 = \sum_{s=2}^{j-1}W_{j,s} + \sum_{s=j+1}^{m-1}X_{j,s} + \sum_{s=3}^{j-1}\sum_{t=2}^{s-1}Y_{j,s,t} + \sum_{s=2}^{j-1}\sum_{t=j+1}^{m-1}Z_{j,s,t} \quad (22c)$$

Here

$$U_{j,s} = \frac{\mathbf{k}_1\delta_{1s}\delta_{sm}}{\mathbf{k}_s\delta_{sj}\delta_{1m}}\left(\frac{\mathbf{k}_1r_1\delta_{jm}}{\mathbf{k}_jr_s\delta_{1m}} - \frac{r_j}{r_s}\right)f_s \quad (22b\text{-}1)$$

$$V_{j,s} = \frac{\mathbf{k}_1\delta_{1j}\delta_{jm}}{\mathbf{k}_j\delta_{sj}\delta_{1m}}\left(\frac{\mathbf{k}_1r_1\delta_{sm}}{\mathbf{k}_sr_j\delta_{1m}} - \frac{r_s}{r_j}\right)f_s \quad (22b\text{-}2)$$

$$W_{j,s} = \frac{\mathbf{k}_1{}^3r_1\delta_{1j}(\delta_{jm})^2\delta_{1s}\delta_{sm}}{\mathbf{k}_j{}^2\mathbf{k}_sr_j(\delta_{1m})^3(\delta_{js})^2}f_jf_s + \frac{\mathbf{k}_1{}^2r_j\delta_{1j}\delta_{1s}(\delta_{sm})^2}{\mathbf{k}_s{}^2r_s(\delta_{1m})^2(\delta_{js})^2}f_s{}^2 + \sum_{\substack{p=2\\p\neq j}}^{m-1}\frac{\mathbf{k}_1{}^3r_1\delta_{1j}\delta_{jm}\delta_{1s}\delta_{sm}\delta_{pm}}{\mathbf{k}_j\mathbf{k}_p\mathbf{k}_sr_s(\delta_{1m})^3\delta_{pj}\delta_{js}}f_pf_s + \sum_{\substack{p=2\\p\neq s}}^{m-1}\frac{\mathbf{k}_1{}^2r_1\delta_{1s}\delta_{sm}\delta_{pm}}{\mathbf{k}_p\mathbf{k}_sr_s(\delta_{1m})^2\delta_{ps}}\left(\frac{\mathbf{k}_1r_1\delta_{jm}}{\mathbf{k}_jr_j\delta_{1m}} - \frac{r_j\delta_{1s}}{r_1\delta_{js}}\right)f_pf_s \quad (22c\text{-}1)$$

$$X_{j,s} = \frac{\mathbf{k}_1{}^2\delta_{1j}(\delta_{jm})^2\delta_{1s}}{\mathbf{k}_j{}^2(\delta_{1m})^2(\delta_{js})^2}\left(\frac{\mathbf{k}_1r_1\delta_{sm}}{\mathbf{k}_sr_j\delta_{1m}} - \frac{r_s}{r_j}\right)f_jf_s - \sum_{\substack{p=2\\p\neq j}}^{m-1}\frac{\mathbf{k}_1{}^2(\delta_{1j})^2\delta_{jm}\delta_{pm}}{\mathbf{k}_j\mathbf{k}_p(\delta_{1m})^2\delta_{jp}\delta_{js}}\left(\frac{\mathbf{k}_1r_1\delta_{sm}}{\mathbf{k}_sr_j\delta_{1m}} - \frac{r_s}{r_j}\right)f_pf_s \quad (22c\text{-}2)$$

$$Y_{j,s,t} = \frac{\mathbf{k}_1{}^2\delta_{sm}\delta_{tm}}{\mathbf{k}_s\mathbf{k}_t(\delta_{1m})^2}\left(\frac{r_j{}^2\delta_{1s}\delta_{1t}}{r_sr_t\delta_{js}\delta_{jt}} - \frac{\mathbf{k}_1r_1{}^3\delta_{jm}}{\mathbf{k}_jr_jr_sr_t\delta_{1m}}\right)f_sf_t \quad (22c\text{-}3)$$

$$Z_{j,s,t} = \frac{\mathbf{k}_1{}^2r_t{}^2\delta_{1j}\delta_{jm}\delta_{1s}\delta_{sm}}{\mathbf{k}_j\mathbf{k}_sr_jr_s(\delta_{1m})^2\delta_{jt}\delta_{ts}}f_sf_t \quad (22c\text{-}4)$$

A sum in equation 22b or 22c is zero if the upper limit is less than the lower limit: *e.g.*, if $j = 2$ then $\sum_{s=2}^{j-1} = 0$.

Equation 22 shows that a plot of the apparent refractometric fraction, F_j, *versus* y extrapolates to $f_j(1 - E_0)/Q$ as $y \rightarrow 0$ rather than to the actual refractometric fraction, f_j, in the starting solution. Clearly the various superimposed concentration differences across a boundary are *not* effects of higher order which vanish more rapidly than the concentration change of the main "protein" com-

ponent across that boundary as $y \rightarrow 0$. Consequently f_j is not in general obtained when F_j is extrapolated to a value of zero for the ratio, y, of total "protein" concentration to "buffer" concentration. For sufficiently small values of y the dependence of F_j on y is given accurately by the terms in equation 22.

Equations for Ascending Boundaries

Figure 2 summarizes the notation used for an ascending boundary system corresponding to the descending boundary system considered above. Each apparent refractometric fraction, F_j, for these ascending boundaries is defined by the same expression, equation 3, which was used for the descending boundary system. To conserve space we will not present steps in the derivation but will give only the final result. This derivation is analogous to that given above; the only additional information needed is supplied by the relation[5]

$$\frac{C_{i,m}}{C_{i,m-1}} = \frac{\omega_m}{\omega_{m-1}} \tag{23}$$

which gives the concentration ratio of each ionic species across the stationary boundary in terms of the values of the regulating function on the two sides of this boundary. After expressing F_j in terms of concentration ratios in the several phases and replacing these ratios by the concentration ratios in phase m by utilizing series expansions for the roots of Dole's equation 33, one arrives finally at the desired result

$$F_{j(\text{asc})} = \frac{f_j}{Q}(1 - G_0 + G_1 y + G_2 y^2 + \ldots) \tag{24}$$

where

$$G_0 = \frac{\mathbf{k}_1 r_1 \delta_{jm}}{\mathbf{k}_j r_j \delta_{1m}} = E_0 \tag{24a}$$

$$G_1 = \sum_{s=j+1}^{m-1} U_{j,s} + \sum_{s=2}^{j-1} V_{j,s} \tag{24b}$$

and

$$G_2 = \sum_{s=j+1}^{m-1} W_{j,s} + \sum_{s=2}^{j-1} X_{j,s} + \sum_{s=j+2}^{m-1} \sum_{t=j+1}^{s-1} Y_{j,s,t} + \sum_{s=j+1}^{m-1} \sum_{t=2}^{j-1} Z_{j,s,t} \tag{24c}$$

Expressions for $U_{j,s}$, etc., are given above, equations 22. It will be observed that G_1 and G_2 are obtained by simple alterations of the limits on the sums in the equations for E_1 and E_2, respectively.

The actual refractometric fractions, f_j, are again defined by equation 4, and the ratios, y, of total "protein" concentration to "buffer" concentration are defined by equation 12; it is to be understood that the concentrations considered are those in the original solution, phase m (Fig. 2). Hence Q (equation 6) and each f_j has the same numerical value for the ascending as for the descending boundary systems of the usual electrophoresis experiment and an important point emerges: because G_0 (equation 24a) is equal to E_0 (equation 22a) *the value of each F_j at $y = 0$ is the same for the ascending and the descending boundary systems.* As pointed out following equation 22, F_j is in general different from f_j at $y = 0$.

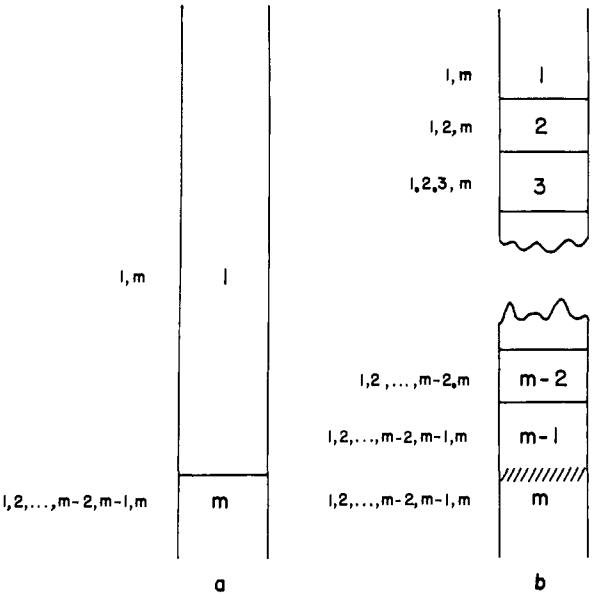

Fig. 2.—The ascending limb of a moving boundary experiment with strong electrolytes, analogous to the electrophoresis of a protein solution: a, before current is passed; b, after current is passed.

Systems Containing Four Ionic Species

This is the simplest type of system involving the determination of an apparent refractometric fraction; the original solution contains two kinds of "protein" ions, 2 and 3, in a "buffer" consisting of ionic species 1 and 4. For this class of systems equations 22 and 24 simplify greatly, and comparisons of theoretical predictions with experimental results can be made readily. Further, certain implications of the theory become evident.

For systems of four ionic species, equation 22 for descending boundaries reduces to

$$F_{2(\text{desc})} = \frac{f_2}{Q}(1 - K_0 + K_1 y + K_2 y^2 + \ldots) \tag{25}$$

where

$$K_0 = \frac{\mathbf{k}_1 r_1 \delta_{24}}{\mathbf{k}_2 r_2 \delta_{14}} \tag{25a}$$

$$K_1 = \frac{\mathbf{k}_1 \delta_{12} \delta_{24}}{\mathbf{k}_2 \delta_{32} \delta_{14}} \left(\frac{\mathbf{k}_1 r_1 \delta_{34}}{\mathbf{k}_3 r_2 \delta_{14}} - \frac{r_3}{r_2} \right) f_3 \tag{25b}$$

and

$$K_2 = K_1 M \tag{25c}$$

Here

$$M = \frac{\mathbf{k}_1 \delta_{13} \delta_{24}}{\mathbf{k}_2 \delta_{32} \delta_{14}} f_2 - \frac{\mathbf{k}_1 \delta_{12} \delta_{34}}{\mathbf{k}_3 \delta_{32} \delta_{14}} f_3 \tag{25c-1}$$

From equation 6

$$Q = 1 - \frac{\mathbf{k}_1 r_1 \delta_{24}}{\mathbf{k}_2 r_2 \delta_{14}} f_2 - \frac{\mathbf{k}_1 r_1 \delta_{34}}{\mathbf{k}_3 r_3 \delta_{14}} f_3 \tag{26}$$

and f_2 and f_3 (where $f_2 + f_3 = 1$) are the actual refractometric fractions of ionic species 2 and 3, equation 4.

For the ascending boundaries equation 24 reduces to

$$F_{2(\text{asc})} = \frac{f_2}{Q}(1 - L_0 + L_1 y + L_2 y^2 + \ldots) \tag{27}$$

where

$$L_0 = \frac{\mathbf{k}_1 r_1 \delta_{24}}{\mathbf{k}_2 r_2 \delta_{14}} = K_0 \qquad (27a)$$

$$L_1 = \frac{\mathbf{k}_1 \delta_{13} \delta_{34}}{\mathbf{k}_3 \delta_{32} \delta_{14}} \left(\frac{\mathbf{k}_1 r_1 \delta_{24}}{\mathbf{k}_2 r_3 \delta_{14}} - \frac{r_2}{r_3} \right) f_3 \qquad (27b)$$

and

$$L_2 = L_1 M \qquad (27c)$$

Here M and Q are defined by equations 25c-1 and 26, respectively.

For the usual electrophoresis experiment, the numerical values of f_2, f_3 and Q are the same for the ascending as for the descending boundary systems. Therefore equations 25 and 27 show directly an important result which was also emphasized above for the general case: in the limit as $y \rightarrow 0$ the same apparent refractometric fraction, $F_{2(y=0)}$, is obtained from both boundary systems.

It is of help in understanding electrophoretic analysis to consider the factors which cause F_2 to differ from f_2, both for the limiting case of $y = 0$ and for non-zero values of y. These factors are readily analyzed by inspection of the equations for 4-ion systems.

(a) **Limiting Case.**—By setting y equal to zero in either equation 25 or 27 and recalling that $f_2 + f_3 = 1$, one obtains

$$F_{2(y=0)} = f_2 \left\{ \frac{1 - \left(\frac{\mathbf{k}_1 r_1}{\delta_{14}}\right)\left[\frac{\delta_{24}}{\mathbf{k}_2 r_2}\right]}{1 - \left(\frac{\mathbf{k}_1 r_1}{\delta_{14}}\right)\left[\frac{\delta_{24}}{\mathbf{k}_2 r_2}\right]\left\{1 + \left[\frac{\mathbf{k}_2 r_2 \delta_{34}}{\mathbf{k}_3 r_3 \delta_{24}} - 1\right] f_3\right\}} \right\} \qquad (28)$$

Here terms which are properties of the "buffer" are enclosed in parentheses and those which are properties of the "proteins" are enclosed in brackets. Except for the trivial case of $f_3 = 0$, it is seen that $F_{2(y=0)}$ equals f_2 only if the two "proteins" have physical properties such that

$$\frac{\mathbf{k}_2 r_2 \delta_{34}}{\mathbf{k}_3 r_3 \delta_{24}} = 1 \qquad (29)$$

For this case the denominator within the large braces of equation 28 equals the numerator. Unless equation 29 is satisfied, the difference between $F_{2(y=0)}$ and f_2 for a given "protein" system cannot be made zero by some advantageous choice of "buffer." The difference between these two values may be minimized, however, by selecting a "buffer" for which the ratio $\mathbf{k}_1 r_1/\delta_{14}$ is as small as possible.

(b) **Non-zero Values of y.**—The dependence of F_2 on y may be considered by examining the coefficients of y and of y^2. It is seen that the coefficient of y^2 in either equation 25 or 27 is zero if the corresponding coefficient of y is zero; this follows because K_2 is proportional to K_1 and L_2 is proportional to L_1. Even if neither K_1 nor L_1 is zero, however, both coefficients of y^2 will be zero for that value of f_2 which makes M equal to zero; then both $F_{2(\mathrm{asc})}$ and $F_{2(\mathrm{desc})}$ depend linearly on y. The condition for M to be zero

$$\frac{C_3}{C_2} = \frac{\delta_{13}\delta_{24}}{\delta_{12}\delta_{34}} \qquad (30)$$

is readily derived by setting $M = 0$ in equation 25c-1 and bearing in mind the definition of f_j (equation 4). It will be noted that equivalent refractions, $\mathbf{k}_i$, are absent from equation 30; the ratio of "protein" equivalent concentrations for which the dependence of F_2 on y becomes linear is determined only by the relative mobilities.

The limiting slope at $y = 0$ of F_2 *versus* y is generally different for the ascending and descending boundaries (K_1 and L_1 are in general unequal). Both slopes increase as $r_3 \rightarrow r_2$, *i.e.*, as $\delta_{32} \rightarrow 0$; this is in agreement with the findings of Svensson, Benjaminsson and Brattsten[12] who have stated that the deviations from the state of "ideal" electrophoresis are greater the smaller the difference in mobility between the components. For descending boundaries it is seen from equation 25b that $K_1 = 0$ only if $\delta_{12} = 0$ or if the term in parentheses is zero. Similarly for ascending boundaries, $L_1 = 0$ only if $\delta_{13} = 0$ or if the term in parentheses is zero.

The variation in apparent analysis with the buffer anion used has been discussed by Longsworth[8] for a hypothetical two-protein case, wherein the proteins are negatively charged ions in 0.1 N solutions of sodium buffer salts. All components were considered to be strong electrolytes. His calculations (made using Dole's theory[5]) for a series of common buffer anions show that as the buffer anion mobility increases the apparent fraction of the faster protein becomes greater, this effect being more pronounced for the ascending than for the descending side. It is of interest to examine the contributions made to this increase by specific terms in the above series expressions for F_2 (for brevity only the descending boundary systems are considered here). This has been done for the same hypothetical systems considered by Longsworth and the results are summarized in Table I. In terms of the notation used in this article component 1 is the buffer anion, components 2 and 3 are the fast and slow protein ions and component 4 is sodium ion. Column 1 lists the buffer anions considered, and the corresponding values[8] of r_1 and $\mathbf{k}_1$ are shown in columns 2 and 3. Numbers in the subsequent columns were computed by substituting into equations 12, 25 and 26 the assumed data,[8] *viz.*, $r_2 = -0.3$, $r_3 = -0.15$, $r_4 = 1$, $C_2 = -0.0036$ equiv./l. and $C_3 = -0.0018$ equiv./l. (for a concentration of 1 g. per 100 ml. for each protein). Finally, taking the refractive index increment for each protein to be 0.00186 (g./100 ml.)$^{-1}$, we have $\mathbf{k}_2 = 0.51667$ (equiv./l.)$^{-1}$ and $\mathbf{k}_3 = 1.03333$ (equiv./l.)$^{-1}$, whence $f_2 = f_3 = 0.5$.

Because the values of $(1 - K_0)/Q$ in column 4 are nearly constant, it is evident from equation 25 that $F_{2(y=0)}$ is almost independent of the buffer anions. For 0.1 N buffer solutions, however, the term $K_1 y/Q$ (column 5 times column 7) has a pronounced effect on $F_{2(\mathrm{desc})}$, with the magnitude of this term being markedly dependent on the anion. This term is counteracted to a slight extent by the much smaller negative term, $K_2 y^2/Q$. It should be observed that the equivalent refraction, $\mathbf{k}_1$, of the buffer plays an important role in determining the magnitude of $K_1 y/Q$. For the K_1/Q part of the term, no regular change is observed as the buffer anion mobility, r_1, increases and $\mathbf{k}_1$ (with

(12) H. Svensson, A. Benjaminsson and I. Brattsten, *Acta Chem. Scand.*, **3**, 307 (1949).

TABLE I

THE DEPENDENCE ON BUFFER ANION OF THE APPARENT REFRACTOMETRIC FRACTIONS, $F_{2(desc)}$, FOR MIXTURES OF TWO HYPOTHETICAL PROTEINS[a]

1 Buffer anion (ionic species 1)	2 $-r_1$	3 k_1	4 $\frac{1 - K_0}{Q}$	5 $\frac{K_1}{Q}$	6 $\frac{K_2}{Q}$	7 For 0.1 N buffers y	8 $F_{2(desc)}$	9 For $y = 0.9174$ $-C_1$	10 $F_{2(desc)}$
Diethylbarbiturate	0.4640	0.04055	0.9931	0.01918	−0.001076	0.9174	0.5049 (.5028)[b]	0.1000	0.5049
Lactate	.5795	.01914	.9964	.01425	− .000443	1.9436	.5112 (.5093)	.2118	.5046
Glycinate	.7098	.01746	.9963	.01761	− .000571	2.1306	.5156 (.5150)	.2323	.5060
Acetate	.7825	.01235	.9973	.01406	− .000342	3.0121	.5182 (.5181)	.3284	.5049
Phosphate (dibasic)	1.1008	.01484	.9960	.02382	− .000842	2.5067	.5252 (.5259)	.2732	.5086
Chloride	1.6810	.01120	.9964	.02428	− .000783	3.3214	.5342 (.5344)	.3621	.5090

[a] To facilitate comparisons, the systems considered here are identical with those discussed by Longsworth (Table IV of ref. 8). Here the buffer anions are denoted by 1, the faster and slower protein ions by 2 and 3 and sodium ion by 4. [b] The values in parentheses were calculated by Longsworth (ref. 8) using Dole's theory (ref. 5).

the exception of phosphate) decreases. However, the value of y, column 7, is inversely proportional to k_1 for a given protein mixture; hence the term K_1y/Q causes a steady progression of values of $F_{2(desc)}$ as the buffer mobility increases. These values (column 8) of $F_{2(desc)}$ which were computed using series expansions of the Dole theory, equations 25, are seen to be in close agreement with the values which Longsworth computed using the complete Dole theory. If the buffer concentrations are chosen to give identical values of y (which amounts to choosing the buffer concentrations inversely proportional to their equivalent refractions), then $F_{2(desc)}$ shows a much smaller variation with change in buffer anion. This is illustrated in columns 9 and 10 of Table I in which are listed buffer anion concentrations, C_1, and the corresponding values of $F_{2(desc)}$, for $y = 0.9174$ (the value for diethylbarbiturate at 0.1 N concentration).

Similar but more pronounced effects can be shown to exist for the ascending system.

It should be emphasized that in general the dependence of F_2 on y is not linear except near $y = 0$. An attempt to draw a straight line through experimental values of F_2 obtained at fairly large values of y may therefore fail to give $F_{2(y = 0)}$. Consequently, even for systems satisfying equation 29 so that $F_{2(y = 0)} = f_2$, the correct analysis is not obtained unless measurements are made at sufficiently small values of y.

Application to Experimental Systems

The above theory will now be applied to some recent data for two systems containing four ionic species in water. The first system demonstrates how the conventional procedures for analyzing electrophoresis experiments may result in large errors; for both systems the rapidity of convergence of the series expressions to the complete Dole theory is indicated.

KCl-$KClO_3$-KIO_3.—For this system we designate chloride, chlorate, iodate and potassium as ionic species 1, 2, 3 and 4, respectively. Preliminary calculations showed that a moving boundary analysis of a solution containing small amounts of $KClO_3$ and KIO_3 ("protein" components) in an aqueous solution of KCl (the "buffer") should yield an apparent refractometric fraction of chlorate, F_2, which differs greatly from its true refractometric fraction, f_2. Therefore this system was chosen for some experiments to test and illustrate the theory; for the case $C_2 = C_3$ these results are presented in Fig. 3. Theoretical curves for F_2 *versus* y are shown which were computed[13] for both the ascending and descending boundary systems using (1) the linear forms of equations 25 and 27 with the y^2 terms omitted, (2) equations 25 and 27 through terms of order y^2 and (3) the complete Dole equations.[14] As y decreases, the two sets of curves for the ascending and descending boundary systems are seen to converge and approach a limiting value of $F_{2(y = 0)} = -0.0719$. This intercept differs not only in magnitude but also in sign from the true refractometric fraction for this case, $f_2 = +0.2916$. For values of y up to about 0.5 (corresponding to a typical experiment with proteins) the deviations of equations 25 and 27 from the complete Dole theory calculations are seen to be negligible.

The values of F_2 in Fig. 3 are negative because the refractive index gradient curve of the leading boundary is small and negative, while that of the following boundary is larger and positive. This inverted gradient is barely visible in the schlieren photograph, Fig. 4a, which was taken at an early stage of the experiment, but it is seen distinctly in the Rayleigh fringe photograph of that figure which was taken at the same time. To conform with established notation[15,16] for experimental work, the phases and boundaries of Fig. 4 are identified using symbols α, β, γ and δ instead of 4, 3, 2 and 1 for the

(13) Data obtained by Longsworth (ref. 15) for 0.2 N solutions at 0° were used for these calculations. His values for the relative mobilities are $r_1 = -1.0231$, $r_2 = -0.8308$, $r_3 = -0.4691$ and $r_4 = 1.0000$; for the refractive increments of the potassium salts (in liters per equivalent for mercury yellow light) he obtained $k_1 = 0.01088$, $k_2 = 0.01142$ and $k_3 = 0.02774$. Although these values will differ somewhat from the correct ones for the present experiments (total salt concentrations of 0.10–0.16 N, and mercury green light), they were the best estimates available.

(14) Dole's equation 33 (ref. 5) was solved numerically, and the roots were substituted into his equation 31 to obtain the required concentrations in the various phases.

(15) L. G. Longsworth, THIS JOURNAL, **67**, 1109 (1945).

(16) R. A. Alberty, *ibid.*, **72**, 2361 (1950).

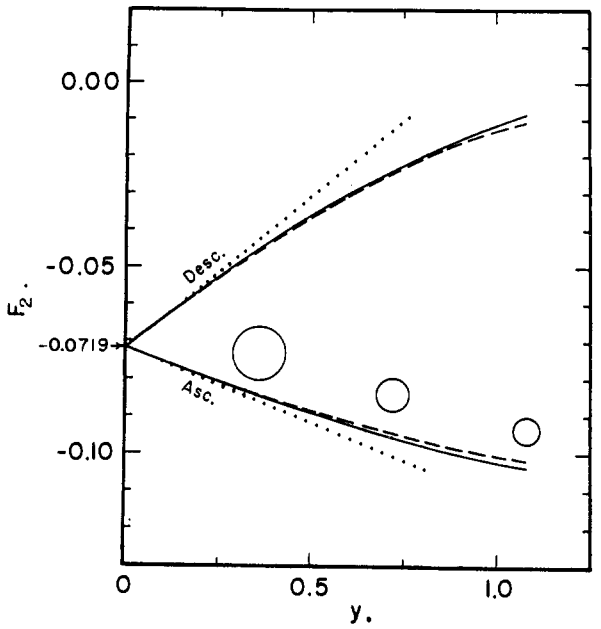

Fig. 3.—Apparent refractometric fraction, F_2, of chlorate for both ascending and descending boundaries of the system KCl–$KClO_3$–KIO_3 as a function of the quantity y (equation 12). The equivalent concentrations of chlorate and iodate are equal ($f_2 = +0.2916$): · · · ·, linear forms of equations 25 and 27 with y^2 terms omitted; – – – –, equations 25 and 27 including y^2 terms; ———, Dole's theory (ref. 5); O, experimental values for the ascending boundaries.

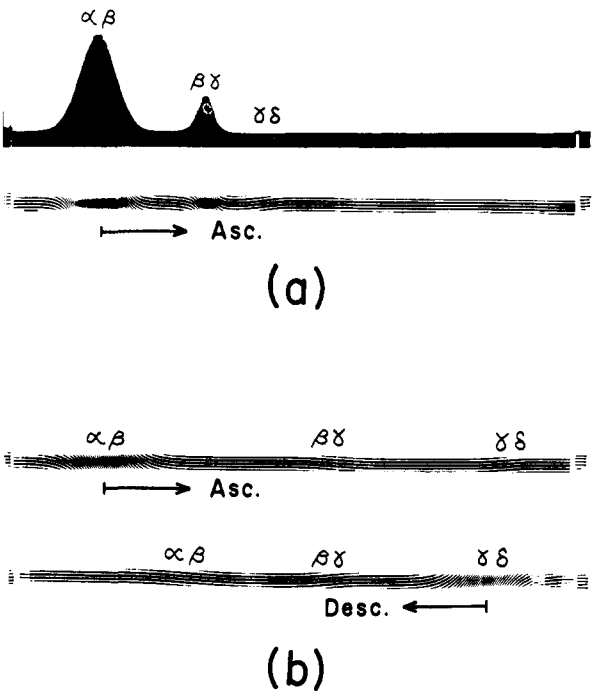

Fig. 4.—Moving boundary patterns for KCl–$KClO_3$–KIO_3 in water at 2°. (a) Schlieren and Rayleigh patterns after 2430 sec. at 16 ma. for an experiment with $f_2 = 0.2916$. In the notation of ref. 16 these ascending boundaries are described by: KCl(0.1)(δ) ← KCl, $KClO_3$(γ) ← KCl, $KClO_3$, KIO_3(β)::KCl(0.1), $KClO_3$(0.03), KIO_3(0.03)(α). (b) Ascending and descending Rayleigh patterns after 5922 and 6012 sec., respectively, at 16 ma. for an experiment with $f_2 = 0.877$. The ascending boundary system of this experiment is described by: KCl(0.1)(δ) ← KCl, $KClO_3$(γ) ← KCl, $KClO_3$, KIO_3(β)::KCl(0.1), $KClO_3$(0.03), KIO_3(0.00173)(α).

ascending phases and 1, 2, 3 and 4 for the descending phases (see Figs. 1 and 2). Measurements[7] of Rayleigh fringe displacements for the different phases after complete boundary separation had occurred were used to obtain the three values of F_2 represented by circles in Fig. 3. The radius of each circle corresponds to an uncertainty of ±0.05 fringe in these Rayleigh measurements. The small discrepancy between the experimental and theoretical values of F_2 for the ascending boundary systems is believed to arise from using values for the r_i and $\mathbf{k}_i$ which are not strictly applicable to this system.[13] No experimental data are shown for the descending boundary systems because the leading boundary was so diffuse that quantitative measurements were not possible.

Because a stable inverted boundary is present, this system is useful for illustrating the unusual values of F_2 which sometimes occur. Therefore $F_{2(y=0)}$ was computed for values of f_2 ranging from zero to unity, using in equation 25 (or 27) Longsworth's data[13] for the r_i and $\mathbf{k}_i$. The results are shown in Fig. 5, where it is observed that there is a discontinuity at $f_2 = 0.860$ ($C_2 = 14.9C_3$). This occurs because the refractive index changes across the negative leading boundary and the positive following boundary are of equal magnitude at this composition, so that the denominator in the equation $F_{2(y=0)} = \Delta n_2/(\Delta n_2 + \Delta n_3)$ becomes zero. In the region $f_2 < 0.860$ the negative values of $F_{2(y=0)}$ occur because Δn_2 is negative but of smaller magnitude than Δn_3, which is positive. For $f_2 > 0.860$, $F_{2(y=0)}$ is positive because Δn_2, though still negative, is of larger magnitude than Δn_3, so that $\Delta n_2 + \Delta n_3$ is negative. When $f_2 = 1$ then $F_{2(y=0)} = 1$, and the only moving boundary should be an inverted one in which chlorate disappears; the existence of an inverted boundary for this case was confirmed experimentally with $y = 0.210$. An experiment also was performed with $f_2 = 0.877$ and $y = 0.359$. The ascending and descending Rayleigh fringe patterns are shown in Fig. 4b. The two moving boundaries, across which the refractive index changes are opposite in sign, are clearly visible. Measurements of the fringe displacements gave values of 1.1 and 0.9 for $-\Delta n_2/\Delta n_3$ for the descending and ascending boundary systems, respectively. These results are in fair agreement with the corresponding values 1.17 and 1.16 predicted by inserting Longsworth's data[13] for the r_i and $\mathbf{k}_i$ into equations 25 and 27.

Sodium Acetate–Aspartate–Glutamate.—The above theory for strong electrolytes also can be applied to certain of the data which are reported in the companion paper[7] for this system. It is applicable to those experiments performed in the *p*H range 7.6–8.0 because in this range only sodium ions and the univalent anion subspecies of the acetate, aspartate and glutamate constituents are present in significant concentration. We will designate acetate, aspartate, glutamate and sodium as ionic species 1, 2, 3 and 4, respectively. Their rela-

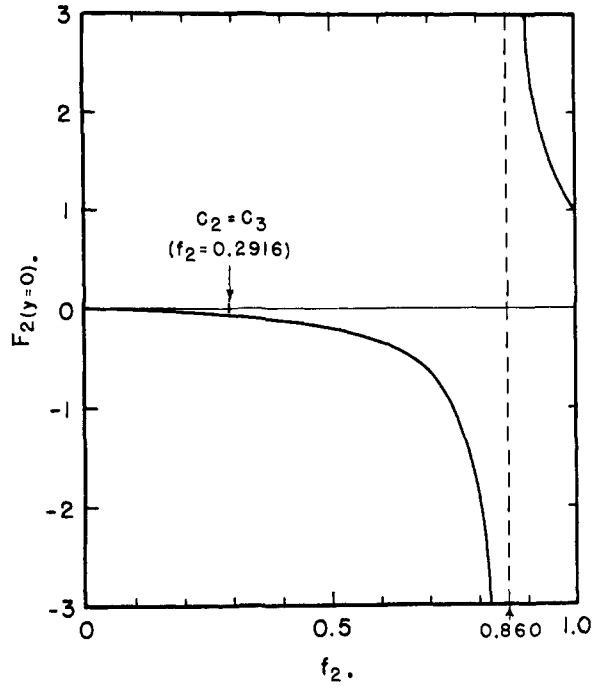

Fig. 5.—Variation of the limiting apparent refractometric fraction of chlorate, $F_{2(y=0)}$, with f_2, the true refractometric fraction for the system KCl–$KClO_3$–KIO_3.

tive mobilities are taken to be[7] $r_1 = -16.7$, $r_2 = -11.1$, $r_3 = -9.8$ and $r_4 = 22.2$, while the refractive increments (in liters per equivalent) for the sodium salts are taken as[17] $\mathbf{k}_1 = 0.01232$, $\mathbf{k}_2 = 0.02960$ and $\mathbf{k}_3 = 0.03239$. In Fig. 6 is plotted the apparent refractometric fraction of aspartate, F_2, as a function of y for both the ascending boundaries (upper three curves) and descending boundaries (lower three curves) when $C_2 = C_3$. The several curves for F_2 were computed in the same manner as those of Fig. 3. Included for comparison are the three experimental values reported in the companion paper[7] for the ascending boundaries of experiments 11, 12 and 13. For values of y up to about 0.5 (corresponding to a typical protein experiment) the maximum deviation of equations 25 and 27 from the complete Dole theory[5] is of the order of one per cent. for this case. It should be noted that as y decreases the curves in Fig. 6 converge and approach the limiting value of 0.4760 at $y = 0$. This value is very near the true refractometric fraction, $f_2 = 0.4775$.[18] It must not be inferred that such close agreement of the limiting values will be observed for all amino acid systems. For this particular system it happens that equation 29 is very nearly satisfied. However, if glycinate is substituted for aspartate as component 2, then for $f_2 = 0.350$ ($C_2 = C_3$) one obtains[19] $F_{2(y=0)} = 0.238$.

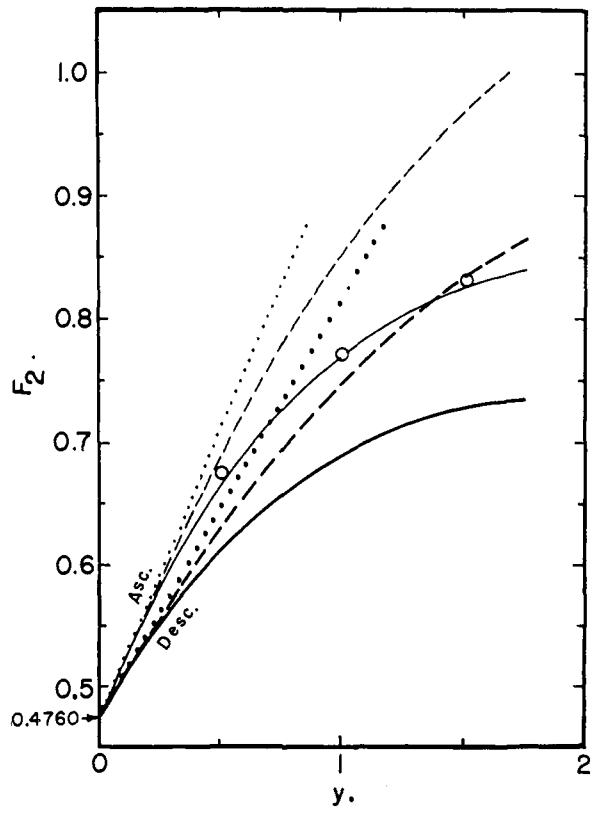

Fig. 6.—Apparent refractometric fraction, F_2, of aspartate for the ascending and descending boundaries as a function of y, the ratio of total aspartate plus glutamate concentration to acetate concentration on the basis of refractive index. The equivalent concentrations of aspartate and glutamate are equal: · · · ·, "limiting law" form of equations 25 and 27 with y^2 terms excluded; – – – –, equations 25 and 27 including y^2 terms; ———, Dole's theory (ref. 5); O, experimental values from ref. 7 for the ascending boundaries.

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(17) E. B. Dismukes and R. A. Alberty, THIS JOURNAL, **75**, 809 (1953).

(18) Four significant figures are retained to illustrate how $F_{2(y=0)}$ would compare with f_2 if the values used for the r_i and $\mathbf{k}_i$ were exact.

(19) Using Longsworth's mobility and refractive index data (ref. 8) for glycinate and the above values for sodium, acetate and glutamate ions.