were laid by Longsworth and MacInnes[19] in their study of the interaction of ovalbumin and yeast nucleic acid. Further information concerning such interactions may be obtained by use of the concept of constituent mobility.[20,21]

Acknowledgments.—The author is indebted to Dr. J. C. Nichol for his experimental tests of various features of the theory and to Dr. H. Svensson of the Institute of Physical Chemistry, Uppsala, for clarifying a number of points and for making available his work before publication. Financial assistance from the Wisconsin Alumni Research Foundation is gratefully acknowledged.

Summary

The application of the moving boundary equation to systems formed by weak electrolytes has been discussed. The constituent mobility of a weak electrolyte may be determined with precision if this constituent disappears in a steady state moving boundary which moves into the original solution. The conditions under which such boundaries are obtained in simple systems formed by weak acids of the uncharged-acid type and by weak bases of the uncharged-base type are discussed.

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[19] Longsworth and MacInnes, *J. Gen. Physiol.*, **25**, 507 (1942).

[20] Alberty and Marvin, *J. Phys. Colloid Chem.*, **54**, 47 (1950).

[21] Smith and Briggs *ibid.*, **54**, 33 (1950).

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF WISCONSIN]

Moving Boundary Systems Formed by Weak Acids and Bases. An Experimental Study[1]

BY J. C. NICHOL[2]

Introduction.—Moving boundary systems containing strong electrolytes, for which relative ion mobilities are constant, can be described by the general theory of Dole.[3] Thus, if the relative ion mobilities of all the species present in the system and the compositions of the two end solutions forming the initial boundary are known, Dole's theory can be applied to calculate the concentrations of the ions in all the newly formed phases, as well as the displacements of the separated boundaries.

If weak electrolytes are present in one or both of the end solutions, the strong electrolyte theory in many cases is inadequate for the description of the system because chemical reactions occur in the moving boundaries.[4,5] This fact is important because the majority of electrophoresis experiments involve weak polyelectrolytes such as proteins and nucleic acids. Studies of simple weak electrolyte systems are required as a preparation for work with the more complicated polyelectrolytes. For example, an understanding of moving boundary systems in which chemical reactions take place is necessary for the interpretation of electrophoresis experiments with antigen–antibody mixtures or protein–nucleic acid mixtures.

A striking illustration of a chemical reaction is provided when an initial boundary is formed between a solution of sodium caproate and a solution of sodium acetate and acetic acid and a potential is applied so that caproate ions descend into the acetate solution (*i. e.*, the caproate ions follow the faster acetate ions). At first a new boundary forms and can be seen moving into the acetate solution. However, the reaction of caproate ions with acetic acid molecules which otherwise would be left behind this boundary soon results in a layer of insoluble caproic acid droplets which completely obscures the boundary position.

If all the products of the reaction in a system similar to the above are soluble, then the velocity of the moving boundary can be determined. The mobility calculated in the ordinary way will be found to be lower than that obtained from similar measurements on systems where none of the acid form is present (Fig. 1).

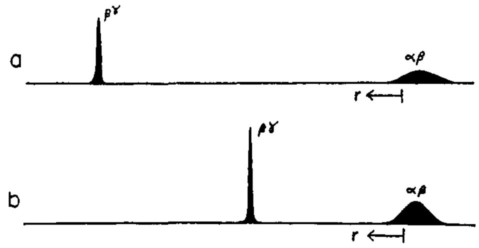

Fig. 1.—Schlieren patterns of the systems (a) NaOAc(0.05)(γ) ← NaCac(β)::NaCac(α), (b) NaOAc(0.05), HOAc(0.05)(γ) ← NaCac, HCac(β)::NaCac(α) 40 coulombs of electricity passed in each experiment.

Another result of the chemical reaction is the formation under certain conditions of a steady state moving boundary even when the faster ion follows the slower. In strong electrolyte experiments the corresponding boundary always spreads with time.

In the preceding paper Alberty[6] has developed a theory on the basis of the weak electrolyte moving

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[1] This work was supported by a grant from the U. S. Public Health Service.

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[3] Dole, THIS JOURNAL, **67**, 1119 (1945).

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

[5] Alberty and Nichol, THIS JOURNAL, **70**, 2297 (1948).

[6] Alberty, *ibid.*, **72**, 2361 (1950).

boundary equation to account for the above behavior of simple moving boundary systems formed by weak electrolytes. For the case in which the chemical reaction in a steady state boundary goes to completion, the theory predicts that the experimental mobility will be equal to that of the ion constituent, which is referred to as the *constituent* mobility.[6] It is the purpose of this paper to describe experiments which test the theory. These experiments are mainly restricted to systems containing not more than two weak acids or bases which form monovalent ions and a total of three ions. A few experiments involving both mono- and divalent phosphate ions are described.

Experimental

The experiments were carried out in the standard 11-ml. Tiselius cell with electrode vessels connected directly to the top section.[7,8] The current was supplied by B batteries connected to the cell through an electronic current regulator,[9] or in some cases without the regulator, and the quantity of electricity was computed from observations of time and the current measured by means of a potentiometer.

The boundary displacements were determined from photographs taken with a diagonal knife edge schlieren optical system designed so that light passed through the cell in a parallel beam. The accuracy of the mobilities is limited by the accuracy with which the cell cross-sectional area and the camera magnification factor could be obtained, as discussed by Longsworth.[10] The cross-sectional area was determined by filling the channel with mercury and measuring the length of the channel with vernier calipers, a procedure which assumes uniformity of the cross section. The magnification factor was determined by photographing a brass bar containing 0.2 mm. diameter holes spaced 1 cm. apart, which was placed in the cell in its usual position in the electrophoresis bath. The distances between the holes in the bar and between the corresponding lines on the photographic plate were determined with a Gaertner comparator. This instrument was also used in measuring boundary displacements on plates used for moving boundary experiments.

When required, samples of the phases produced by the passage of current were removed from the cell at the conclusion of the experiments by means of a syringe equipped with a long stainless steel needle and clamped in a device for raising or lowering the needle and withdrawing the barrel of the syringe.[10,11] The specific conductances of these solutions were determined at 0° in a conductance cell with conical platinum electrodes[12] having a volume of 1.8 ml., using a Jones conductance bridge. Mobilities were calculated by using these conductances at 0° and the boundary displacements measured at 1° in the electrophoresis apparatus.

Reagent or C. P. quality chemicals were used for the preparation of solutions. Solutions of weak acids and bases and their salts were prepared by titrating solutions of the acids or bases with standard sodium hydroxide or hydrochloric acid. The solutions were made up to volume at 0° in calibrated volumetric flasks. The *p*H values were measured at 25°, using a Beckman *p*H meter.

Mobility Data.—The mobilities of the ions involved in various experiments are given in Table I along with the pK_a' values for the acids, which are included because the dissociation constants play an important part in determining the type of moving boundary system obtained. The abbreviations for the ions used in describing the various moving boundary systems are also indicated.

Systems Involving Weak Acids which Form Monovalent Anions.—Detailed experiments with the following moving boundary types were carried out to illustrate the weak acid systems shown in Fig. 2 of the preceding paper:[6] *Type 1* fast ion and corresponding weak acid followed by slow ion which forms an acid of higher pK_a' than the leading acid; *Type 2,* fast ion and corresponding weak acid followed by slow ion which forms an acid of lower pK_a' than the leading acid (1 or 2 *pK* units lower); *Type 3,* slow ion and corresponding weak acid followed by a fast ion which forms an acid of higher pK_a' than the leading acid; *Type 4,* fast ion and corresponding weak acid followed by a slow ion which forms an acid of very much lower pK_a' than the leading acid (3 or 4 *pK* units lower). Since the Tiselius cell has two channels, two such experiments were sometimes carried out simultaneously, the anion with the higher mobility leading in one channel and following the slower ion in the other.

Type 1: NaOAc, HOAc(γ) $\leftarrow$ NaCac, HCac(β)::NaCac(α)[13] (Fig. 1).—In this system the chemical reaction occurring in the $\beta\gamma$ boundary is $\mathrm{Cac^- + HOAc \rightleftarrows OAc^- + HCac}$. The pK_a' of cacodylic acid is considerably higher than that of acetic acid (Table I) and the reaction goes to completion because the acetate ion produced is carried out of the region in which the reaction occurs. Since no acetic acid is left behind the moving boundary, the acetate constituent[6] mobility in the γ solution, $\bar{u}^{\gamma}_{\mathrm{OAc}}$, is obtained from the boundary velocity by means of the expression

$$\bar{u}^{\gamma}_{\mathrm{OAc}} = v^{\beta\gamma}\kappa^{\gamma} \tag{1}$$

where $v^{\beta\gamma}$ is the volume in ml. swept through by the boundary on passage of 1 coulomb of electricity and κ^{γ} is the specific conductance of the γ phase. The cacodylate constituent mobility in the β solution may be calculated from the expression

$$\bar{u}^{\beta}_{\mathrm{Cac}} = v^{\beta\gamma}\kappa^{\beta} \tag{2}$$

if a sample of the β solution is removed from the cell after the experiment for a conductance measurement. Equations 1 and 2 are the forms to which the moving boundary equation (equation 3 of the preceding paper[6]) for the acetate and cacodylate constituents reduces in this instance. The acetate constituent mobility is given by the equation

$$\bar{u}^{\gamma}_{\mathrm{OAc}} = u^{\gamma}_{\mathrm{OAc}} \frac{c^{\gamma}_{\mathrm{OAc}}}{c^{\gamma}_{\mathrm{OAc}} + c^{\gamma}_{\mathrm{HOAc}}} \tag{3}$$

if the mobility of acetic acid is zero. The quantities $c^{\gamma}_{\mathrm{HOAc}}$ and $c^{\gamma}_{\mathrm{OAc}}$[14] represent the molar concentrations of acetic acid and acetate ions and $u^{\gamma}_{\mathrm{OAc}}$ is the mobility of acetate ion in the γ solution. Similarly

$$\bar{u}^{\beta}_{\mathrm{Cac}} = u^{\beta}_{\mathrm{Cac}} \frac{c^{\beta}_{\mathrm{Cac}}}{c^{\beta}_{\mathrm{Cac}} + c^{\beta}_{\mathrm{HCac}}} \tag{4}$$

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[7] Longsworth, THIS JOURNAL, **65**, 1755 (1943).

[8] Alberty, *J. Phys. Colloid Chem.*, **53**, 114 (1949).

[9] This regulator was constructed by Mr. H. H. Marvin.

[10] Longsworth, THIS JOURNAL, **67**, 1109 (1945).

[11] Mr. E. Hanson constructed this sampling device.

[12] Longsworth, Shedlovsky and MacInnes, *J. Exp. Med.*, **70**, 379 (1939).

[13] The conventions used are the same as those in the preceding paper.

[14] Molar concentrations are given the sign of the ion, *e. g.*, c_{OAc} is negative and c_{HOAc}, which is one form or subspecies of the acetate constituent, is also taken as negative. The acetate constituent concentration is $c_{\mathrm{OAc}} + c_{\mathrm{HOAc}} = \bar{c}_{\mathrm{OAc}}$.

According to the theory the mobilities calculated by means of equations 1 and 2 should check those obtained using equations 3 and 4, respectively, within the limitations of our knowledge of $u^{\gamma}_{\mathrm{OAc}}$, $c^{\gamma}_{\mathrm{OAc}}$, $c^{\gamma}_{\mathrm{HOAc}}$, u^{β}_{Cac}, c^{β}_{Cac}, and $c^{\beta}_{\mathrm{HCAc}}$.[15]

Table II gives data for experiments with this system which verify the theory. The concentrations in the original solutions α and γ, the boundary displacements $v^{\beta\gamma}$, the specific conductances κ^{γ} and κ^{β}, and $p\mathrm{H}^{\beta}_{\mathrm{obs.}}$ are given in the footnotes and in columns 1, 2, 3, 4 and 10. Columns 5 and 6 list, respectively, the acetate mobilities calculated from the experimental data $v^{\beta\gamma}\kappa^{\gamma}$ and the acetate constituent mobilities calculated from equation 3 by using the known concentrations $c^{\gamma}_{\mathrm{OAc}}$ and $c^{\gamma}_{\mathrm{HOAc}}$ and taking $u^{\gamma}_{\mathrm{OAc}} = -17.6 \times 10^{-5}$ cm.^{2} volt^{-1} sec.$^{-1}$ (Expt. 1). The agreement between the data of these two columns verifies equation 1.

The mobilities $v^{\beta\gamma}\kappa^{\beta}$ (column 7) also agree with the cacodylate constituent mobilities within the experimental error (column 8). The values in column 8 were calculated from equation 4, $c^{\beta}_{\mathrm{HCac}}$ being taken equal to $c^{\gamma}_{\mathrm{HOAc}}$, and c^{β}_{Cac} (column 9), being computed from the conductance of the β solution by using a graph of conductance *versus* concentration of sodium cacodylate on the assumption that the cacodylic acid in the β solution has no effect on the conductance.

It is interesting to note that for strong electrolyte systems the $\alpha\beta$ boundary remaining near the site of the initial boundary is always a dilution boundary, all ion species being diluted in the same ratio. In the weak electrolyte system under consideration, cacodylic acid is formed in the β phase whether or not it is present in the α phase, and since it is part of the cacodylate constituent, the $\alpha\beta$ boundary is not a dilution boundary.

If acetic acid is omitted from the γ phase (Expt. 1 of Table II), we have the strong electrolyte moving boundary system NaOAc (γ)$\leftarrow$NaCac (β)::NaCac(α), for which the expressions $v^{\beta\gamma}\kappa^{\gamma}$ and $v^{\beta\gamma}\kappa^{\beta}$ give the ion mobilities, $u^{\gamma}_{\mathrm{OAc}}$ and u^{β}_{Cac}. Figure 1a shows the electrophoretic pattern for this experiment while Fig. 1b illustrates the decrease by one-half observed in the $\beta\gamma$ boundary velocity when acetic acid and sodium acetate are present in equal concentration in the γ phase. Since κ^{γ} (column 3) changes by only 0.3% when the acetic acid concentration is increased from zero to 0.05 *M*, the mobility $v^{\beta\gamma}\kappa^{\gamma}$ decreases by one-half within the limits of accuracy of the data in column 5.

Figure 1 shows that the $\alpha\beta$ boundary remaining near the site of the initial boundary undergoes a small backward displacement. The moving boundary equation predicts this movement which results from the change in relative mobilities of sodium ion and cacodylate constituent across the boundary.

TABLE I

MOBILITIES OF ANIONS AND CATIONS AT 0° AND TITRATION CONSTANTS (pK'_a) FOR THE CORRESPONDING ACIDS AT 25°

Ion	Symbol in text	$u \times 10^5$ cm.^{2} volt^{-1} sec.$^{-1}$			
		0.1 equivs./l.	0.05	0.01	pK'_a
Sodium[a]	Na	22.23	23.20	24.84	...
Chloride[a]	Cl	−37.03	−38.25	−40.40	...
Formate[b]	Form	−25.0	−26.1 (−26.3)[c]	−28.3	3.6
Acetate[b]	OAc	−16.6	−17.7 (−17.6)[c]	−19.5	4.7
Monochloroacetate[b]	M	−16.3	−17.3	−19.2	2.9
Trichloroacetate[b]	T	−13.6	−14.6	−16.2	0.9
Lactate[b]	L	−13.1	−14.2 (−14.3)[c]	−16.3	3.8
Cacodylate[b]	Cac	−10.1	−11.4	−13.2	6.2
Phosphate[c] (monobasic)	H_2PO_4	−12.0	−13.4	−15.7	6.8[d]
Phosphate[c] (dibasic)	HPO_4	−20.2	−22.3	.....	12.3[e]
Diethanolamine H[b]	DEH	11.4	13.0	16.3	9.0
Triethanolamine H[b]	TEH	9.1	11.0	13.6	7.9

[a] Moving boundary method, E. A. Anderson, Dissertation, University of Wisconsin, 1949. [b] Assuming additivity of ion conductances of sodium or chloride salt. [c] Moving boundary method. [d] Constant for ionization of monovalent phosphate ion. [e] Constant for ionization of divalent phosphate ion.

If cacodylic acid is included in the α phase (Expt. 2, Table II), we have the system NaOAc(γ)$\leftarrow$NaCac(β)::NaCac, HCac(α). As in Expt. 1, ion mobilities are obtained from the $\beta\gamma$ boundary displacement. No cacodylic acid appears in the β solution and hence a large pH gradient occurs across the $\alpha\beta$ boundary. Similar results have been found in other experiments of this type involving acetate–butyrate and acetate–propionate systems. These experiments show that by means of a potential gradient it is possible to obtain a pure solution of the ion form of the constituent from a solution containing the ion

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(15) In assigning values to the mobilities of the acetate and cacodylate ions and to the concentrations of these ions and of acetic and cacodylic acids, the following assumptions are made. In the first place, for solutions containing both the ion and uncharged acid forms of the acetate (or cacodylate) constituent, it is assumed that an individual acetate moiety in the ion form has the same mobility as in the absence of the acid form, *i. e.*, that the viscosity effect of the acid is negligible, and that the acid form has zero mobility. Thus, in the solution 0.05 molar in both sodium acetate and acetic acid, if hydrolysis is ignored, an acetate moiety on the average is assumed to spend half of its time as an ion with a mobility of -17.6×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ (the mobility determined from moving boundary experiments on 0.05 *M* sodium acetate solutions at pH 8–9) and the remaining time in combination with hydrogen as an acetic acid molecule with mobility zero. Although we have no means for measuring the mobility of an ion in the presence of the uncharged acid, the conductance data give an indication of the viscosity increase when the acid is present. The conductances listed in column 3 of Table II for 0.05 *M* sodium acetate solutions decrease by roughly 1% when the acetic acid concentration is varied from 0.0–0.25 *M*. Finally in assigning values to $c^{\beta}_{\mathrm{HCac}}$ it is assumed that for every molecule of acetic acid in the volume of the γ solution replaced by the β solution, a molecule of cacodylic acid is produced, whence $c^{\beta}_{\mathrm{HCac}} = c^{\gamma}_{\mathrm{HOAc}}$.

TABLE II

DATA FOR THE SYSTEM NaOAc,[a] HOAc(γ) ← NaCac,[a] HCac(β)::NaCac(HCac)[b](α)

Expt.	1 C^{γ}_{HOAc}	2 $v^{\beta\gamma} \times 10^3$	3 $\kappa^{\gamma} \times 10^3$	4 $\kappa^{\beta} \times 10^3$	5 $\bar{u}^{\alpha}_{\text{OAc}} \times 10^5$ obsd.	6 $\bar{u}^{\alpha}_{\text{OAc}} \times 10^5$ theor.	7 $\bar{u}^{\beta}_{\text{Cac}} \times 10^5$, obsd.	8 $\bar{u}^{\beta}_{\text{Cac}} \times 10^5$ theor.	9 c^{β}_{Cac} obsd.	10 $p\text{H}^{\beta}$ obsd.	11 $p\text{H}^{\beta}$ theor.
1	0	$-89._0$	1.978	1.310	−17.6	−17.6	−11.7	−11.7	-0.038_7	8.5	...
2[b]	0	$-89._0$	1.978	1.310	−17.6	−17.6	−11.7	−11.7	$-\ .038_7$	8.5	...
3	−0.01	$-74._5$	1.976	1.235	−14.7	−14.7	− 9.2	− 9.3	$-\ .036_8$	6.7	6.7
4	− .05	$-44._5$	1.972	0.963	− 8.8	− 8.8	− 4.3	− 4.4	$-\ .027_8$	5.9	5.9
5	− .10	$-30._0$	1.967	0.686	− 5.9	− 5.9	− 2.1	− 2.1	$-\ .019_4$	5.5	5.3
6	− .15	$-23._0$	1.959	...	− 4.5	− 4.4	...	....	......	...	...
7	− .25	$-15._0$	1.953	...	− 2.9	− 2.9	...	....	......	...	...

[a] $C^{\gamma}_{\text{NaOAc}} = 0.05\ M$ and $C^{\alpha}_{\text{NaCac}} = 0.15\ M$ in all experiments. [b] $C^{\alpha}_{\text{HCac}} = -0.15\ M$ in Experiments 2 and 7. In all other experiments $C^{\alpha}_{\text{HCac}} = 0$.

and uncharged acid forms in equilibrium.[16]

From the weak electrolyte theory it is possible to predict the composition of the β solution and hence $p\text{H}^{\beta}$. One cacodylic acid molecule is produced for each acetic acid molecule in the volume of solution which is replaced by the β solution, and, therefore, $c^{\beta}_{\text{HCac}} = c^{\gamma}_{\text{HOAc}}$, the latter value being known.[15] Furthermore, c^{β}_{Cac} may be calculated from the expression (see equation (11) of the preceding paper)

$$c^{\beta}_{\text{Cac}} = \frac{c^{\gamma}_{\text{HOAc}}(u^{\gamma}_{\text{OAc}}u^{\beta}_{\text{Na}} - u^{\beta}_{\text{Cac}}u^{\gamma}_{\text{Na}}) + c^{\gamma}_{\text{OAc}}(u^{\gamma}_{\text{OAc}} - u^{\gamma}_{\text{Na}})u^{\beta}_{\text{Cac}}}{(u^{\beta}_{\text{Cac}} - u^{\beta}_{\text{Na}})u^{\gamma}_{\text{OAc}}} \qquad (5)$$

where u^{γ}_{OAc}, u^{β}_{Cac}, u^{γ}_{Na} and u^{β}_{Na} are ion mobilities.

To evaluate c^{β}_{Cac} by means of equation 5 a method of successive approximations must be used. As an example, suppose that $c^{\gamma}_{\text{HOAc}} = c^{\gamma}_{\text{OAc}} = -0.05$. The relative mobilities of sodium, acetate and cacodylate ions at 0.05 M concentration are used to obtain an approximate value for c^{β}_{Cac}. The calculation is repeated by using the mobilities of sodium and cacodylate ions at this concentration relative to that of sodium in a 0.05 M solution to obtain a second value for c^{β}_{Cac}. The process is repeated until a constant concentration is obtained.

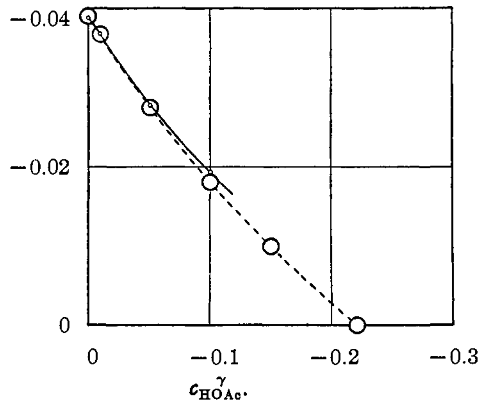

Fig. 2.—Plot of dependence of c^{β}_{Cac} upon c^{γ}_{HOAc} for the system NaOAc(0.05), HOAc(γ) ← NaCac, HCac(β):: NaCac(α) experimental curve, —; theoretical curve, -------.

In Fig. 2 c^{β}_{Cac}, determined by conductance measurements, is plotted against c^{γ}_{HOAc} for $c^{\gamma}_{\text{OAc}} = -0.05$ (solid line). This plot may be compared with the curve constructed from equation 5 in the manner just described (dotted line). The size of the circles around the points on the curves indicates the probable accuracy of the data. Equation 5 contains terms which are differences of mobilities, and the error in the calculated value of c^{β}_{Cac} is approximately 5% if the error in the mobility values is approximately 1%. When this is taken into consideration, it is apparent that for acetic acid concentrations up to about 0.10 M, the experimental and theoretical curves are in agreement. An examination of the electrophoretic patterns reveals that the β phase is not homogeneous at concentrations above 0.1 M and therefore, c^{β}_{Cac} calculated from κ^{β} would have no significance. A diffuse boundary, barely detectable when $c^{\gamma}_{\text{HOAc}} = -0.15$ but quite pronounced when $c^{\gamma}_{\text{HOAc}} = -0.25$ appears between the $\alpha\beta$ and $\beta\gamma$ boundaries. This suggests convection in part of the β region. In Table II the values of $v^{\beta\gamma}$, κ^{γ} and the acetate constituent mobility (columns 5 and 6) agree within the experimental error even at high acetic acid concentrations and in all experiments a steady state moving boundary is formed. This is still possible as long as the inhomogeneity in the β phase does not extend right up to the moving boundary.

Columns 10 and 11 of Table II list, respectively, the measured values of $p\text{H}^{\beta}$ and those calculated from the predicted composition of the β phase by means of the relation

$$p\text{H}^{\beta} = pK'_{\text{HCac}} + \log\,(c^{\beta}_{\text{Cac}}/c^{\beta}_{\text{HCac}}) \qquad (6)$$

Values for c^{β}_{Cac} from the dotted curve of Fig. 2 which is based on equation 5 are used.

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[16] *Cf.* Consden, Gordon and Martin, *Biochem. J.*, **40**, 33 (1946). The authors point out that movement of the charged forms of amino acids away from the uncharged forms under a potential gradient will complicate the separation of individual acids from mixtures during ionophoresis in silica jelly if the system is not well buffered throughout to maintain a constant pH.

From equation 5 it is predicted that c^{β}_{Cac} becomes zero at a definite concentration of acetic acid (dotted curve, Fig. 2). However, in the derivation of the equation it is assumed that the ionization of cacodylic acid is zero. Actually there will always be some cacodylate ions in the β phase because of the ionization of cacodylic acid.

In order that boundaries of the type discussed above may move in a steady state, the conditions illustrated by the graph in Fig. 3 of the preceding paper must be fulfilled. These conditions which are expressed by equation 16[6] depend upon the ratio of ion to acid concentration in the phase into which the boundary moves, the ionization constants of the two acids, and the relative mobilities of the cation and of the two anions. The graph may be considered to consist of four quadrants divided by lines $r_{\mathrm{R}}/r_{\mathrm{S}} = 1$ and $pK'_{\mathrm{HS}} - pK'_{\mathrm{HR}} = 0$. The leading and indicator anions are designated as R^- and S^-. For the acetate–cacodylate system, $pK'_{\mathrm{HOAc}} - pK'_{\mathrm{HCac}} = 1.5$ and $r_{\mathrm{OAc}}/r_{\mathrm{Cac}} \sim 1.5$. These values are represented by a point in the upper right hand quadrant in which one would always expect a steady state boundary yielding the constituent mobilities. Experimentally, this is found to be true for acetic acid in the concentration range zero to 0.25 *M*. Similar results were obtained for the system NaForm, HForm (γ)←NaCac, HCac(β)::NaCac(α). If acetate–cacodylate boundaries are formed in both cell channels, the system obtained in the second channel is NaCac, HCac(α)←NaOAc, HOAc(β)::NaOAc(γ). In this example $pK'_{\mathrm{HOAc}} - pK'_{\mathrm{HCac}} = -1.5$ and $r_{\mathrm{Cac}}/r_{\mathrm{OAc}} \sim 0.7$ (lower left quadrant). As predicted by the graph, a steady state boundary yielding constituent mobilities is not obtained.

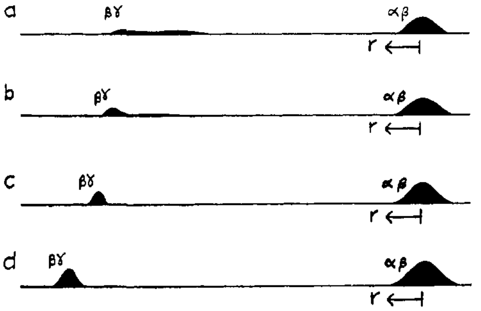

Fig. 3.—Patterns for the system NaOAc, HOAc(γ) ← NaL, HL, HOAc(β)::NaL(α): $c^{\gamma}_{\mathrm{OAc}} = -0.05$ and $c^{\alpha}_{\mathrm{L}} = -0.10$ *M* for all experiments. $c^{\gamma}_{\mathrm{HOAc}}$ is (a) -0.03, (b) -0.01, (c) -0.005, (d) 0.0 *M*. 40 coulombs of electricity passed in each experiment.

Type 2: NaOAc, HOAc(γ)←NaL, HL(β)::NaL(α).—Here $pK'_{\mathrm{HL}} - pK'_{\mathrm{HOAc}} = -0.9$ and $r_{\mathrm{OAc}}/r_{\mathrm{L}} \sim 1.2$ (lower right quadrant). Equation 16 indicates that if the ratio $c^{\gamma}_{\mathrm{OAc}}/c^{\gamma}_{\mathrm{HOAc}}$ is less than 29, a steady state boundary will not be obtained. Experimentally, it is difficult to say for exactly what ratio spreading occurs with time because there is a gradual change from a sharp to a diffuse boundary as $c^{\gamma}_{\mathrm{HOAc}}$ is increased (Fig. 3). However, the boundary quite definitely spreads when $c^{\gamma}_{\mathrm{OAc}}/c^{\gamma}_{\mathrm{HOAc}} \lesseqgtr 5$ (Figs. 3a and 3b) and moves as a steady state when $c^{\gamma}_{\mathrm{OAc}}/c^{\gamma}_{\mathrm{HOAc}} \gtreqless 10$ (Figs. 3c and 3d). Similar results are observed for the system NaForm, HForm(γ)←NaM, HM(β)::NaM(α), which also falls in the lower right quadrant. Spreading definitely occurs when $c^{\gamma}_{\mathrm{Form}}/c^{\gamma}_{\mathrm{HForm}} \lesseqgtr 5$. This observation is in qualitative agreement with equation 16 which predicts spreading when the ratio is $\lesseqgtr 8.4$. For this type of experiment, if the concentration of the leading acid is high enough, one would expect an incomplete chemical reaction in the β phase and hence an inhomogeneous solution because $pK'_{\mathrm{HR}} > pK'_{\mathrm{HS}}$. In Figs. 3a and 3b the very diffuse trailing edge of the moving boundary looks like a partially separated secondary boundary. It is possibly a result of the incomplete reaction of lactate ions with acetic acid.

Type 3: NaL, HL(α)←NaOAc, HOAc(β)::NaOAc(γ).—For this system $pK'_{\mathrm{HOAc}} - pK'_{\mathrm{HL}} = 0.9$ and $r_{\mathrm{L}}/r_{\mathrm{OAc}} \sim 0.8$ (upper left quadrant). Equation 16 predicts that $c^{\alpha}_{\mathrm{L}}/c^{\alpha}_{\mathrm{HL}}$ should be less than 2.6 if a steady state boundary is to be obtained. Again, experimentally the exact transition point is hard to determine because of the gradual change from a sharp to a diffuse boundary as c^{α}_{HL} is decreased. Figure 4 shows the schlieren patterns for four experiments over a range of c^{α}_{HL} values. The boundary moves in a steady state yielding the lactate and acetate constituent mobilities when $c/^{\alpha}_{\mathrm{L}}c^{\alpha}_{\mathrm{HL}} \lesseqgtr 5$ (Figs. 4a and 4b) although when the ratio is 5, a shoulder is apparent on the trailing edge of the peak (Fig. 4b). Spreading is observed when $c^{\alpha}_{\mathrm{L}}/c^{\alpha}_{\mathrm{HL}} \gtreqless 10$ (Figs. 4c and 4d). Figure 4d ($c^{\alpha}_{\mathrm{HL}} = 0$) is the pattern for a strong electrolyte experiment in which the faster ion follows the slower with the result that the boundary rapidly spreads. The system NaM, HM(α)←NaForm, HForm(β)::

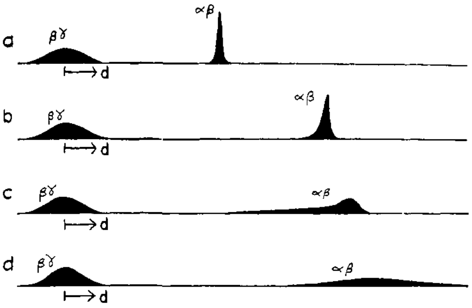

Fig. 4.—Patterns for the system NaL, HL(α) ← NaOAc, HOAc(β)::NaOAc(γ) $c^{\alpha}_{\mathrm{NaL}} = 0.05$ and $c^{\gamma}_{\mathrm{NaOAc}} = 0.025$ in each experiment: c^{α}_{HL} is (a) -0.05, (b) $-$ 0.01, (c) -0.005, (d) 0.0 *M*; 40 coulombs of electricity passed in each experiment.

NaForm(γ) is of the same type, and the boundary moves in a steady state when $c^{\alpha}_{M}/c^{\alpha}_{HM} \gtrless 2$. As in the preceding example these results are in qualitative agreement with equation 16, which predicts sharp boundaries when the ratio is $\gtrless 0.8$.

In Table III is found a comparison of the constituent mobilities, c^{β}_{OAc} and $p\text{H}^{\beta}$ obtained in the experiment of Fig. 4a with the values calculated by using equations 3, 4, 5 and 6. The agreement verifies the theory for this case. It is interesting that although the conductance of the β phase is higher than that of the α phase (and hence that the acetate constituent mobility is greater than the lactate constituent mobility) a steady state moving boundary is obtained. This illustrates the point that for such weak electrolyte systems the quantity which will determine whether or not a boundary will spread is the relative magnitude of the product of constituent mobility and potential gradient in the two phases rather than that of the constituent mobilities themselves.

Table III

Data for the System NaL(0.05), HL(0.05)(α) ← NaOAc, HOAc(β)::NaOAc(γ)

	Obsd.	Theor.
$\bar{u}^{\beta}_{OAc} \times 10^5$	−9.4	−9.4
$\bar{u}^{\alpha}_{L} \times 10^5$	−7.2	−7.2
c^{β}_{OAc}	−0.061	−0.062
$p\text{H}^{\beta}$	4.8	4.8

Table IV

Data for the System NaOAc(0.05), HOAc(δ) ← NaT, HOAc(NaOAc)(γ) ← NaT(β)::NaT(α)

Expt.	c^{δ}_{HOAc}	$v^{\gamma\delta}\kappa^{\delta} \times 10^5$	$u^{\delta}_{OAc} \times 10^5$ theor.	$\bar{u}^{\delta}_{OAc} \times 10^5$ theor.
1	0	−17.6	−17.6	...
2	−0.05	−16.5	−17.6	−8.8

Type 4: NaOAc, HOAc(δ)←NaT, HOAc(NaOAc)(γ)←NaT(β)::NaT(α).—In the examples considered up to this point, the ionization constants of the acids involved have been such that complete or at least partially complete chemical reactions can be expected. However, for this system, $pK'_{HT} - pK'_{HOAc} = -3.8$, and therefore the chemical reaction proceeds to an insignificant extent. The behavior of the $\gamma\delta$ moving boundary can be explained on this basis. A steady state moving boundary is obtained (Fig. 5a) and the value for the $v^{\gamma\delta}\kappa^{\delta}$ product is lower than the acetate ion mobility but higher than the constituent mobility (Table IV, Expt. 2). If no acetic acid is present (Expt. 1), the acetate ion mobility is obtained. In Expt. 2 the trichloroacetate ion can be considered to move through the β phase without reacting appreciably with acetic acid. The resulting system is somewhat analogous to strong electrolyte systems in which the slower ion follows the faster and a steady state moving boundary is obtained.

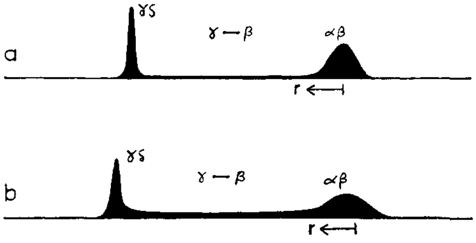

Fig. 5.—Patterns for the systems (a) NaOAc (0.05), HOAc(0.05)(δ) ← NaT, HOAc, (NaOAc)(γ) ← NaT (β)::NaT(α), (b) NaForm(0.05) HForm(0.05)(δ) ← NaT, HForm, (NaForm)(γ) ← NaT(β)::NaT(α); 30 coulombs of electricity passed in each experiment.

The resemblance to a strong electrolyte system is, however, not complete. In the first place, the acetate constituent does not disappear across the moving boundary and therefore the $v^{\gamma\delta}\kappa^{\delta}$ product does not represent the mobility of the acetate ion. If the moving boundary equation is solved, it is seen that the amount by which the experimentally measured $v^{\gamma\delta}\kappa^{\delta}$ is lower than u^{δ}_{OAc} depends on c^{γ}_{OAc}. This concentration is very small and the velocity of the $\gamma\delta$ boundary is about 5% lower than that of the acetate ions (Table IV). In the analogous experiment with formate constituent leading (Fig. 5b), c^{γ}_{Form} is higher because of the higher degree of ionization of formic acid, and the velocity of the $\gamma\delta$ boundary is 30% lower than that of formate ions. It should be noted that in both experiments one constituent, trichloroacetate, does disappear across the $\gamma\delta$ boundary. Consequently its mobility is given by $\bar{u}^{\gamma}_{T} = v^{\gamma\delta}\kappa^{\gamma}$. This value should closely approach the trichloroacetate ion mobility if the chemical reaction with the leading acid is negligible.

Another difference between these systems and the strong electrolyte three ion case is that because of the dissociation of the weak acid in the γ region, a second boundary (the $\beta\gamma$ boundary) might be expected to form and move with the acetate or formate constituent mobility. Experimentally, a very diffuse area extending the whole distance between the $\gamma\delta$ and $\alpha\beta$ boundaries is observed in both the acetate and formate experiments (Fig. 5). This indicates that the boundary forms, but that it diffuses very rapidly. The inhomogeneous solution between the end solutions is indicated as γ-β in Fig. 5.

Systems Involving both Monovalent and Divalent Anions: KI(0.15) (α)←K_2HPO_4(β)←K_2HPO_4, KH_2PO_4(γ)::K_2HPO_4(0.025), KH_2PO_4(0.025) (δ).—In this system two moving boundaries are observed (Fig. 6a). The faster boundary is very sharp and in Fig. 6a the upper part of the peak is cut off. The leading ion, iodide, is the anion of a strong acid and has a higher mobility than divalent phosphate ion. The value of $v^{\alpha\beta}\kappa^{\beta}$ is -22×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ which corresponds to the mobility of divalent phosphate ion in a pure potassium dibasic phosphate solution of a concentration equal to that calculated from κ^β on the assumption that only the divalent form is present in the β solution. The *p*H of a sample withdrawn from the β phase is about 8.7 which indicates that 99% of the phosphate constituent in this phase is in the divalent form. It is likely that the β solution in the cell contains only divalent phosphate ions, the low *p*H being accounted for by the absorption of carbon dioxide from the air into the poorly buffered sample during its withdrawal and transfer to the *p*H cup.

Since the phosphate constituent does not disappear across the $\beta\gamma$ boundary, the value of $v^{\beta\gamma}\kappa^\gamma$ is not a constituent mobility. Experimentally, this value is about 15% higher than would be expected for the monovalent phosphate form. In all experiments the monovalent and divalent phosphate ions were present in equimolar concentration in the δ phase, and it was observed that $p\text{H}^\gamma = 6.8 = p\text{H}^\delta$, which indicates that the $\gamma\delta$ boundary is a dilution boundary.

This experiment illustrates the same type of behavior as Expt. 2 of Table II. In the phosphate experiment the application of a potential results in the formation of a phase of potassium dibasic phosphate from an equilibrium mixture of the divalent and monovalent forms, while in Expt. 2 a phase of sodium cacodylate is formed from an equilibrium mixture of ion and uncharged acid.

If potassium iodide is present in all phases, two moving boundaries are still observed (Fig. 6b). This system is analogous to an ascending protein boundary. The value of $v^{\alpha\beta}\kappa^\beta$ is -19.6×10^{-5} cm.² volt⁻¹ sec.⁻¹ which is the mobility to be expected for divalent phosphate at the ionic strength of the β phase. The *p*H of a sample of the β phase is 8.7, as found in the preceding system.

Figures 6c and 6d show the schlieren patterns for experiments with solutions containing monovalent and divalent succinate and oxalate ions, respectively. In these cases the refractive index gradient does not parallel the density gradient at all of the boundaries, with the result that inverted peaks are observed.

Systems Involving Weak Bases.—These systems are the same as the weak acid systems except that cations instead of anions take part in the chemical reactions. Therefore, the weak electrolyte theory should apply to them equally well. Experimentally, this is found to be the case. The following experiment, involving diethanolamine (DE) and triethanolamine (TE) illustrates the behavior of a weak base system: $\text{TEHCl}(\alpha)::\text{TE},\ \text{TEHCl}(\beta)\rightarrow\text{DE},\ \text{DEHCl}(\gamma)$. The reaction $\text{DE} + \text{TEH}^+ \rightleftarrows \text{DEH}^+ + \text{TE}$ goes to completion because the pK'_a of the leading cation, DEH^+, is considerably higher than that of TEH^+ (Table I). In contrast, for the analogous weak acid systems, it is desirable that the uncharged acid form of the *following* constituent have the higher pK'_a.

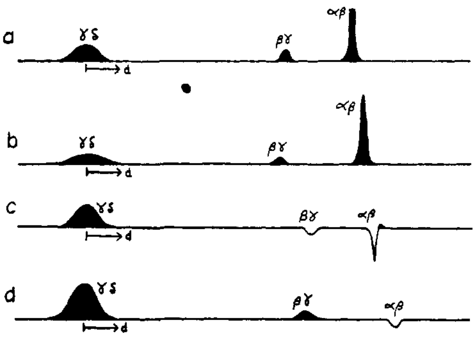

Fig. 6.—Patterns of the systems: (a) $\text{KI}(0.15)(\alpha) \leftarrow \text{K}_2\text{HPO}_4(\beta) \leftarrow \text{K}_2\text{HPO}_4,\ \text{KH}_2\text{PO}_4(\gamma)::\text{K}_2\text{HPO}_4(0.025),\ \text{KH}_2\text{PO}_4(0.025)(\delta)$; (b) $\text{KI}(0.20)(\alpha) \leftarrow \text{K}_2\text{HPO}_4,\ \text{KI}(\beta) \leftarrow \text{K}_2\text{HPO}_4,\ \text{KH}_2\text{PO}_4,\ \text{KI}(\gamma)::\text{K}_2\text{HPO}_4(0.025),\ \text{KH}_2\text{PO}_4(0.025)\ \text{KI}(0.05)(\delta)$; (c) $\text{Na}_2\text{SO}_4(0.10)(\alpha) \leftarrow \text{Na}_2\text{Su}(\beta) \leftarrow \text{Na}_2\text{Su},\ \text{NaHSu}(\gamma)::\text{Na}_2\text{Su}(0.03),\ \text{NaHSu}(0.03)(\delta)$, (Su, divalent succinate ion); (d) $\text{Na}_2\text{SO}_4(0.10)(\alpha) \leftarrow \text{Na}_2\text{C}_2\text{O}_4(\beta) \leftarrow \text{K}_2\text{C}_2\text{O}_4,\ \text{KHC}_2\text{O}_4(\gamma)::\text{K}_2\text{C}_2\text{O}_4(0.04),\ \text{KHC}_2\text{O}_4(0.04)(\delta)$.

When diethanolamine is present only as the hydrochloride in 0.05*M* concentration in the γ solution, the mobility calculated from the boundary velocity and κ^γ is 13×10^{-5} cm.² volt⁻¹ sec.⁻¹. When the solution contains free diethanolamine in 0.05*M* concentration, the mobility is 6.5×10^{-5} cm.² sec.⁻¹ volt⁻¹ (constituent mobility). The values of c^β_{TEH} calculated from the equation

$$c^\beta_{\text{TEH}} = \frac{c^\gamma_{\text{DE}}(u^\gamma_{\text{DEH}}u^\beta_{\text{Cl}} - u^\beta_{\text{TEH}}u^\gamma_{\text{Cl}}) + c^\gamma_{\text{DEH}}(u^\gamma_{\text{DEH}} - u^\gamma_{\text{Cl}})u^\beta_{\text{TEH}}}{(u^\beta_{\text{TEH}} - u^\beta_{\text{Cl}})u^\gamma_{\text{DEH}}} \quad (7)$$

are 0.045 and 0.040 for the above cases where free diethanolamine is present in the γ phase in zero and 0.05*M* concentrations, respectively, as compared to the values 0.045 and 0.040 for c^β_{TEH} calculated from conductance measurements on the β phases. The assumption is made that $c^\gamma_{\text{DE}} = c^\beta_{\text{TE}}$ (*cf.* the acetate–cacodylate experiments discussed above).

The theoretical triethanolamine ion and constituent mobilities in the β phases formed in the two experiments are 11.3×10^{-5} and 5.2×10^{-5} cm.² volt⁻¹ sec.⁻¹, respectively, as compared to the values 11.4×10^{-5} and 5.3×10^{-5} cm.² volt⁻¹ sec.⁻¹ obtained from the boundary velocity and κ^β. The observed value of 8.0 for $p\text{H}^\beta$ in the experiment where $c^\gamma_{\text{DE}} = 0.05$ agrees with the theoretical value calculated from c^β_{TE}.

Discussion

The experimental results show that the weak electrolyte theory developed by Alberty[6] predicts the behavior of moving boundary systems involving two uncharged acids or bases and their monovalent salts. For cases in which a chemical reaction goes to completion in the moving boundary with the result that each constituent disappears across it, the mobility values calculated from the boundary velocity and the conductances of the phases separated by the boundary agree with the theoretical constituent mobilities within the experimental error. Thus the moving boundary method can be used for the experimental determination of constituent mobilities as long as a steady state boundary is formed in which the chemical reaction is complete. It should be noted that the theoretical constituent mobilities are obtained by substituting the mobility of the ion and the concentrations of ion and uncharged form into the defining equation (*e. g.*, equation 3 for acetate constituent). This assumes that the mobility of the ion form of the constituent is not affected by the presence of the uncharged molecules.

The conditions under which steady state boundaries are expected may be obtained from the theory. The experimental results agree qualitatively with the theory, although the specific experimental conditions for which the boundary ceases to move in a steady state do not exactly coincide with those predicted theoretically. This is not surprising, since the titration constants are assumed to be independent of concentration and since the theory assumes constant relative ion mobilities.

Acknowledgments.—The author is indebted to Dr. J. W. Williams for his interest in this work. He is also grateful to Dr. R. A. Alberty for many helpful discussions by way of instruction in moving boundary theory.

Summary

The moving boundary equation for weak electrolytes has been verified for systems containing weak acids and bases which dissociate to give monovalent ions. Depending upon the relative values of the ionization constants and of the ion mobilities concerned, chemical reactions may occur in the moving boundary systems. If these reactions go to completion, the experimentally determined mobilities ($v\kappa$ products) are identical with the constituent mobilities. Thus for the system NaOAc, HOAc(γ)←NaCac, HCac(β)::NaCac(α), the experimental value, $v^{\beta\gamma}\kappa^{\gamma}$, is identical with the acetate constituent mobility given by the equation $\bar{u}^{\gamma}_{\mathrm{OAc}} = u^{\gamma}_{\mathrm{OAc}}c^{\gamma}_{\mathrm{OAc}}/(c^{\gamma}_{\mathrm{OAc}} + c^{\gamma}_{\mathrm{HOAc}})$. In contrast to the strong electrolyte case, the theory predicts and the experimental results confirm that, for weak electrolyte systems, steady state moving boundaries may be obtained even when the boundary is followed by the ion of higher mobility.

MADISON, WIS. RECEIVED OCTOBER 22, 1949

[CONTRIBUTION FROM THE JOHN HARRISON LABORATORY OF CHEMISTRY OF THE UNIVERSITY OF PENNSYLVANIA]

Hydrogen Cycle Cation Exchange for Heteropoly Acids and Salts*

BY LOUIS C. W. BAKER, BERNARD LOEV AND THOMAS P. MCCUTCHEON

This investigation was carried out to determine the feasibility of preparing free heteropoly acids from their salts by use of a cation exchange resin. It was expected that the behavior of the heteropoly anions in the presence of a cation exchanger would throw some light on the equilibria existing in the solutions. In addition to their well-known uses[1,2,3] free heteropoly acids are, because of their great solubility, frequently advantageous intermediates in the preparation of heteropoly salts.[3,4,5]

A high capacity sulfonic acid type cation exchange resin was chosen because heteropoly acids are usually strong and often easily reduced. This resin possesses only strongly acidic functional groups, has practically no reducing power, and, if used properly, does not store anions from the regenerant for later release to the solutions being put through the column.

Several well-known heteropoly salts, representative of types suggested by their possible behaviors in the presence of a cation exchange resin, were prepared. Analyses, to establish the identity of the heteropoly ions, were performed in most cases. Following the suggestion of Illingworth and Keggin,[2] powder X-ray patterns of all salts were taken to enable easy identification when they were re-formed in later stages of the work.[6]

Solutions or suspensions of the salts were passed through a column of exchange resin. The *p*H's of the resulting heteropoly acid solutions were determined, and aliquots of the solutions were analyzed for the constituents of the heteropoly anions. In each case an attempt was made to re-form the original heteropoly salt from the acid solution by metathesis. The powder X-ray patterns of the salts so formed were compared with those of the parent salts. The general absence of

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* We wish to thank the Committee on Advancement of Research of the University of Pennsylvania for a grant which helped to make this work possible.

(1) H. Wu, *J. Biol. Chem.*, **43**, 189 ff. (1920).

(2) Illingworth and Keggin, *J. Chem. Soc.*, 575 ff. (1935).

(3) M. Jean, *Ann. Chim.*, [12], **3**, 470 ff. (1948).

(4) G. Spacu and V. Nicholaescu, *Bull. Soc. Stiinte Cluj*, **9**, 45 ff. (1938).

(5) *Ibid.*, **9**, 347 ff. (1939).

(6) These X-ray patterns will appear in the third supplement to the A. S. T.M. Powder X-Ray File.