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## A THEORETICAL STUDY ON THE ZONE MOBILITY-pH CURVE IN PAPER ELECTROPHORESIS OF LOW MOLECULAR WEIGHT COMPOUNDS WITH A DISSOCIABLE PROTON AND ITS APPLICATION TO PHOSPHORUS COMPOUNDS

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### SUMMARY

It was found that the zone mobility of a compound with dissociable proton plotted against the pH value of the background solution is approximately expressed by a hyperbolic tangent function. By the use of this relation, we can easily draw the zone mobility-pH curve of migrating species and estimate the consecutive dissociation constants. The relationships between the mobility and the molecular weight or the hydrated ionic radius were also discussed.

JOKL's equation is only applicable to the first dissociation of a migrating substance and the deviation increases with increasing charge number because of the hydration increment. After considering the hydration ratio, we proposed a modification of the equation.

The observed relative zone mobility-pH curves of phosphorus compounds such as phosphorus oxyacids, phenylphosphorus compounds and phosphoric esters of hexoses or inosines were in fair agreement with the calculated ones.

The equation proposed is useful in practice for the estimation of the dissociation constant and/or molecular weight of an unknown sample.

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### INTRODUCTION

Extensive studies<sup>1</sup> on the separation or the theoretical treatment of high polymers have been published. Recent reviews on the electrophoresis of proteins<sup>2</sup> also show the advances in high polymer studies. However, there is very little on the theoretical treatment of low molecular weight compounds.

CONSDEN *et al.*<sup>3</sup> derived a relationship between the mobility and the pH of the background buffer solution, and discussed the separation of two compounds having different  $pK$  values.

By analysing the observed zone mobility-pH curves obtained by paper electrophoresis, a few workers have attempted to estimate the dissociation constants

of weak acids or bases of low molecular weight<sup>4</sup> or the stability constants of metal complexes<sup>5-10</sup>.

Recently JOKL<sup>11</sup> found an interesting and important relationship between the zone mobilities of migrating substances on paper strips and their molecular weights. Improving upon JOKL's results, EDWARD AND WALDRON-EDWARD<sup>12</sup> calculated the mobilities of organic compounds based on their size, shape and number of charges in free solution, and compared the calculated mobilities with the observed ones.

However, no generally available equation for the zone mobility for weak acids of low molecular weight has yet been derived in terms of the many factors such as charge, dissociation constant, molecular weight of migrating substance and pH value of migrating medium. Although a mathematical expression giving the relationship between the zone mobility, dissociation constant and pH has already been derived, we cannot easily connect any zone mobility-pH curve with this particular mathematical form. Hence, we have derived an approximate equation which is useful in practice for analysing zone mobility-pH curves, in terms of dissociation constant, molecular weight, charge and degree of hydration of a given migrating ion. The validity of our equation was tested by drawing the relative zone mobility-pH curves of various phosphorus compounds after correcting for the electroosmotic flow.

#### THEORETICAL

In order to simplify the subsequent discussion, the following assumptions were made: (1) the migrating species are spherical; (2) the  $\zeta$ -potential is neglected because the molecules treated here are not colloidal particles, but ionic species of low molecular weight; (3) ion-pair formation<sup>13</sup> between a migrating anion and other cations in the background solution is negligibly small; (4) the absorptive interaction between the migrating ion and the surface of paper as a supporting medium is negligibly small.

#### *Relationship between zone mobility and dissociation constant*

Let us consider a localized zone consisting of the migrating species in a migrating medium as shown in Fig. 1.

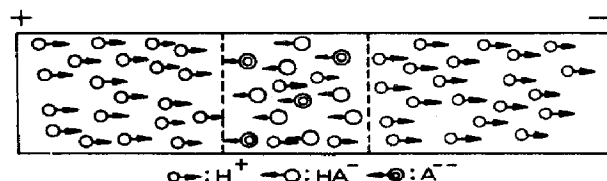


Fig. 1. Equilibrium between protons and anions in a migrating zone.

The concentration of hydrogen ions in the localized zone is constantly equivalent to that in the other parts of the migrating medium (this situation may be easily produced by the use of a suitable buffer solution). When an electric field is applied to this migrating medium as shown in Fig. 1, the ionic species migrate toward each electrode. The anionic species present in the initial localized zone migrate toward the anode while hydrogen ions, present together with the anions, migrate toward the cathode. Consequently, the initial equilibrium between hydrogen ions and anions in the zone is disturbed. However, as hydrogen ions are continuously supplied to the

zone from the rest of the solution, all the migrating species present in the zone will usually maintain the same dynamic equilibrium as the initial one, provided that the exchange reactions as shown in eqn. (1) are very rapid and reversible.



$$\frac{[H_{n-i}A^{-i}][H^+]}{[H_{n-(i-1)}A^{-(i-1)}]} = k_i \quad (i = 1 \sim n) \quad (1')$$

where  $H_{n-i}$  shows the form of the various migrating species and  $k_i$  is the consecutive dissociation constant.

The zone mobility obtained by paper electrophoresis is the weighted mean value of the mobilities of all ion species which are present together in the migrating zone. Thus, the zone mobility  $U$  is given by

$$U = \frac{u_0[H_nA^0] + u_1[H_{n-1}A^{-1}] + \dots + u_i[H_{n-i}A^{-i}] + \dots + u_n[A^{-n}]}{[H_nA^0] + [H_{n-1}A^{-1}] + \dots + [H_{n-i}A^{-i}] + \dots + [A^{-n}]} \quad (2)$$

where  $u_i$  is the mobility of the migrating species  $H_{n-i}A^{-i}$  and  $[H_{n-i}A^{-i}]$  is the molar concentration of  $H_{n-i}A^{-i}$ .

Substitution of eqn. (1') into eqn. (2) yields eqn. (3).

$$U = \frac{u_0 + u_1k_1/[H^+] + u_2k_1k_2/[H^+]^2 + \dots + u_ik_1k_2 \dots k_i/[H^+]^i + \dots + u_nk_1k_2 \dots k_n/[H^+]^n}{1 + k_1/[H^+] + k_1k_2/[H^+]^2 + \dots + k_1k_2 \dots k_i/[H^+]^i + \dots + k_1k_2 \dots k_n/[H^+]^n} \quad (3)$$

Since  $u_0$  corresponds to the mobility of  $H_nA^0$ , it is often omitted<sup>4</sup>. As the calculation of zone mobilities using eqn. (3) is complicated, we attempted to simplify eqn. (3).

If we choose only two successive ion species, that is,  $H_{n-(i-1)}A^{-(i-1)}$  and  $H_{n-i}A^{-i}$  from all the species in the zone, the zone mobility  $U_i$  (a component zone mobility) composed from these species is given by

$$U_i = u_{i-1} \left\{ \frac{1 + \alpha_i k_i/[H^+]}{1 + k_i/[H^+]} \right\} = u_{i-1} \left\{ \frac{1 + \alpha_i}{2} + \frac{1 - \alpha_i}{2} \frac{1 - k_i/[H^+]}{1 + k_i/[H^+]} \right\} \quad (4)$$

where

$$\alpha_i = u_i/u_{i-1} \quad (\alpha_i > 1)$$

Now, when we put

$$k_i/[H^+] = e^{-2y} \quad (6)$$

eqn. (7) is obtained

$$y = \frac{2.303}{2} (pk_i - pH) \quad (7)$$

Furthermore, by combining the well-known mathematical relation, eqn. (8),

$$\frac{1 - e^{-2y}}{1 + e^{-2y}} = \tanh y \quad (8)$$

with eqns. (6) and (7), eqn. (4) is altered to eqn. (9)

$$\begin{aligned} U_i &= u_{i-1} \left\{ \frac{1 + \alpha_i}{2} + \frac{1 - \alpha_i}{2} \frac{1 - e^{-2y}}{1 + e^{-2y}} \right\} \\ &= u_{i-1} \left\{ \frac{\alpha_i + 1}{2} + \frac{\alpha_i - 1}{2} \tanh \left[ \frac{2.303}{2} (\text{pH} - \text{p}k_i) \right] \right\}^* \quad (9) \end{aligned}$$

The zone mobility is schematically represented as a function of pH in Fig. 2A. The first term,  $u_{i-1}(\alpha_i + 1)/2$ , in eqn. (9), shows the distance of the inflexion point of the sigmoidal curve from the pH-axis,  $u_{i-1}(\alpha_i - 1)/2$  in the second term the amplitude of the curve, and  $\text{p}k_i$  the distance of the inflexion point of the curve from the  $U$ -axis, respectively.

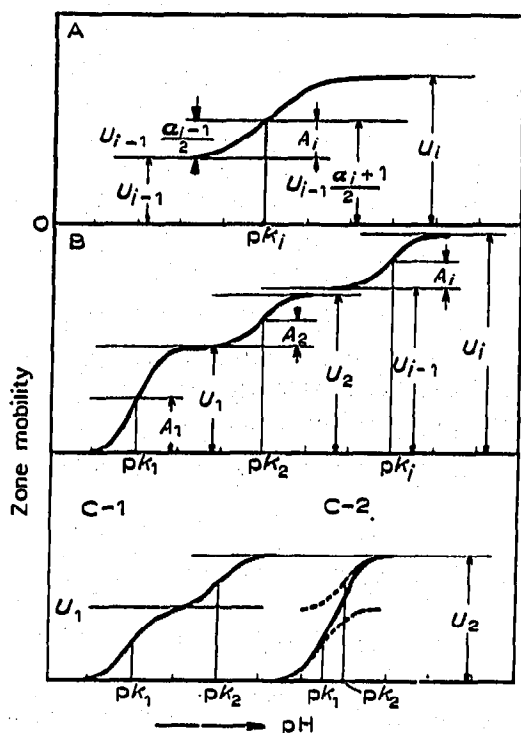


Fig. 2. Schematic zone mobility-pH curves of ionic species with dissociable proton. (A) Component zone mobility-pH curves; (B) zone mobility-pH curve calculated from eqn. (2); (C) zone mobility-pH curve combined with two component curves, C-1 ( $\text{p}k_2 - \text{p}k_1 = 4$ ), C-2 ( $\text{p}k_2 - \text{p}k_1 = 2$ ).

Eqn. (9) shows only one component of the zone mobility resulting from two successive ionic species; the zone mobility resulting from various ionic species in eqn. (3) as a function of pH is shown in Fig. 2B. An equation representing this con-

\* In order to make the term  $(1 - \alpha_i)/2$  positive, the order of pH and  $\text{p}k_i$  in the hyperbolic tangent was reversed.

tinuous  $U$ -pH curve is not the simple sum of eqn. (9) from  $i = 1$  to  $n$ . A modification of the first term must be made.

$$U_{\tanh} = \sum_{i=1}^n \left\{ u_{i-1} \frac{\alpha_i + 1}{2} - u_{i-1} + u_{i-1} \frac{\alpha_i - 1}{2} \tanh \left[ \frac{2.303}{2} (\text{pH} - \text{p}k_i) \right] \right\} \\ = \sum_{i=1}^n \left\{ A_i + A_i \tanh \left[ \frac{2.303}{2} (\text{pH} - \text{p}k_i) \right] \right\} \quad (10)$$

where

$$A_i = u_{i-1} \frac{\alpha_i + 1}{2} - u_{i-1} = u_{i-1} \frac{\alpha_i - 1}{2} = \frac{u_i - u_{i-1}}{2} \quad (11)$$

If the difference between  $\text{p}k_i$  and  $\text{p}k_{i-1}$  is larger than 2,  $U_{\tanh}$  becomes a good approximate value of  $U$  with an error of less than 1.2%.\* In other words, the zone mobility is usually dependent on two main ionic species.

We can easily understand from the schematic diagram the mathematical meaning of the modification in the  $U$ -pH curve in Fig. 2B. The shape of the zone mobility-pH curve for  $i = 1$  to  $i = n$  is a chain of sigmoidal curves. WALDRON-EDWARD<sup>4</sup> treated only one unit curve of the chain shown in Fig. 2B. At each inflexion point of the chain of sigmoidal curves, the second term of eqn. (10) must be zero.

$$\text{pH} - \text{p}k_i = 0 \quad (12)$$

The pH value corresponding to each point of inflexion is equivalent to that of each consecutive dissociation constant  $\text{p}k_i$ .  $A_i$  is easily estimated from eqn. (11) when the pH value of the migrating medium is equal to the  $\text{p}k_i$  of the migrating species. Substituting eqn. (12) into eqn. (10), we obtain the zone mobility at  $\text{pH} = \text{p}k_i$

$$U_{(\text{pH}=\text{p}k_i)} = \frac{u_i + u_{i-1}}{2} \quad (13)$$

In order to make the meaning of the notation clearer, we repeat that  $u$  is the mobility of each ionic species and this is not usually observable, while  $U$  represents the zone mobility which is observable in any case. In the case where all the ions present in the system are of the same ionic species,  $U$  is equal to  $u$ . Accordingly, in the precise discussion of the radius or molecular weight of migrating ions given later  $u$  must be used.

When the difference between two consecutive dissociation constants is less than 4 in  $\text{p}k$  value, the two sigmoidal curves superimpose as shown in Fig. 2C-2. In such a case, neither in the inflexion points nor the mobility of the ionic species  $u$  can easily be determined from the zone mobility-pH curve.

#### *Relationship between mobility and ionic radius*

If Stokes' law is applied to the migrating species, the mobility  $u_i$  of the ionic species  $\text{H}_{n-i}\text{A}^{-i}$  is given by

$$u_i = \frac{Z_i e}{6\pi\eta r_i 300} = k \frac{Z_i}{r_i} \quad (14)$$

\* When  $0.5 < \text{p}k_i - \text{p}k_{i-1} < 1$ ,  $5.1 > [(U_{\tanh} - U)/U] \times 100 > 3.8$

where  $e$  is the electrostatic unit,  $Z_t$  the charge of the migrating ionic species  $H_{n-t}A^{-t}$ ,  $r_t$  the radius of the species and  $\eta$  the viscosity of the migrating medium. From eqn. (15), we can evaluate the relative radius of the migrating species  $H_{n-t}A^{-t}$  relative to the species  $H_{n-(t-1)}A^{-(t-1)}$ .

$$R_t = \frac{r_t}{r_{t-1}} = \frac{Z_t}{Z_{t-1}} \frac{u_{t-1}}{u_t} = \frac{Z_t}{Z_{t-1}} \frac{1}{\alpha_t} \quad (15)$$

As the loss of two or three hydrogen atoms in a molecule does not have much effect in varying the size of the migrating species when the molecular weight is above 50,  $R_t$  is almost unity. Accordingly, the mobility ratio  $\alpha_t$  is equal to the ratio of the charge number of  $Z_t$  to  $Z_{t-1}$ . On the other hand, the degree of hydration of an ionic species in aqueous solution generally increases with the increment of the charge number of the species. Thus, in the case of the ionic species in aqueous solution it is assumed that the actual  $R_t$  (hydrated radius) will be greater than unity. Although EDWARD AND WALDRON-EDWARD<sup>12</sup> estimated the radii of organic ions by using the van der Waals radii of the component atoms, this treatment cannot be applied to mobilities of hydrated ions, especially inorganic ions of low molecular weight.

Since there is no satisfactory method, in a mixed system of various ion species, of measuring the hydrated radii of the ions, we compared  $u_t$  with  $u_{t-1}$  in order to obtain some useful knowledge about the hydration. Eqn. (16) is a modification of eqn. (15).

$$\frac{u_t}{u_{t-1}} = \frac{Z_t}{Z_{t-1}} \frac{r_{t-1}}{r_t} = \frac{Z_t}{Z_{t-1}} \frac{r_{t-1}}{r_{t-1} + \Delta r_t} \quad (16)$$

where  $\Delta r_t$  represents the increment of the hydrated radii of  $H_{n-(t-1)}A^{-(t-1)}$  caused by an increment of unit charge. Thus, from eqn. (16), we can derive an incremental ratio for the hydrated radius as follows;

$$\frac{\Delta r_t}{r_{t-1}} = \frac{Z_t}{Z_{t-1}} \frac{u_{t-1}}{u_t} - 1 = \frac{Z_t}{Z_{t-1}} \frac{1}{\alpha_t} - 1 \quad (17)$$

The incremental ratio ( $\Delta r_t/r_{t-1}$ ) of hydrated radius may be estimated from eqn. (17) in which  $Z_{t-1}$  and  $Z_t$  correspond to the charge number of  $H_{n-(t-1)}A^{-(t-1)}$  and  $H_{n-t}A^{-t}$  ions, respectively, while  $\alpha_t$  is obtained experimentally. Further discussion of eqn. (17) and the degree of hydration is given in the experimental part.

#### *Relationship between mobility and molecular weight*

JOKL<sup>11</sup> derived a relation between zone mobilities of migrating species and their molecular weights based on theoretical considerations and experimental results. The equation is

$$\frac{U}{Z} = a \frac{1}{\sqrt{m}} + b \quad (18)$$

where  $U$  is the zone mobility,  $Z$  the charge number,  $m$  the molecular weight of the migrating species, and  $a$  and  $b$  are constants. Since  $U/Z$  in eqn. (18) shows the mean

mobility per unit charge of ionic species, the variation of ionic radius with hydration, caused by increasing the charge, is not considered. Although the validity of eqn. (18) was tested for many organic compounds and proved, experiments on ionic species which have the same kind of molecule and only differ in the number of charges were not made.

In general, as the degree of hydration of ions depends on the kind of ion and the number of charges, the expression of equivalent mobility  $U/Z$  is not applicable.

If the effects of hydration on the mobility are taken into account, eqn. (18) should be changed into eqn. (19).

$$u_i = \left( a_i \frac{1}{\sqrt{m}} + b \right) Z_i \quad (19)$$

with

$$a_{i-1} > a_i \quad (19')$$

where  $a_i$  is a constant for the ionic species with the given dissociation number  $i$ , and  $b$  is a constant depending only on the migrating conditions used. From eqns. (11) and (19), eqn. (20) can be obtained

$$A_i = \frac{Z_i a_i - Z_{i-1} a_{i-1}}{2} \frac{1}{\sqrt{m}} \quad (20)$$

with

$$A_{i-1} > A_i \quad (20')$$

Substitution of eqn. (20) into eqn. (10), gives eqn. (21).

$$U = \sum_{i=1}^n \frac{Z_i a_i - Z_{i-1} a_{i-1}}{2} \frac{1}{\sqrt{m}} \left\{ 1 + \tanh \left[ \frac{2.303}{2} (\text{pH} - \text{p}k_i) \right] \right\} \quad (21)$$

When  $a_i$  is equal to  $a_{i-1}$ , eqn. (21) is simplified to

$$U = \frac{1}{2} \sum_{i=1}^n \frac{a_i}{\sqrt{m}} \left\{ 1 + \tanh \left[ \frac{2.303}{2} (\text{pH} - \text{p}k_i) \right] \right\} \quad (22)$$

## EXPERIMENTAL

### Apparatus

The apparatus for the determination of the migration pattern has been described in a previous paper<sup>14</sup>. The essential part of the equipment was the arrangement of the cathode and the anode, which consisted of a series of chambers, each of which is filled with a background electrolyte (50 ml) of different pH value. All ten chambers had a platinum wire electrode, and a diaphragm plate to prevent a change of pH value of migrating medium (paper strip) during migration. The center of the migration chamber was filled with carbon tetrachloride. Each of the ten anodic chambers was connected with the corresponding cathodic chamber by a strip of Toyoroshi No. 50 paper strip (size 2 × 40 cm), onto which a flowing-water jacket (glass tube) was fixed. The cooling water was circulated through the jacket by a commercial cooling

apparatus to keep the temperature constant, with a deviation of  $\pm 1^\circ$ . The potential gradient was 500 V/34 cm, the duration of electromigration 1–1.5 hours, and the total current intensity amounted to about 30–40 mA.

#### *Substances investigated*

Among the substances studied were the following: orthophosphoric acid,  $H_3PO_4$ ; phosphite,  $Na_2HPO_3$ ; hypophosphite,  $NaH_2PO_2$ ; pyrophosphate,  $Na_4P_2O_7$ ; phenylphosphinic acid,  $C_6H_5P(=O)H(OH)$ ; phenylphosphonic acid,  $C_6H_5P=O(OH)_2$ ; inosine-5'-phosphate; inosine-5'-diphosphate; inosine-5'-triphosphate; glucose-1-phosphate; glucose-6-phosphate; fructose-6-phosphate; fructose-1,6-diphosphate. All the substances were prepared in a concentration of 0.05–0.1 M.

#### *Background buffer solution*

The background buffer solution plays an important role in electrophoresis. For example, MAZZEI AND LEDERER<sup>13</sup> recently reported ion-pair formation between the migrating substance and the buffer electrolyte. In our experiments, to lessen the interaction between the phosphorus compound and buffer electrolyte as far as possible, hydrochloric acid, ammonium acetate and aqueous ammonia were used. Background buffer solutions of different pH values were prepared by mixing 0.1 M hydrochloric acid, 0.1 M ammonium acetate and 0.1 M aqueous ammonia. The ionic strength of the solution ( $\mu$ ) was 0.1.

#### *Procedure*

First a paper strip was uniformly dipped in the background buffer solution. The excess of the solution in the paper was removed with another filter paper, then about 2–5  $\mu$ l of the sample solution was placed on the starting line marked on the strip. The width of the sample solution placed on the strip was 1–2 mm. After setting up the ten strips of paper in the migrating chamber, and the water-cooled jacket, a constant stabilized voltage (500 V) was applied between the two electrodes. After migration, the strips were taken out of the chambers, and dried as quickly as possible by an infrared lamp to prevent spreading of the migrating zone.

#### *Detection of zone mobility of phosphorus compounds*

Although oxyacids of phosphorus are usually localized by the molybdenum-blue method, in the present method this was carried out by exposure of the electropherogram to neutrons followed by scanning with a GM-counter or by autoradiography. For reactor irradiation, the dried strip was rolled up with a sheet of polyethylene. The rolled papers were wrapped in another sheet of polyethylene, put into a pneumatic capsule and irradiated in a pneumatic tube of KUR\* for 30 min at a neutron flux of  $5 \cdot 10^{12}n/cm^2 \cdot sec$ . After removal from the reactor, the paper was allowed to "cool" for some 5–10 days to reduce the radioactive background of the paper strip itself. Then the strip was autoradiographed, using Fuzi medical X-ray film. Exposure time was 2–3 days.

#### *Correction for electroosmotic transport*

In this experiment, the observed movement of the phosphorus compound was

\* Kyoto University Reactor.



corrected for electroosmotic transport which was determined with a separate zone of hydrogen peroxide as a flow indicator<sup>16</sup>. The electroosmotic flow indicated by hydrogen peroxide varied with the pH of the background buffer solution and was very small below pH = 4.<sup>16</sup> The maximum of the flow was about 1.5 cm under given conditions (potential gradient: 500 V/34 cm; migration time: 60 min at 20°). The uncorrected actual movements of hypophosphorous, phosphorous and phosphoric acids are shown by solid lines in Fig. 3 while the dotted lines show the movements of the phosphorus compounds corrected for electroosmotic transport. The relative mobility ( $U_p$ ) of each compound was calculated from the corrected mobility (see the following section).

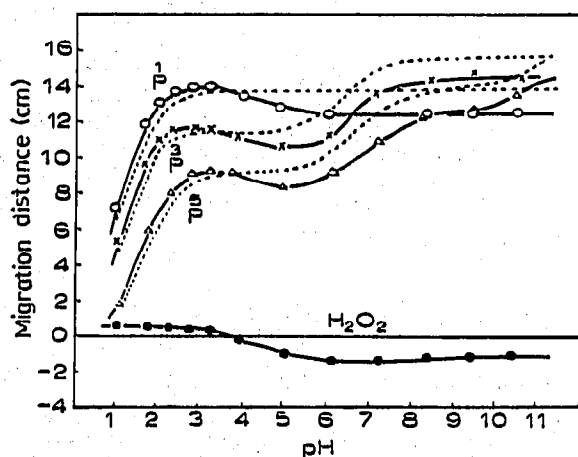


Fig. 3. Correction for electroosmotic transport. (—) observed migration distance; (----) corrected migration distance; (—●—) electroosmotic transport of  $H_2O_2$ .  $\overset{1}{P} = H_3PO_2$ ;  $\overset{3}{P} = H_3PO_3$ ;  $\overset{5}{P} = H_3PO_4$ .

#### APPLICATION OF THE THEORETICAL EQUATION TO PHOSPHORUS COMPOUNDS

In the theoretical part, we derived a relationship between the mobility, molecular weight, charge number and dissociation constant at different pH values of the migrating medium. If all the consecutive dissociation constants and the mobilities of the migrating species are known, we can easily draw a zone mobility-pH curve based on eqns. (10) and (11). The drawing of a  $U_p$ -pH curve using the original relation eqn. (3) is a laborious task.

In order to estimate approximately the mobility of a migrating species having a known molecular weight, we can use JOKL's empirical equation<sup>9</sup> which was derived from the measurement of the relative zone mobility ( $U_t$ ) of forty organic and twenty metal complex ions on Whatman No. 1 paper

$$U_t = \left( 14.7 \frac{1}{\sqrt{m}} - 0.29 \right) Z \quad (23)$$

with tetraethylammonium ion as a standard.

As phosphorus compounds are being studied in this experiment, we used hypo-

phosphorous acid as a standard because it has only one inflexion point and shows constant mobility over the range of  $\text{pH} = 2-13$ . When using the hypophosphorous anion as a standard, eqn. (23) can be altered to eqn. (24)

$$U_p = (7.75 \frac{I}{\sqrt{m}} - 0.12) Z_1 \quad (24)$$

The relative mobilities  $U_p$  are listed in Table I.

Substituting the values of the mobility  $u_1$ ,  $\alpha$  (arbitrary) and the known dissociation constant  $\text{pk}$  given in Table I, into eqn. (10), we can draw the calculated zone mobility-pH curve of each of the oxyacids of phosphorus as shown in Fig. 4A-E.

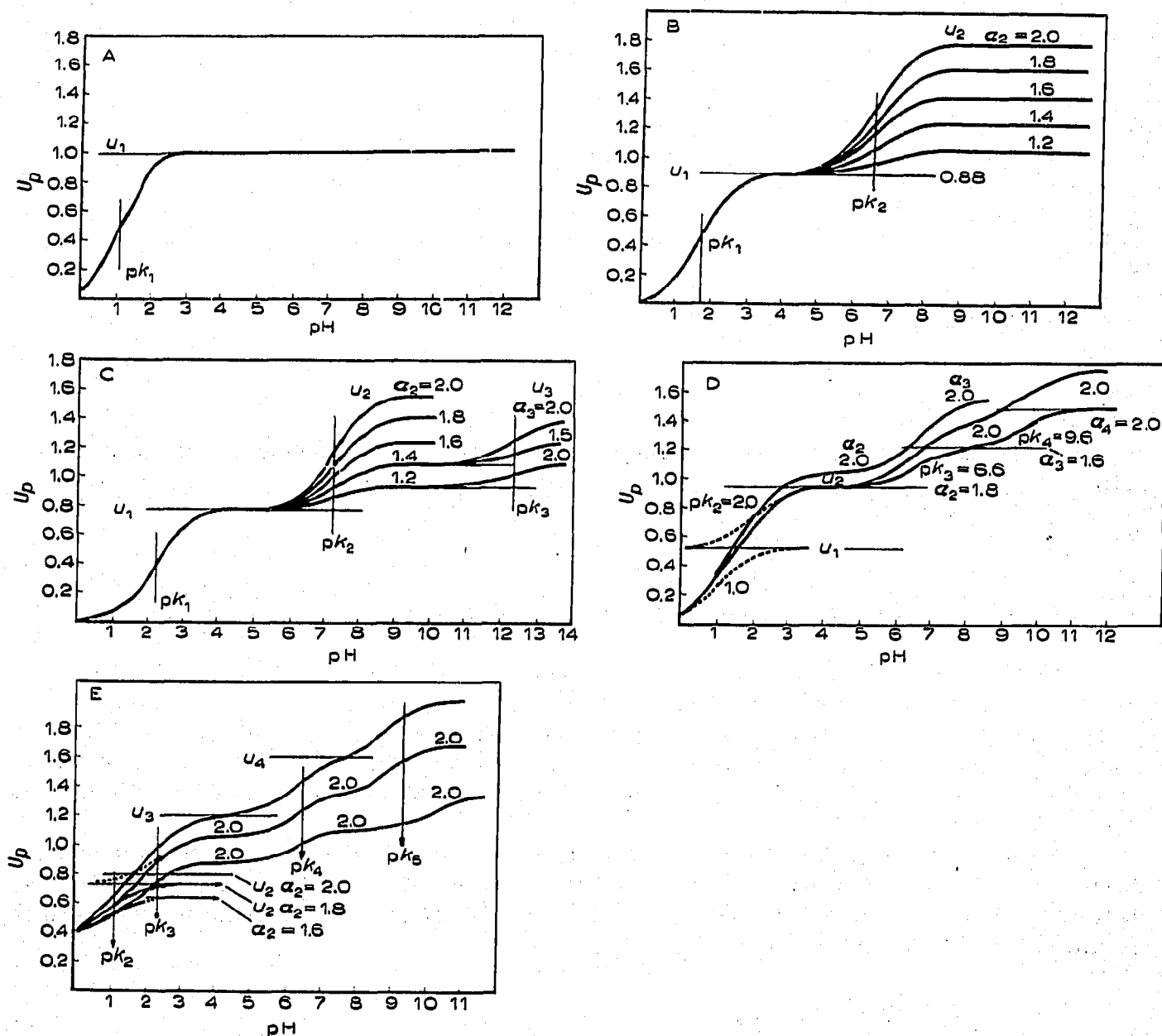


Fig. 4. Calculated zone mobility-pH curves of phosphorus oxyacids. (A) Hypophosphorous acid ( $\text{P}^{\text{I}}$ ); (B) phosphorous acid ( $\text{P}^{\text{III}}$ ); (C) phosphoric acid ( $\text{P}^{\text{V}}$ ); (D) pyrophosphoric acid ( $\text{P}_2^{\text{V}}$ ); (E) triphosphoric acid ( $\text{P}_3^{\text{V}}$ ).  $\alpha$  in the figure is an arbitrary value!

In the case of univalent anions, the calculated mobilities were in fairly good agreement with the observed values. However, as we pointed out in the theoretical part, the observed mobility of a bivalent anion  $u_2$  or a trivalent anion  $u_3$  was not twice or thrice that of  $u_1$ . Thus, we introduced a parameter  $\alpha_i$  which changed with the degree of hydration. If the hydration of the migrating species is not changed by an increment in their charge numbers, the mobility of the bivalent anion must be twice that of the univalent anion, and the parameter  $\alpha_2$  must be 2. The  $\alpha_2$  values, as shown in Table I, were usually lower than 2.

Fig. 5 shows the observed relative zone mobility-pH curves of inorganic oxyacids of phosphorus. The dotted lines in Fig. 5 show the calculated curves. The experimental curves were in agreement with those calculated with some of the parameters  $\alpha_2$  and  $\alpha_3$ , which are given in Table I and in Fig. 4. But there are a few differences between the two curves at low pH values of the background buffer solution. Even if the paper strip used as migrating medium was cooled to keep the temperature constant, the temperature of the paper itself, which contains a high concentration of hydrogen ions, was not quickly equilibrated with that of the cooling medium and so has a higher temperature than that observed. This error may be minimized by the use of a lower voltage (below 250 V). The mobilities obtained at lower pH must be corrected.

The  $pK$  values of the inflexion points of  $U_p$ -pH curves were in agreement with data measured by the other conventional methods as shown in Table I. Unknown dissociation constants of compounds were determined by drawing the observed  $U_p$ -pH curves.

The observed  $U_p$ -pH curves of phenylphosphinic and phenylphosphonic acids are shown in Fig. 6. By comparing two curves, we determined the chemical form of phenylphosphinic acid<sup>14</sup>. This treatment can be applied to other compounds.

Figs. 7 and 8 show the observed  $U_p$ -pH curves of phosphoric esters of glucose, fructose and inosines. By analysing these curves, we can determine the dissociation constant of the phosphoric ester, the molecular weight or the chemical form.

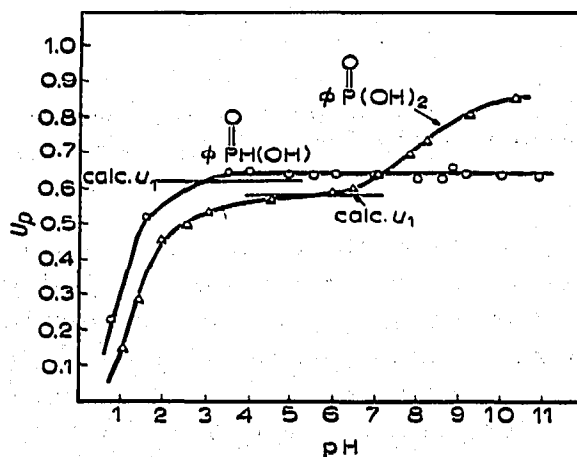
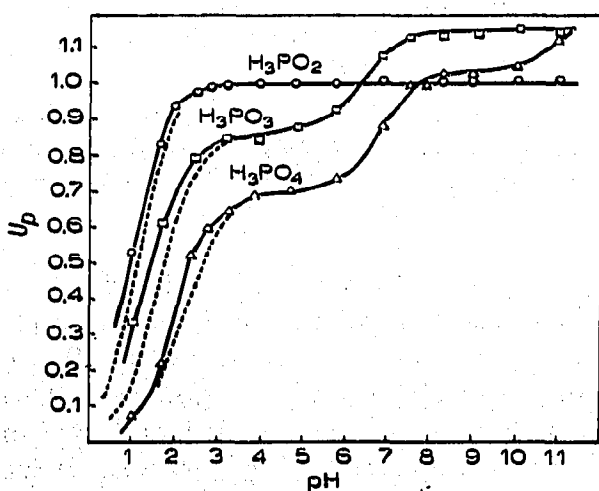


Fig. 5. Observed zone mobility-pH curves of inorganic oxyacids of phosphorus. Dotted lines: corrected curves. Migration conditions: 500 V/34 cm;  $20 \pm 1^\circ$ ;  $\mu = 0.1$ ; 1 h.

Fig. 6. Observed zone mobility-pH curves of phenyl-phosphinic and phenylphosphonic acids.

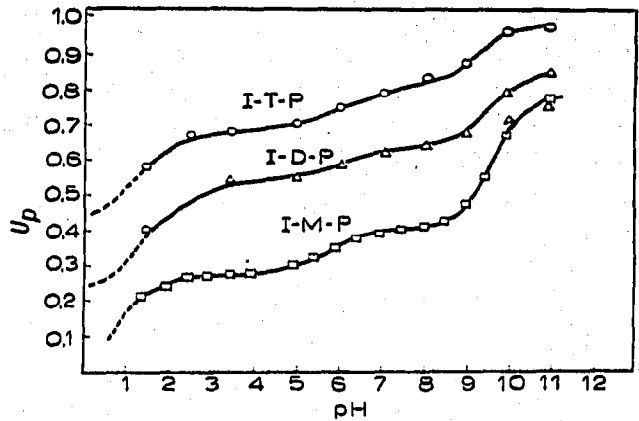
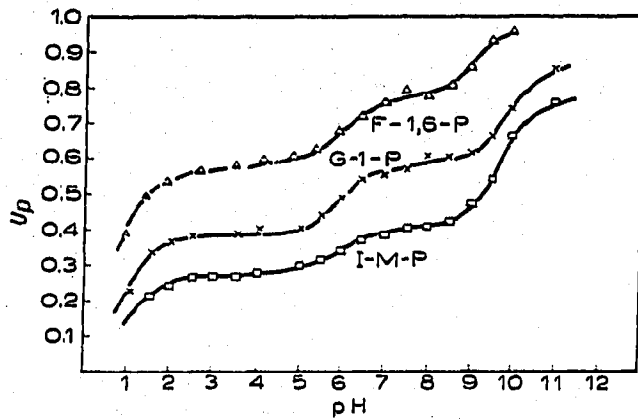


Fig. 7. Observed zone mobility-pH curves of phosphoric esters of hexoses. G-1-P = glucose-1-phosphates F-1,6-P = fructose-1,6-diphosphate; I-M-P = inosine-5'-monophosphate. The observed zone mobilities of fructose-6-phosphate (F-6-P) and glucose-6-phosphate (G-6-P) are similar to that of G-1-P.

Fig. 8. Observed zone mobility-pH curves of phosphoric esters of inosines. I-M-P = inosine-5'-monophosphate; I-D-P = inosine-5'-diphosphate; I-T-P = inosine-5'-triphosphate.

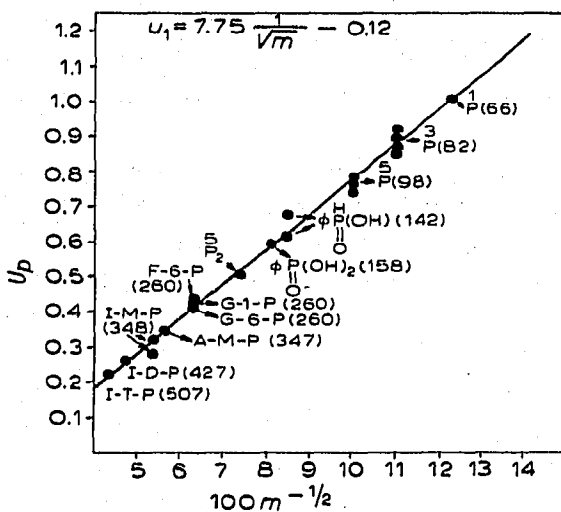


Fig. 9. Relationship between the relative mobility of monoanionic species of phosphorus compounds and molecular weight. The numbers in parentheses are the molecular weights of the monoanions.

Fig. 9 shows the comparison of the relative zone mobility observed for  $i = 1$  with that calculated from eqn. (24). Judging from the fact that the deviation from a straight line was small, we can consider that  $a_1$  and  $b$  are nearly constant for all the phosphorus compounds. In other words, if a compound similar to the compound in question is used as a standard, complex factors such as obstruction and absorption by the filter paper affecting the mobility could be compensated. Furthermore, if we know the relative mobility of a phosphorus compound, the molecular weight can be approximately evaluated from eqn. (24).

When the ratio  $\Delta r_i/r_{i-1}$  was calculated from eqn. (17), the values of  $\Delta r_2/r_1$  were within 0.38-0.49 over the range of molecular weight = 66-347. This shows that the

TABLE I

RELATIVE ZONE MOBILITIES AND DISSOCIATION CONSTANTS OF PHOSPHORUS COMPOUNDS

Substance	$u_1$		$u_2$ Obs.	$\alpha_2$ Obs.	$\Delta r_2/r_1$ Obs.	$pk_1(\text{Obs.})$				$pk_1(\text{Ref.})$				Ref. No.	
	Calcd.	Obs.				1	2	3	4	1	2	3	4		
H <sub>3</sub> PO <sub>2</sub> <sup>a</sup>	1.00	1.00				1.1					1.1				17
											1.1				18
H <sub>3</sub> PO <sub>3</sub>	0.88	0.86	1.15	1.34	0.49	1.3	6.6				1.1	6.7			17
											1.3	6.5			18
H <sub>3</sub> PO <sub>4</sub>	0.78	0.73	1.04	1.42	0.41	2.1	7.1				2.2	7.2	12.3		19
											2.1	7.1	12.3		17
H <sub>4</sub> P <sub>2</sub> O <sub>7</sub>	0.52	(0.41) <sup>b</sup>	0.81	1.62 <sup>c</sup>	0.23 <sup>c</sup>	1.0	2.0	6.6	9.6		1.0	1.9	6.6	9.6	19
											1.0	2.0	6.6	9.6	17
C <sub>6</sub> H <sub>5</sub> P=O.H(OH)	0.62	0.65				1.1									
C <sub>6</sub> H <sub>5</sub> P=O(OH) <sub>2</sub>	0.58	0.60	0.81	1.35	0.48	1.5	7.5								
G-1-P	0.40	0.39	0.57	1.46	0.47	1.2	6.4				1.1	6.1			20
											1.2	6.5			21
F-6-P	0.40	0.41	0.58	1.41	0.42	1.0	6.2				1.0	6.1			22
F-1,6-P	0.35	(0.29)	0.58				0.9		6.2					6.3	23
I-M-P	0.32	0.29	0.42	1.45	0.38	1.2	6.0				2.4	6.4			24
I-D-P	0.26	(0.27)	0.54	1.22 <sup>c</sup>	0.22 <sup>c</sup>	—	1.2								
							~ 1.6								
I-T-P	0.22	(0.23)	0.70 <sup>d</sup>	1.14 <sup>d</sup>	0.17 <sup>d</sup>	—	—			1.2					
										~ 1.6					

<sup>a</sup>  $u_1$  of H<sub>3</sub>PO<sub>2</sub> is a standard.<sup>b</sup> The number in parentheses shows the equivalent mobility.<sup>c</sup> Values of  $\alpha_3$  and  $\Delta r_3/r_2$ .<sup>d</sup> Values of  $u_3$ ,  $\alpha_4$  and  $\Delta r_4/r_3$ .

radius of a bivalent anion is larger than that of a univalent anion by about 40%. The ratio  $\Delta r_3/r_2$  obtained from the zone mobility of pyrophosphate or inosine-5'-diphosphate was about 0.2. The ratio  $\Delta r_4/r_3$  of inosine-5'-triphosphate was 0.17.

From these results, we concluded that the increase of the radius of the migrating species decreased with increasing charge and did not largely depend on the kind of migrating substance.

All the theoretical and experimental results of zone mobility, parameter  $\alpha$ , increment ratio of ionic radius and dissociation constant are listed in Table I. The parentheses in Table I show the equivalent zone mobility because the zone mobility of univalent anions was not determined experimentally.

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