

SEPARATION OF LOWER OXO ACIDS OF PHOSPHORUS BY GRADIENT ELUTION CHROMATOGRAPHY USING AN ANION EXCHANGE RESIN

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INTRODUCTION

A scheme of gradient elution for anion-exchange chromatography, first developed by GRANDE AND BEUKENKAMP¹, has been applied to the separation of lower oxo acids of phosphorus by POLLARD and his coworkers^{2,3}. However, no theoretical basis has so far been given for the separation of these acids. RIEMAN *et al.*⁴ have derived equations for the positions of the elution peaks, from the gradient elution theory studied by ALM *et al.*⁵, and employing chloride, bromide, and oxalate ions, obtained good agreement between theory and practice, although only univalent and bivalent ions have been taken as examples. The authors have attempted (1) to establish anion exchange sorbabilities and charges for these phosphate species in neutral ammonium acetate solution, and (2) to find a general method to predict the position of the elution peaks in gradient elution from batch equilibration data. POLLARD *et al.*^{2,3} used a mixed aqueous solution of potassium chloride and ammonium acetate as an eluent. The use of eluent containing only ammonium acetate seems more convenient, because it is easily removed from phosphate species by heating the effluent after elution. Furthermore, theoretical treatment of chromatography may also become easier than when a mixed eluent is used.

THEORY

The device with a constant volume mixing bottle is sketched in Fig. 1. Connections A and B are initially filled with the reservoir solution and the eluent of the mixing bottle, respectively, small volumes of liquids remaining on the top or at the end of the resin bed while the elution takes place can be neglected in calculations. A sorption band peak moves down with changing rate due to the concentration gradient of the eluent. If v (defined in a convenient dimension as ml resin bed per ml eluent) is a velocity of the peak at a particular concentration of eluent, with which the sorption peak contacts momentarily, the distance L (in ml resin bed) which the peak moved is expressed as a function of the flow volume V of eluent as follows:

$$L = \int_0^V v dV = \int_0^{V_b} (1/D_0) dV + \int_{V_b}^V (1/D) dV \quad (1)$$

where D is the volume distribution ratio of the sample ion for a concentration C of

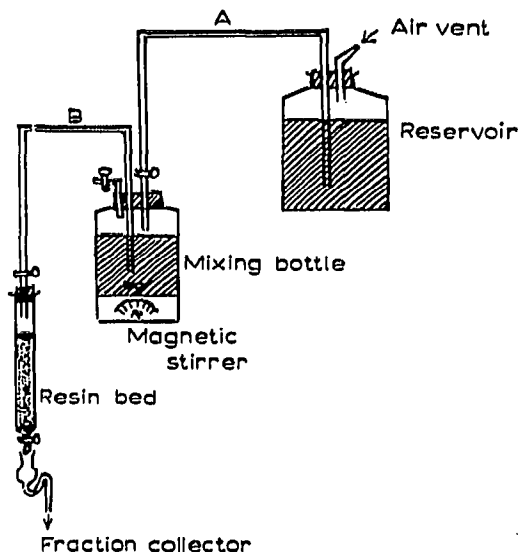


Fig. 1. Device with constant-volume mixing bottle for gradient elution. A and B, see text.

eluent, D_0 is the ratio for the initial concentration C_0 of the eluent in the mixing bottle, and V_b is the volume of the solution in the tube B. These D values are obtained from batch equilibration data. The first term on the right side of eqn. (1) corresponds to constant concentration elution from glass tube B initially filled with V_b ml of the eluent of concentration C_0 . The concentration C of the eluent when V ml have flowed through the column is denoted as follows:

$$C = C_r - (C_r - C_0) \exp \{-(V - V_b)/V_m\} \quad (2)$$

where C_r is the concentration of the eluent in the reservoir and V_m is the volume of the solution in the mixing bottle.

In most cases of simple ions, *i.e.*, when neither complexation nor polymerization occurs over a whole range of eluent concentration, the following relation holds between D and C ,

$$D = aC^{-n} \quad (3)$$

where a and n are constants. The same type of equation can be derived by the ideal treatment of DONNAN's equations. Thus, eqn. (1) becomes

$$L = (V_b/D_0) + (C_r^n/a) \int_{V_b}^V [1 - \alpha \exp \{-(V - V_b)/V_m\}]^n dV \quad (4)$$

where α is $(C_r - C_0)/C_r$. When L reaches the resin bed volume S , the solute concentration in the effluent becomes maximum.

The effluent volume V_{\max} of this point is substituted in eqn. (4), then:

$$\{S - (V_b/D_0)\} (a/C_r^n) = \int_{V_b}^{V_{\max}} [1 - \alpha \exp \{-(V - V_b)/V_m\}]^n dV \quad (5)$$

When n is an integer, eqn. (5) can be developed as follows:

$$D_r \{S - (V_b/D_0)\} = V_{\max} - V_b + V_m \sum_{j=1}^n (-1)^j n C_j (1/j) \alpha^j [1 - \exp \{-j(V_{\max} - V_b)/V_m\}] \quad (6)$$

where D_r is the distribution ratio for the reservoir concentration C_r , and the symbol \mathbf{C} means combination. Or in another form,

$$D(C/C_r)^n \{S - (V_b/D_0)\} = V_{\max} - V_b + V_m \sum_{j=1}^n (-1)^j n C_j (1/j) \alpha^j [1 - \exp \{-j(V_{\max} - V_b)/V_m\}] \quad (7)$$

If V_b/D_0 is very small compared with S , eqns. (6) and (7) are somewhat simplified as follows:

$$D_r S \text{ or } D(C/C_r)^n S = V_{\max} + V_m \sum_{j=1}^n (-1)^j n C_j (1/j) \alpha^j \{1 - \exp(-jV_{\max}/V_m)\} \quad (8)$$

When pure water is used as an initial eluent in the mixing bottle,

$$C_0 = 0 \text{ and } D_0 = \infty, \text{ and } D_r S \text{ or } D(C/C_r)^n S = V_{\max} - V_b + V_m \sum_{j=1}^n (-1)^j n C_j (1/j) [1 - \exp \{-j(V_{\max} - V_b)/V_m\}] \quad (9)$$

In eqns. (5) to (9), S , V_m , α and V_b are constant for a given elution system, and D_r and n are considered constant for the ion. For each n value (integer), eqn. (6) can be expanded as follows:

$$n = 0: D_r \{S - (V_b/D_0)\} = V_{\max} - V_b \quad (10)$$

$$n = 1: D_r \{S - (V_b/D_0)\} = V_{\max} - V_b - V_m \alpha [1 - \exp \{- (V_{\max} - V_b)/V_m\}] \quad (11)$$

$$n = 2: D_r \{S - (V_b/D_0)\} = V_{\max} - V_b - 2V_m \alpha [1 - \exp \{- (V_{\max} - V_b)/V_m\}] + (1/2)V_m \alpha^2 [1 - \exp \{- 2(V_{\max} - V_b)/V_m\}] \quad (12)$$

$$n = 3: D_r \{S - (V_b/D_0)\} = V_{\max} - V_b - 3V_m \alpha [1 - \exp \{- (V_{\max} - V_b)/V_m\}] + (3/2)V_m \alpha^2 [1 - \exp \{- 2(V_{\max} - V_b)/V_m\}] - (1/3)V_m \alpha^3 [1 - \exp \{- 3(V_{\max} - V_b)/V_m\}] \quad (13)$$

etc.

Although these equations are implicit with respect to V_{\max} , they can readily be solved graphically. Typical curves are shown in Fig. 2. In preparing these curves values of $D_r S$ are calculated by inserting proper values of V_{\max} into eqn. (8) with

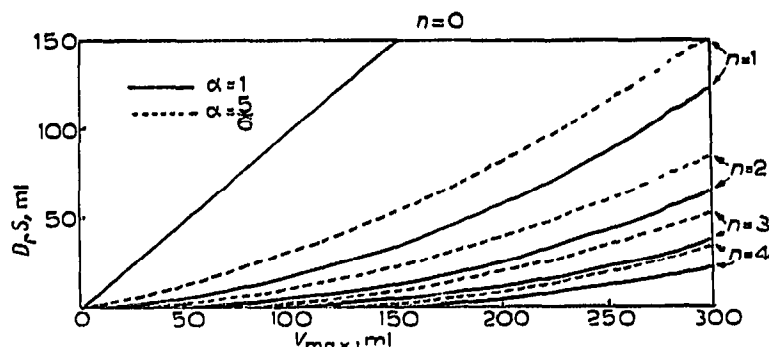


Fig. 2. Relation between V_{\max} and $D_r S$ calculated from eqn. (8). $V_{\max} = 250$ ml.

fixed conditions for the other factors. In most cases this procedure is not very difficult.

When n is not an integer, interpolation should be made between two curves. In any case, batch data are necessary to predict behavior in gradient elution. An experimental value of n should be used for the calculation, because experience shows that even simple ions give non-integral n values because of the partial dissociation or activity coefficient problems.

The distribution ratio can be expressed in the following form,

$$D = \{[P^0] + [P^{1-}] + \cdots + [P^{b-}]\} / \{(P^0) + (P^{1-}) + \cdots + (P^{a-})\} \quad (14)$$

where $[]$ and $()$ represent the concentrations in the resin and the solution, respectively' and the number superscribed on P represents the negative charge of each phosphate species. The highest negative charge is represented by $a-$ or $b-$. For example, P^0 is a neutral acid molecule and P^{1-} means an anion produced by removing one proton from P^0 , etc. The dissociation constant (concentration basis) is defined for an i -charged ion as,

$$k_i = (P^{i-}) (H^+)^i / (P^0) \text{ or } (P^{i-}) / (P^0) = k_i / (H^+)^i \quad (15)$$

DONNAN'S equilibrium gives the following equations for each combination of positive and negative ions,

$$[NH_4^+]^i [P^{i-}] = K_1 (NH_4^+)^i (P^{i-}) \quad (16)$$

$$[NH_4^+] [OAc^-] = K_2 (NH_4^+) (OAc^-) \quad (17)$$

where K_1 and K_2 are activity coefficient quotients. From eqns. (16) and (17),

$$[P^{i-}] / (P^{i-}) = K'_i [OAc^-]^i / (OAc^-)^i \quad (18)$$

where K'_i equals the ratio of K_1 to K_2^i . From eqns. (14), (15) and (18),

$$D = \bar{K} [K'_0 + \{k'_1 / (OAc^-)\} + \{k'_2 / (OAc^-)^2\} + \cdots + \{k'_b / (OAc^-)^b\}] \quad (19)$$

where

$$\bar{K} = 1 / [1 + \{k_1 / (H^+)\} + \{k_2 / (H^+)^2\} + \cdots + \{k_a / (H^+)^a\}] = \text{const.}$$

$$k'_i = K'_i \{k_i / (H^+)^i\} [OAc^-]^i = \text{const.}$$

Eqn. (19) can be rewritten as follows:

$$\begin{aligned} \ln D &= \ln \bar{K} + \ln [K'_0 + \{k'_1/(\text{OAc}^-)\} + \{k'_2/(\text{OAc}^-)^2\} + \cdots + \\ &+ \{k'_b/(\text{OAc}^-)^b\}] \\ (\partial \ln D)/\{\partial \ln (\text{OAc}^-)\} &= \\ &= [-\{k'_1/(\text{OAc}^-)^2\} - \{2k'_2/(\text{OAc}^-)^3\} - \cdots - \{bk'_b/(\text{OAc}^-)^{b+1}\}] (\text{OAc}^-)/[K'_0 + \\ &+ \{k'_1/(\text{OAc}^-)\} + \{k'_2/(\text{OAc}^-)^2\} + \cdots + \{k'_b/(\text{OAc}^-)^b\}] \end{aligned} \quad (20)$$

Therefore,

$$\begin{aligned} (\partial \log D)/\{\partial \log (\text{OAc}^-)\} &= -\{[P^{1-}] + 2[P^{2-}] + \cdots + b[P^{b-}]\}/\{[P^0] + \\ &+ [P^{1-}] + \cdots + [P^{b-}]\} \equiv \bar{i} \end{aligned} \quad (21)$$

In eqn. (21) \bar{i} is the mean charge of the phosphate species in this medium. The values D in ammonium acetate solutions of various concentrations can be determined experimentally. Then, the \bar{i} is equal to the slope n of the curve obtained by plotting $\log D$ versus $\log (\text{OAc}^-)$, assuming that activity coefficient problems can be ignored.

EXPERIMENTAL

Resin

The anion exchange resin Dowex 1-X8 (100 ~ 200 mesh) was converted to the acetate form before use. Resins were roughly air-dried under suction before weighing. Slight variations in the resin moisture content in separate series do not cause any serious errors in calculations. The conversion factor between the resin bed volume and the resin gravity (air-dried) were determined experimentally.

Reagents

For the separation of lower oxo acids of phosphorus, ammonium acetate solutions of various concentrations were used as eluents. The phosphates employed in this investigation were as follows: sodium salt of $\overset{1}{P}$ -acid (hypophosphite) $\text{NaPH}_2\text{O}_2 \cdot 2\text{H}_2\text{O}$; disodium salt of $\overset{3}{P}$ -acid (phosphite) $\text{Na}_2\text{PHO}_3 \cdot 5\text{H}_2\text{O}$; disodium salt of $\overset{3}{P}$ -O- $\overset{3}{P}$ -acid (pyrophosphite) $\text{Na}_2\text{P}_2\text{H}_2\text{O}_5$; trisodium salt of $\overset{3}{P}$ - $\overset{4}{P}$ -acid $\text{Na}_3\text{P}_2\text{HO}_5 \cdot 12\text{H}_2\text{O}$; hexapotassium-sodium salt of $(-\overset{3}{P}-)_6$ -ring acid $[\text{K}(\text{Na})\text{PO}_2]_6 \cdot x\text{H}_2\text{O}$; pentasodium salt of $\overset{4}{P}$ - $\overset{3}{P}$ - $\overset{4}{P}$ -acid $\text{Na}_5\text{P}_3\text{O}_8 \cdot 14\text{H}_2\text{O}$; disodium dihydrogen salt of $\overset{4}{P}$ - $\overset{4}{P}$ -acid (hypophosphate) $\text{Na}_2\text{H}_2\text{P}_2\text{O}_6 \cdot 6\text{H}_2\text{O}$; and disodium hydrogen salt of $\overset{5}{P}$ -acid (orthophosphate). The salts of $\overset{1}{P}$ -, $\overset{3}{P}$ -, and $\overset{5}{P}$ -acid were commercial reagents of the purest grade, while the others were supplied by Dr. B. BLASER and Dr. K. H. WORMS.

Colorimetric determination of the oxo acids of phosphorus

The molybdenum(V)-molybdenum(VI) reagent method⁶ was applied. Procedure III recommended by YOZA AND OHASHI⁷ was used for all of the oxo acids.

Determination of distribution ratios

One gram of air-dried resin was put in a stoppered Erlenmeyer flask, and 20 ml of a solution containing 0.03 *M* to 0.8 *M* ammonium acetate and a definite amount of a phosphate species (0.5 mg as P) was added. After the mixture had been shaken mechanically for 60 min, 10 ml of the liquid phase was withdrawn and its volume was made up to 100 ml with water. A 5 ml aliquot of this solution was heated on a water bath to dryness to remove ammonium acetate. The sample was then dissolved in water and determined colorimetrically. All equilibrations were done at room temperature $18^{\circ} \pm 2^{\circ}$. The distribution ratio *D* is calculated as follows:

$$D = \frac{\text{millimoles of phosphorus per ml resin}}{\text{millimoles of phosphorus per ml solution}}$$

Separation procedure

The apparatus is shown in Fig. 1. The capacity of the reservoir and mixing bottle were 1 l and 250 ml, respectively. The resin bed volume for the first or second elution run was about 5 ml and for the third about 10 ml (I.D. 0.8 to 0.9 cm). The ammonium acetate concentrations (pH \approx 6.8) in the reservoir and mixing bottle for the three elutions are given in Fig. 4. Two ml of an aqueous solution containing each phosphate sample (0.1 mg P for each species) was added to the top of the resin bed. When no liquid was left above the bed, 2 ml of water was added to wash the inside wall. Then, the apparatus was connected up and the elution carried out continuously. The flow rate was maintained at 0.5 to 0.6 ml per min. The solution volume in the mixing bottle thus remained constant at 250 ml. Five ml fractions of the effluent were collected with a fraction collector. Phosphate concentrations in the fractions were determined colorimetrically.

RESULTS AND DISCUSSION

When logarithms of the distribution ratios were plotted against logarithms of the concentrations of ammonium acetate (Fig. 3), straight lines were obtained except in the case of the $\overset{4}{\text{P}}-\overset{3}{\text{P}}-\overset{4}{\text{P}}$ -acid and the $(-\overset{3}{\text{P}}-)_{\text{6}}$ -ring acid. The plots of these two acids were straight lines only in the concentrated range of the acetate ion. Such behavior may be due to the fact that the molecules of these acids are too large to penetrate the resin phase. Further studies have not been made on this problem. Fig. 3 shows that the order of the sorbability of the oxo acids of phosphorus depends on the concentration of the ammonium acetate solution; for example, the sorbabilities increase in the order $\overset{1}{\text{P}} < \overset{5}{\text{P}} < \overset{3}{\text{P}} < \overset{4}{\text{P}}-\overset{4}{\text{P}} < \overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}} < \text{P}-\overset{4}{\text{P}}$ at 0.2 *M*, and $\overset{1}{\text{P}} < \overset{3}{\text{P}} < \overset{5}{\text{P}} < \overset{4}{\text{P}}-\overset{4}{\text{P}} < \overset{2}{\text{P}}-\overset{4}{\text{P}} < \overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}}$ at 0.6 *M*. This phenomenon is naturally expected, since the individual *D* slopes of the oxo acids of phosphorus are each different. Therefore, these slopes should have important significance in explaining the elution behavior of these ions. Under ideal conditions, these slopes must agree with the values of \bar{i} expressed in eqn. (21). These values of \bar{i} determined by graphical analysis are given in Table I, where for comparison, the values of \bar{i} calculated from the known dissociation constants are also given. Table I shows that experimental values are in good agreement with

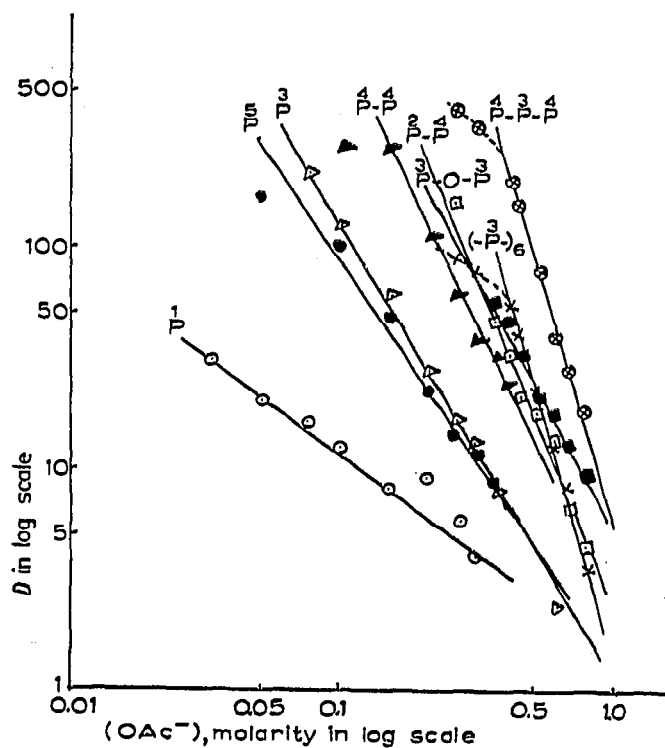


Fig. 3. Volume distribution ratios of oxo acids of phosphorus in ammonium acetate solutions of various concentrations.

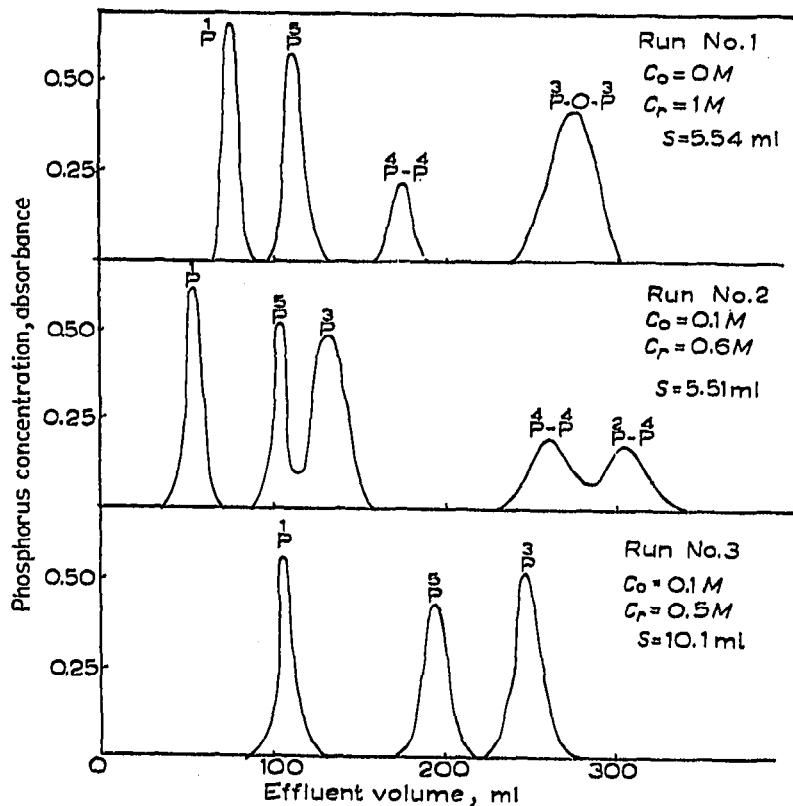


Fig. 4. Gradient elution of oxo acids of phosphorus with ammonium acetate. $V_{\max} = 250$ ml; flow rate = $0.5 \sim 0.6$ ml/min.

TABLE I

CALCULATED AND OBSERVED VALUES OF \bar{i} The calculated values of \bar{i} were obtained from the dissociation constants. The observed ones were obtained from batch equilibration data.

Oxo acid of phosphorus	\bar{i}	
	Calc.	Obs.
$\overset{1}{\text{P}}$	1.0	0.88
$\overset{5}{\text{P}}$	1.5	1.7
$\overset{3}{\text{P}}$	1.7	2.0
$\overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}}$		2.1
$\overset{4}{\text{P}}-\overset{4}{\text{P}}$	2.1	2.4
$\overset{2}{\text{P}}-\overset{4}{\text{P}}$		2.8
$(-\overset{3}{\text{P}}-)_{\theta}$		3.9
$\overset{4}{\text{P}}-\overset{3}{\text{P}}-\overset{4}{\text{P}}$		4.0

those obtained from the dissociation constants although the former are always slightly higher than the latter. The following reasons for this fact are possibly that: the term containing the activity coefficients in eqn. (21) could not be maintained constant within a series of measurements; the \bar{i} obtained from the slope should correspond to the dissociated state of the species present in the resin, which may not be lower than that in the solution, because the hydrogen ion concentration in the resin phase is generally lower than in the solution. The positions of the elution peaks can be predicted from these data according to eqn. (7). Elution curves are given in Fig. 4. In Table II, the positions of the elution peaks observed are compared with those calculated from the curves in Fig. 2, using the experimental values of D and \bar{i} . The observed values agree fairly well with the calculated values. It can be said that the

TABLE II

CALCULATED AND OBSERVED VALUES OF V_{\max}

Oxo acid of phosphorus	Run No.	1		2		3	
	$C_0 \sim C_r (M)$	0 ~ 1		0.1 ~ 0.6		0.1 ~ 0.5	
	$S (ml)$	5.54		5.51		10.1	
	$V_{\max} (ml)$	$V_{\max} (ml)$		$V_{\max} (ml)$		$V_{\max} (ml)$	
	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	
$\overset{1}{\text{P}}$	88	76	60	54	93	108	
$\overset{5}{\text{P}}$	138	113	136	105	206	198	
$\overset{3}{\text{P}}$			158	135	238	249	
$\overset{4}{\text{P}}-\overset{4}{\text{P}}$	202	178	260	265			
$\overset{2}{\text{P}}-\overset{4}{\text{P}}$			313	311			
$\overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}}$	245	275					

proposed method for the prediction of the position of the elution peaks in gradient elution chromatography is valid, except for large ions which give complicated patterns on the D curves. The elution conditions employed in this work are not necessarily the best ones for the separation of the lower oxo acids of phosphorus. Depending on the purpose of the separation, the best conditions have to be selected from the method described above.

LIST OF SYMBOLS EMPLOYED

A, B	= Tubes shown in Fig. 1.
a	= Constant in eqn. (3).
C	= Concentration of eluent.
C_0	= Initial concentration of eluent in the mixing bottle.
C_r	= Concentration of eluent in the reservoir.
D, D_0, D_r	= Volume distribution ratio of the sample ion at concentration C, C_0 and C_r of eluent, respectively.
\bar{i}	= Mean charge of sample ion.
K	= Activity coefficient quotient.
k	= Dissociation constant of oxo acids of phosphorus.
L	= Distance moved by the peak (ml).
n	= Constant in eqn. (3).
S	= Resin bed volume (ml).
V	= Flow volume of eluent (ml).
V_b	= Volume of the solution in tube B (ml).
V_m	= Volume of the solution in the mixing bottle (ml).
V_{\max}	= Position of the solution peak (ml).
v	= Velocity of the adsorption band peak (ml resin bed per ml eluent).
α	= $(C_r - C_0)/C_r$.
[], ()	= Concentration of ion in the resin and the solution, respectively.

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SUMMARY

A method has been proposed to predict the positions of the elution peaks of some lower oxo acids of phosphorus and orthophosphoric acid in gradient elution chromatography through an anion exchange resin. If the relation between logarithms of the distribution ratios and of the concentration of ammonium acetate as eluent is linear, one can calculate the position of the elution peak by the method presented here. The calculated positions of the elution peaks of $\overset{1}{\text{P}}^-$, $\overset{5}{\text{P}}^-$, $\overset{3}{\text{P}}^-$, $\overset{4}{\text{P}}-\overset{4}{\text{P}}^-$, $\overset{2}{\text{P}}-\overset{4}{\text{P}}^-$, and $\overset{3}{\text{P}}-\overset{3}{\text{P}}^-$ acid agreed approximately with the observed ones. The order of the sorbabilities of phosphate ions is dependent upon the concentrations of ammonium acetate as eluent.

REFERENCES

- 1 J. A. GRANDE AND J. BEUKENKAMP, *Anal. Chem.*, 28 (1956) 1497.
- 2 F. H. POLLARD, D. E. ROGERS, M. T. ROTHWELL AND G. NICKLESS, *J. Chromatog.*, 9 (1962) 227.
- 3 F. H. POLLARD, G. NICKLESS AND M. T. ROTHWELL, *J. Chromatog.*, 10 (1963) 212.
- 4 H. SCHWAB, W. RIEMAN AND P. A. VAUGHAN, *Anal. Chem.*, 29 (1957) 1357.
- 5 R. S. ALM, R. J. P. WILLIAMS AND A. TISELIUS, *Acta Chem. Scand.*, 6 (1952) 826.
- 6 F. LUCENA-CONDE AND L. PRAT, *Anal. Chim. Acta*, 16 (1957) 473.
- 7 N. YOZA AND S. OHASHI, *Bull. Chem. Soc. Japan*, 37 (1964) 33, 37.

J. Chromatog., 25 (1966) 398-407