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GEL CHROMATOGRAPHIC BEHAVIOR OF THE OXO ACIDS OF PHOSPHORUS

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SUMMARY

A number of oxo anions of phosphorus which contain one to four phosphorus atoms in the molecule were eluted with potassium chloride solutions on Sephadex columns. The K_d values of the oxo anions of phosphorus were measured as functions of the concentration and pH of the eluents, sample concentration and gel porosity. K_d values obtained by both the column and batch method were compared to each other.

The main conclusions are as follows. (1) When pure water was used as an eluent, extremely low K_d values were observed because of the electrostatic repulsion between the sample anions and the negatively charged gel matrix. (2) The K_d values increased with increasing concentration of potassium chloride. This may be due to the variation in the degree of hydration of sample anions. (3) At sample concentrations lower than 10^{-2} gram atom P per l the elution curves were symmetrical, while unsymmetrical elution curves were obtained at the higher sample concentrations. (4) The K_d values did not depend on the pH values of the eluent. (5) The most satisfactory separation of ortho-, di- and triphosphate anions was carried out on a Sephadex G-25 column. (6) The effective sizes of the oxo anions of phosphorus in aqueous solution were estimated from the gel chromatographic data.

INTRODUCTION

Some recent studies¹⁻⁸ have shown that gel chromatography is useful for the fractionation of macromolecules as well as for the separation of small inorganic ions. Although there are many arguments concerning the separation mechanism of inorganic species on a gel column, the molecular-sieve effect appears to be the major factor in many cases.

Since the family of oxo acids of phosphorus contains a number of compounds with different degrees of polymerization, investigation of their behavior in gel chromatography seems to be very appropriate for clarifying the separation mechanism of inorganic species on a gel column.

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In a previous paper¹ the present authors have demonstrated that oligophosphates can be eluted on a Sephadex G-25 column in order of decreasing molecular dimensions. ROGERS and his coworkers²⁻⁴ have pointed out that both sample concentration and background electrolyte concentration play important roles in the gel chromatographic behavior of oligophosphates. FELTER *et al.*⁵ and BOMBAUGH *et al.*⁶ have fractionated some highly-polymerized linear phosphates using gel columns.

The purpose of the present investigation is to obtain fundamental information on the gel chromatographic behavior of a number of oxo acids of phosphorus which contain one to four phosphorus atoms in a molecule. The gel chromatographic investigation for a series of linear phosphates with degrees of polymerization higher than four will be reported in a subsequent paper⁹. In general the distribution coefficient K_d in gel chromatography is given by eqn. 1,

$$V_e = V_o + K_d \cdot V_i \quad (1)$$

where V_e is the elution volume of a given solute, V_o the void volume outside the gel particles and V_i the internal volume within the gel phase. Although the molecular-sieve effect is a major factor for the separation mechanism, side effects such as ion exclusion^{3,7}, adsorption⁸ and diffusion¹⁰ must be considered in some cases. In order to estimate the contribution of these side effects the present authors measured K_d values for oxo anions of phosphorus as functions of concentration and pH of eluent, sample concentration and gel porosity, using Sephadex (a cross-linked dextran) as gel material and potassium chloride as the eluting agent, *i.e.*, background electrolyte. As has been pointed out in a preceding paper¹¹, potassium chloride is an appropriate background electrolyte for the gel chromatographic investigation of relatively large anions such as oxo anions of phosphorus. In the present study, the effective sizes of the oxo anions of phosphorus in aqueous solution were estimated from their gel chromatographic data as well.

Abbreviated notations proposed by BLASER AND WORMS¹² such as $\overset{1}{P}$ and $\overset{3}{P}$ are used in this paper. Ortho-, di-, tri-, tetra-, trimeta- and tetrametaphosphate are represented as P_1 , P_2 , P_3 , P_4 , P_{3m} and P_{4m} , respectively.

EXPERIMENTAL

Sample solutions

Sample solutions of seventeen oxo acids of phosphorus were prepared by dissolving the following salts in a solution of the same composition as that of the eluent used for the column operation:

$\text{NaPH}_2\text{O}_2 \cdot \text{H}_2\text{O}$ was used for $\overset{1}{P}$ -acid; $\text{Na}_2\text{PHO}_3 \cdot 5\text{H}_2\text{O}$ for $\overset{3}{P}$ -acid; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and KH_2PO_4 for P_1 -acid; $\text{Na}_3\text{P}_2\text{HO}_5 \cdot 12\text{H}_2\text{O}$ for $\overset{2}{P}-\overset{4}{P}$ -acid; $\text{Na}_2\text{H}_2\text{P}_2\text{O}_6 \cdot 6\text{H}_2\text{O}$ for $\overset{4}{P}-\overset{4}{P}$ -acid; $\text{Na}_2\text{P}_2\text{H}_2\text{O}_5$ for $\overset{3}{P}-\text{O}-\overset{3}{P}$ -acid; $\text{Na}_3\text{P}_2\text{HO}_5 \cdot 4\text{H}_2\text{O}$ for $\overset{3}{P}-\text{O}-\overset{5}{P}$ -acid; $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ for P_2 -acid; $\text{Na}_5\text{P}_3\text{O}_8 \cdot 14\text{H}_2\text{O}$ for $\overset{4}{P}-\overset{3}{P}-\overset{4}{P}$ -acid; $\text{Na}_4\text{P}_3\text{HO}_8 \cdot \text{H}_2\text{O}$ for $\overset{3}{P}-\text{O}-\overset{4}{P}-\overset{4}{P}$ -acid; $(\text{NH}_4)_5\text{P}_3\text{O}_9 \cdot x\text{H}_2\text{O}$ for $\overset{5}{P}-\text{O}-\overset{4}{P}-\overset{4}{P}$ -acid; $\text{Na}_5\text{P}_3\text{O}_{10} \cdot 6\text{H}_2\text{O}$ for P_3 -acid; $\text{Na}_3\text{P}_3\text{O}_9 \cdot 6\text{H}_2\text{O}$ for P_{3m} -acid; $\text{Na}_6\text{P}_4\text{O}_{11} \cdot x\text{H}_2\text{O}$ for $\overset{4}{P}-\overset{4}{P}-\text{O}-\overset{4}{P}-\overset{4}{P}$ -acid; $\text{Na}_4\text{P}_4\text{O}_{10} \cdot 4\text{H}_2\text{O}$

for $(-\overset{4}{\text{P}}-\overset{4}{\text{P}}-\text{O}-)_2$ -acid; a sodium tetrphosphate solution was used for P_4 -acid and $\text{Na}_4\text{P}_4\text{O}_{12}\cdot 4\text{H}_2\text{O}$ for P_{4m} -acid.

The salts of $\overset{1}{\text{P}}-$, $\overset{3}{\text{P}}-$, P_1- , P_2- and P_3 -acids were commercial reagents, while the salts of $\overset{4}{\text{P}}-\overset{4}{\text{P}}-$ (ref. 13), $\overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}}-$ (ref. 14), $\overset{3}{\text{P}}-\text{O}-\overset{5}{\text{P}}-$ (ref. 15), $\overset{3}{\text{P}}-\text{O}-\overset{4}{\text{P}}-\overset{4}{\text{P}}-$ (ref. 16), P_{3m} - (ref. 17), P_4- (ref. 18) and P_{4m} -acid (ref. 19) and potassium Kurrol's salt (ref. 20) were prepared in the authors' laboratory. The salts of $\overset{2}{\text{P}}-\overset{4}{\text{P}}-$, $\overset{4}{\text{P}}-\overset{3}{\text{P}}-\overset{4}{\text{P}}-$, $\overset{5}{\text{P}}-\text{O}-\overset{4}{\text{P}}-\overset{4}{\text{P}}-$, $\overset{4}{\text{P}}-\overset{4}{\text{P}}-\text{O}-\overset{4}{\text{P}}-\overset{4}{\text{P}}-$ and $(-\overset{4}{\text{P}}-\overset{4}{\text{P}}-\text{O}-)_2$ -acid were supplied by BLASER AND WORMS.

Solutions of Blue Dextran 2000 (Pharmacia Fine Chemicals) and tritiated water (Radiochemical Center, Amersham, Great Britain), prepared by dissolving them in a solution of the same composition as that of the eluent were used as standard materials of $K_a = 0$ and 1, respectively. Potassium Kurrol's salt, $(\text{KPO}_3)_n$, which is a linear phosphate with an extremely high degree of polymerization (estimated to be about 10,000), was employed in place of Blue Dextran 2000 in some experiments. Potassium Kurrol's salt was dissolved in a dilute aqueous solution of lithium chloride and then added to the eluent employed, because it is difficult to dissolve it directly in an aqueous solution of potassium chloride²¹.

Eluents

Pure water and 0.1, 0.5 and 1.0 *M* aqueous solutions of potassium chloride were used as eluents. Some of the potassium chloride eluent solutions were adjusted to definite pH values with suitable buffer solutions.

Columns

Several types of Sephadex, G-10 (particle size, 40–120 μ), G-15 (40–120 μ), G-25 (20–80 μ) and G-50 (20–80 μ), were used. Three glass tubes (Shoei Glass) of 1.5 \times 60, 1.2 \times 130 and 1.5 \times 90 cm filled with 100, 120 and 150 ml of Sephadex gels, respectively, were used as columns. Gel beds were prepared by the method described in a preceding paper¹¹.

Procedure for elution

One milliliter of a sample solution was placed on top of the gel column and chromatographed by the procedure described in a preceding paper¹¹. Sample concentrations were adjusted to 2×10^{-3} – 5×10^{-3} gram atom P per l, unless otherwise stated. The concentrations of the oxo acids of phosphorus in the effluents were determined colorimetrically by means of a molybdenum(V)–molybdenum(VI) reagent^{22,23}.

Determination of K_a by the batch method

In a 50 ml erlenmeyer flask with a glass stopper, 3.0 g of dry gel powder and 25.0 ml of a potassium chloride solution were mixed. The gel was allowed to swell for 24 h with occasional shaking. Then, 5.0 ml of a sample solution was added. The suspension was stirred for 1 h with a mechanical agitator. Preliminary experiments showed that no detectable concentration differences were found when the stirring was continued for 0.5, 1, 2, 3, 4 and 24 h. The gel was allowed to settle, and an aliquot of the solution was then withdrawn from the flask. Its phosphorus concentration was

determined colorimetrically. A similar method was employed to determine the void volume with Blue Dextran 2000 and potassium Kurrol's salt.

RESULTS AND DISCUSSION

Standardization of the columns

In gel chromatography the standardization of columns has a great advantage because results obtained with different columns can readily be compared with one another. Blue Dextran 2000 and tritiated water have been widely used to standardize gel chromatographic columns. Blue Dextran 2000 was weakly adsorbed on a highly cross-linked dextran gel to give an unsymmetrical elution curve. The maximum position of its elution peak at a concentration lower than 0.2% (w/w) is comparable with that of potassium Kurrol's salt, which is large enough to be excluded completely from the gel phase. In the present work Blue Dextran 2000 was usually employed because of the facility of its determination. When pure water was used as an eluent, however, the elution curve of Blue Dextran 2000 was so broad that its peak position could not be determined exactly. In such a case potassium Kurrol's salt was used as a standard material.

TABLE I

THE EFFECT OF ELUENT CONCENTRATION ON THE K_d VALUES
Gel, Sephadex G-25; bed volume, 150 ml.

Sample	K_d			
	Pure water	KCl		
		0.1 M	0.5 M	1.0 M
¹ P		0.80	0.83	0.83
³ P		0.78	0.83	0.84
P ₁	0.36	0.78	0.83	0.84
³ P- ⁴ P		0.64	0.74	0.76
⁴ P- ⁴ P		0.67	0.77	0.79
³ P-O- ³ P		0.59	0.66	0.69
³ P-O- ⁵ P		0.61	0.70	0.73
P ₂	0.30	0.62	0.71	0.75
⁴ P- ³ P- ⁴ P		0.61	0.73	0.76
³ P-O- ⁴ P- ⁴ P		0.50	0.62	0.70
⁵ P-O- ⁴ P- ⁴ P		0.51	0.67	0.70
P ₃	0.21	0.52	0.62	0.69
P _{3m}		0.52	0.67	0.70
⁴ P- ⁴ P-O- ⁴ P- ⁴ P		0.46	0.62	0.64
(- ⁴ P- ⁴ P-O-) ₂		0.48	0.62	0.65
P _{4m}		0.46	0.60	0.65

Tritiated water was used to determine the total liquid volume in a column. However, some investigators^{24,25} have demonstrated that its elution volume is slightly larger than the true total liquid volume, because isotope exchange of hydrogen atoms takes place very rapidly between water and dextran when one employs a highly cross-linked dextran gel. However, no special calibration due to this phenomenon was carried out in this work, because the correction term is negligibly small. Precision for the K_d values obtained in this work is within the range of ± 0.02 .

Effect of eluent concentration

The K_d values of the oxo anions of phosphorus eluted with pure water and 0.1, 0.5 and 1.0 *M* solutions of potassium chloride from a Sephadex G-25 column are shown in Table I. The K_d values of the P_1^- , P_2^- and P_3^- anions eluted with pure water are extremely low, as compared with those obtained by the elutions with the potassium chloride solutions. Since there are no appreciable differences between the V_o and V_i values obtained in the presence and the absence of potassium chloride as a background electrolyte, the low K_d values obtained in the absence of potassium chloride cannot be ascribed to the variation in the column parameters. The low K_d values may be explained, as has been pointed out by earlier workers^{3,7}, on the basis of the Donnan ion exclusion effect between the anionic solute species and a small number of fixed negative charges in the gel matrix which are believed to be carboxylate groups. In other words the penetration of the oxo anions of phosphorus into the gel phase is restricted by electrostatic repulsion between these oxo anions and the gel matrix.

Since the amount of fixed negative charges on the gel matrix is very small, the ion exclusion effect caused by the negative charges may be eliminated by the addition of potassium chloride to an eluent. NEDDERMEYER AND ROGERS³, who observed the elution behavior of sodium chloride on a Sephadex column, have shown that a concentration of background electrolyte higher than 0.01 *M* is sufficient to eliminate the ion exclusion effect due to the gel matrix.

As shown in Table I, the K_d values of the oxo anions of phosphorus increase with increasing concentrations of potassium chloride. It is evident that the variations in the K_d values are not due to the column parameters, because both the V_o and V_i values did not depend upon potassium chloride concentrations, at least for the gel columns composed of Sephadex G-25. The fact that the extent of the variations in the K_d values for the monophosphorus species was small in comparison with those for the other species supports the above conclusion. If the variations in the K_d values are caused by the gel bed itself, for instance by the deformation of the gel particles, the same degree of variation will be observed. According to preliminary experiments in our laboratory the V_i values for the columns composed of Sephadex G-10 increased with increasing concentrations of potassium chloride in eluents. Therefore, one must take the dependence of the column parameters on the eluent concentration into consideration when a highly cross-linked gel is employed.

The K_d values of both the potassium and the chloride ion on a Sephadex G-15 column were higher than those of any of the oxo anions of phosphorus. Therefore, the oxo anions of phosphorus in the gel phase are always accompanied by the background electrolyte. In terms of phases I and II described in the preceding paper¹¹ the oxo anions of phosphorus can penetrate into phase II but not into phase I. This means that there may be no direct interaction between the oxo anions of phosphorus and the gel matrix.

TABLE II

EFFECT OF SAMPLE CONCENTRATION ON THE K_d VALUESGel, Sephadex G-25; bed volume, 100 ml for P^3 and P_3 , 150 ml for the others; eluent, 0.1 M KCl.

Sample	K_d						
	Concn. of samples (gram atom P/l)						
	0.001	0.005	0.01	0.05	0.1	0.5	1
P^3	0.78	0.78	0.78		0.79		0.80
P_1	0.78	0.79	0.79		0.80		0.80
$\text{P}^4\text{-P}^4$	0.66	0.66	0.66	0.69	0.71		
P_2	0.62	0.63	0.63		0.69		
P_3	0.52	0.50	0.51	0.55	0.57	0.66	0.70
P_{3m}	0.52	0.52	0.52		0.58		0.69
$\text{P}^4\text{-P}^4\text{-O-P}^4\text{-P}^4$	0.46	0.46	0.46		0.50		0.66
$(\text{-P}^4\text{-P}^4\text{-O-})_2$	0.48	0.48	0.47				
P_{4m}	0.46	0.46	0.45		0.50		0.63

Finally one must consider the deformation of the solute molecules. The degree of hydration in the oxo anions of phosphorus could decrease with increasing concentrations of potassium chloride in the eluents. This assumption leads to the conclusion that the K_d values of the oxo anions of phosphorus would become higher at the higher

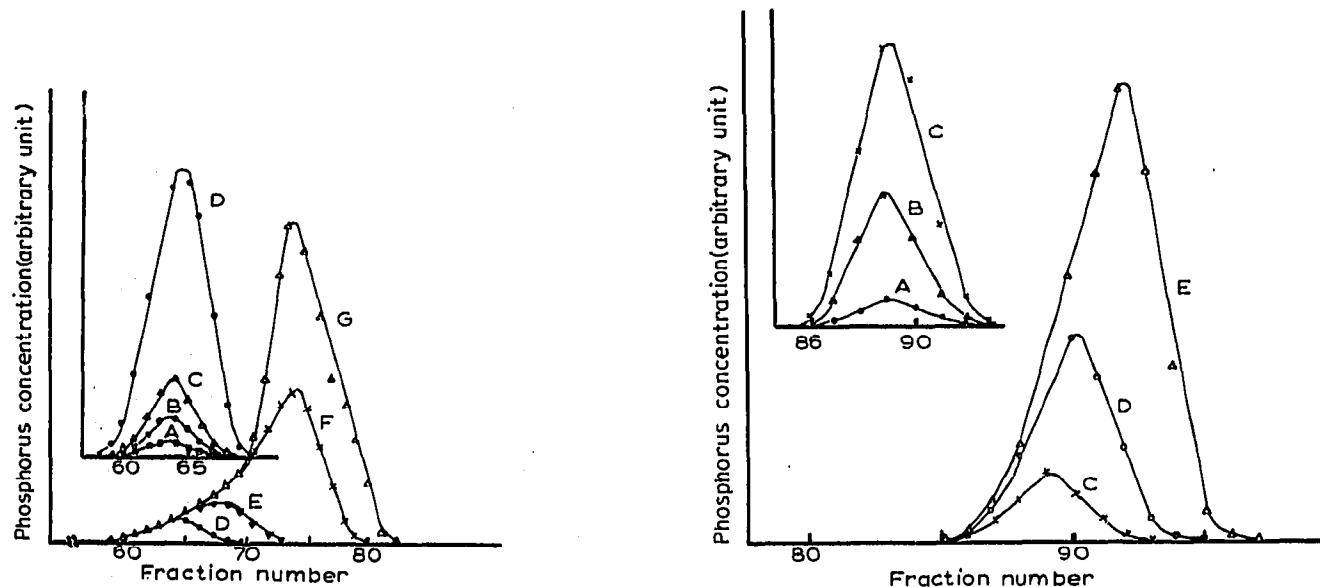


Fig. 1. Effect of sample concentration on the elution curves of the P_3 -anion. Gel, Sephadex G-25; bed volume, 100 ml; eluent, 0.1 M KCl. Sample concentration (gram atom P per l): A = 0.001; B = 0.005; C = 0.01; D = 0.05; E = 0.1; F = 0.5; G = 1.0. One fraction = 1.03 ml.

Fig. 2. Effect of sample concentration on the elution curves of the $^4\text{P-}^4\text{P}$ -anion. Gel, Sephadex G-25; bed volume, 150 ml; eluent, 0.1 M KCl. Sample concentration (gram atom P per l): A = 0.001; B = 0.005; C = 0.01; D = 0.05; E = 0.1. One fraction = 1.14 ml.

concentrations of potassium chloride. It has been pointed out²⁶ that the extent of the variations in the K_d values for non-electrolytes such as glucose, at different concentrations of a background electrolyte, are smaller than those for K_d values of ionic species. The K_d values of the oxo anions of phosphorus as well as alkaline earth metal ions²⁷ are dependent not only on the concentration of the eluents but also on the concentration of the sample solutions. These facts also support the above conclusion.

Effect of sample concentration

The effect of the sample concentration on the K_d values is given in Table II. This kind of effect is also of great importance in gel chromatography in evaluating the contribution of the molecular-sieve effect and other side effects, such as adsorption, to the separation process of solutes. The elution curves of the oxo anions of phosphorus tested all change in the same manner with the sample solution concentration. Two examples for the P_3^- and P_4^{4-} -anion are illustrated in Figs. 1 and 2. When the sample concentrations were lower than 0.01 gram atom P per l, the elution curves were symmetrical, and the elution peak positions did not change. However, when the concentrations were higher than 0.01 gram atom P per l, unsymmetrical elution curves with skewed leading edges were observed. The K_d values for these elution curves given in Table II were measured at the maximum concentrations. Elution patterns of this type are analogous to a concave adsorption isotherm in adsorption chromatography. However, the explanation based on adsorption seems to be unreasonable, because the K_d values of the oxo anions of phosphorus increase with increase of the sample concentrations, *i.e.*, the sample concentration dependence is contrary to that usually observed in adsorption chromatography. It seems to be more appropriate to explain the sample concentration dependence for the oxo anions of phosphorus in terms of the existence of oxo anions with different degrees of hydration.

Effect of pH of eluent solutions

The K_d values of the oxo anions of phosphorus eluted with 0.1 *M* potassium chloride solutions adjusted to pH 4.6, 7.0 and 9.2 from a Sephadex G-25 column are summarized in Table III. There is no significant pH dependence for the K_d values in columns a, b and c of Table III, but the K_d values obtained with the eluents buffered with borate are, in general, somewhat lower as shown in columns d and e. LINDQVIST²⁸ has demonstrated that the borate ion is strongly adsorbed on dextran gel. This phenomenon may be ascribed to the chemical reaction between the borate ion and dextran, because it is well known that the borate ion reacts with polyalcohols such as mannitol to form a strong acid. The electrostatic repulsion between the oxo anions of phosphorus and the borate anions adsorbed on the dextran gel leads to the decrease in the K_d values. This suggests that the buffering agent for the eluent should be selected carefully when one wants to separate solute molecules on the basis of the molecular-sieve effect.

The K_d values of ammonia and hydrochloric acid, when eluted with pure water through a Sephadex G-25 column, were reported to be 0.8 (ref. 29). This indicates that neither ammonium nor chloride ion is strongly adsorbed on the dextran gel. It has also been observed²⁹ that aromatic compounds are adsorbed to some extent on gel materials when eluted with pure water. However, it can be expected from earlier work²⁰ that the adsorption of aromatic compounds on dextran gel can be eliminated by the use of

TABLE III

THE EFFECT OF THE pH OF THE ELUENT ON THE K_d VALUES
Gel, Sephadex G-25; bed volume, 150 ml; eluent, 0.1 M KCl.

Sample	K_d				
	<i>a</i> pH 4.6 phthalate- NaOH buffer	<i>b</i> pH 7.0 Tris buffer	<i>c</i> pH 9.2 $\text{NH}_3\text{-NH}_4\text{Cl}$ buffer	<i>d</i> pH 7.0 borate buffer	<i>e</i> pH 9.2 borate buffer
P^1	0.79	0.78	0.79	0.73	0.61
P^3	0.78	0.78	0.78	0.73	0.57
P_1	0.78	0.78	0.78	0.73	0.59
$\text{P}^2\text{-P}^4$	0.64	0.64	0.65	0.57	0.40
$\text{P}^4\text{-P}^4$	0.67	0.68	0.68	0.62	0.52
$\text{P}^3\text{-O-P}^3$	0.59	0.59	0.58	0.52	0.41
$\text{P}^3\text{-O-P}^5$	0.61	0.60	0.60	0.50	0.38
P_2	0.62	0.62	0.63	0.54	0.41
$\text{P}^4\text{-P}^3\text{-P}^4$	0.61	0.60	0.61	0.52	0.39
$\text{P}^3\text{-O-P}^4\text{-P}^4$	0.50	0.49	0.49	0.45	0.35
$\text{P}^5\text{-O-P}^4\text{-P}^4$	0.51	0.50	0.51	0.49	0.36
P_3	0.52	0.50	0.51	0.42	0.33
P_{3m}	0.52	0.52	0.52	0.44	0.33
$\text{P}^4\text{-P}^4\text{-O-P}^4\text{-P}^4$	0.46	0.46	0.47	0.42	0.32
$(\text{-P}^4\text{-P}^4\text{-O-})_2$	0.48	0.48	0.48	0.42	0.30
P_{4m}	0.46	0.47	0.45	0.39	0.27

a background electrolyte. When P_1 -, P_2 - and P_3 -anions were eluted with 0.1 M potassium chloride solutions containing 0.001, 0.005 and 0.01 M phthalate buffer from a Sephadex G-25 column, the K_d values of these anions did not change. This suggests that the phthalate anion is not adsorbed on the dextran gel. Since the K_d values listed in column b of Table III coincide well with those in columns a and c, it appears that the Tris buffer is also not adsorbed on the dextran gel.

The above discussion leads one to the conclusion that pH dependence of the K_d values of the oxo anions of phosphorus cannot be observed, when appropriate buffer agents are employed, in spite of the different dissociation states of the phosphorus oxo acids. The electrostatic effect appears to be a minor factor in the gel chromatographic behavior of the phosphorus oxo anions.

Effect of solute molecule diffusion

Experiments under static conditions were carried out to evaluate the contri-

TABLE IV

K_d VALUES OBTAINED BY THE BATCH AND COLUMN METHOD

Gel, Sephadex G-25; bed volume, 150 ml; eluent, 0.1 M KCl.

Sample	<i>K_d</i>	
	Batch	Column
P ₁	0.78	0.78
P ₂	0.61	0.62
P ₃	0.51	0.52
P _{3m}	0.52	0.52
P _{3m}	0.46	0.46

bution of the diffusion of the solute molecules to the separation process on the column. If the separation of the solute molecules is only based on the molecular-sieve effect, the distribution coefficient *K_d* calculated from eqn. 2 should be equal to that obtained by the column method. Eqn. 2 can be derived on the assumption that the solute concentration within the gel phase is essentially the same as that in the external liquid phase. Then,

$$K_d = 1 - \frac{C_2 - C_1}{C_2} \cdot \frac{V_o' + V_i'}{V_i'} \quad (2)$$

where *C₂* is the concentration of the solute in equilibrium with a certain amount of Sephadex, *C₁* is the concentration when no Sephadex is added, *V_o'* is the void volume of the solution and *V_i'* the internal volume. *V_o'* + *V_i'* is considered to be equal to the total volume of the solution added. *V_i'* is calculated from the data for Blue Dextran 2000 or potassium Kurrol's salt as a standard material of *K_d* = 0.

The *K_d* values of some oxo anions of phosphorus obtained by the column and the batch method are shown in Table IV. There are no significant differences between the *K_d* values obtained by either method. This means that the effect of diffusion on *K_d* can be neglected in the gel chromatographic process under the conditions used in the present investigation.

TABLE V

K_d VALUES CLASSIFIED BY THE NUMBER OF PHOSPHORUS ATOMS IN A MOLECULE

Gel, Sephadex G-25; bed volume, 150 ml; eluent: 0.1 M KCl.

	Number of phosphorus atoms in a molecule			
	1	2	3	4
<i>K_d</i>	0.80-0.78	0.68-0.58	0.61-0.49	0.48-0.45

The elution order of the solute molecules and the effect of gel porosity

From all the results mentioned above it can be concluded that when the oxo anions of phosphorus are chromatographed on a Sephadex G-25 column with a 0.1 M

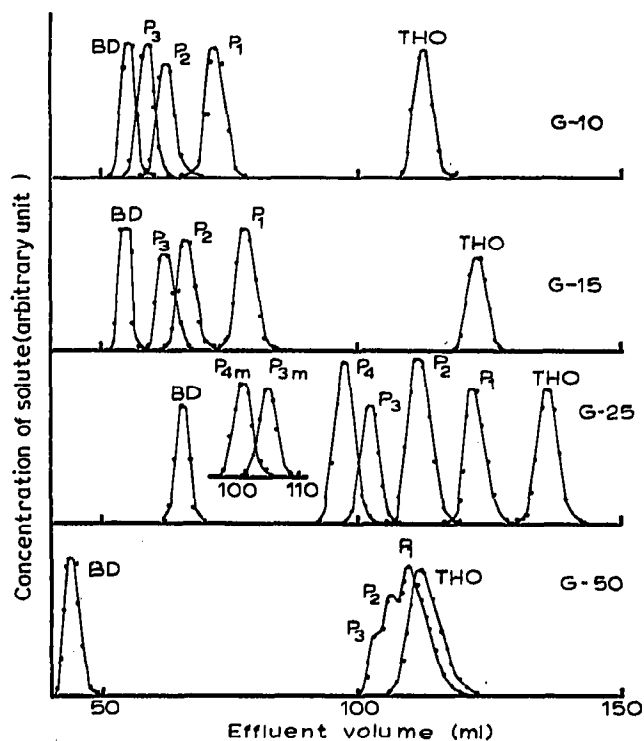


Fig. 3. Effect of gel porosity on the elution curves of P_1^- , P_2^- , P_3^- , P_4^- , P_{3m}^- and P_{4m}^- -anions. Bed volume, 150 ml for G-10, G-15 and G-25 columns; 120 ml for the G-50 column. Eluent: 0.1 M KCl. BD = Blue Dextran 2000; THO = tritiated water.

potassium chloride solution, the separation mechanism is based only on the molecular-sieve effect. The order of the K_d values of the oxo anions of phosphorus obtained under the above conditions is roughly in agreement with that expected from their molecular dimensions as shown in Table V. This gel chromatographic method has been successfully applied to the group separation of ^{32}P -labeled oxo acids of phosphorus produced by neutron-irradiation of orthophosphates³⁰.

P_1^- , P_2^- and P_3^- -anion were eluted from Sephadex G-10, G-15, G-25 and G-50 columns with 0.1 M potassium chloride solutions. As shown in Fig. 3, the elution volume of each phosphate increases with increasing porosity of the gels. The Sephadex G-25 column gives the most satisfactory separation of P_1^- , P_2^- and P_3^- -anions.

Estimation of the size of the solute molecules

In recent years relationships between distribution coefficients and the molecular size of a solute have been studied by many investigators. As has been described in a preceding paper¹¹, LAURENT AND KILLANDER's model³¹ can be expressed by eqn. 3.

$$(-\log K_{av})^{\dagger} = A \cdot R_s + B \quad (3)$$

where A and B are constants for a given gel column and R_s is the radius of a solute molecule. K_{av} is defined as

$$K_{av} = \frac{V_e - V_o}{V_t - V_o} = K_d \cdot \frac{V_t}{V_t + V_o} \quad (4)$$

where V_t is the total volume of the gel bed, and V_g the volume occupied by the gel matrix. For a Sephadex G-25 column $V_t/(V_t + V_g)$ was found to be equal to 0.862. If the elution process is controlled only by the molecular-sieve effect, one can calculate the R_s values of the solutes using eqn. 3.

As shown in Fig. 6 of the preceding paper¹¹, there is a linear relationship between the $(-\log K_{av})^{\frac{1}{2}}$ and the R_s values of potassium, chloride, sodium, nickel and magnesium ions. The K_{av} values of these ions were determined from an elution with a 0.1 M potassium chloride solution on a Sephadex G-15 column. The R_s values of these ions were taken from NIGHTINGALE's paper³². On the assumption that the linear relationship mentioned above can be used for the estimation of the molecular sizes of the oxo anions of phosphorus, the R_s values shown in Table VI were obtained from the K_{av} values of the oxo anions of phosphorus observed during an elution with a 0.1 M potassium chloride solution on a Sephadex G-15 column. Another set of the R_s values shown in Table VI was obtained by a similar method from the data for a Sephadex G-25 column. The results indicate that the oxo anions of phosphorus are considerably hydrated in aqueous solution.

TABLE VI

 K_{av} VALUES AND MOLECULAR SIZE

Bed volume, 150 ml (* average K_{av} values obtained with both columns of bed volume 100 and 150 ml). Eluent, 0.1 M KCl.

Sample	Sephadex G-15		Sephadex G-25	
	K_{av}	R_s (\AA)	K_{av}	R_s (\AA)
$\overset{1}{\text{P}}$	0.39*	4.3	0.69	4.3
$\overset{3}{\text{P}}$	0.37	4.4	0.67	4.4
P_1	0.35*	4.5	0.67	4.4
$\overset{2}{\text{P}}-\overset{4}{\text{P}}$	0.22	5.6	0.55	5.5
$\overset{4}{\text{P}}-\overset{4}{\text{P}}$	0.24*	5.3	0.58	5.6
$\overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}}$	0.19*	5.7	0.51	5.8
$\overset{5}{\text{P}}-\text{O}-\overset{3}{\text{P}}$	0.20	5.7	0.52	5.7
P_2	0.20*	5.7	0.53	5.7
$\overset{4}{\text{P}}-\overset{3}{\text{P}}-\overset{4}{\text{P}}$			0.52	5.7
$\overset{3}{\text{P}}-\text{O}-\overset{4}{\text{P}}-\overset{4}{\text{P}}$	0.10	6.8	0.43	6.5
$\overset{5}{\text{P}}-\text{O}-\overset{4}{\text{P}}-\overset{4}{\text{P}}$	0.13*	6.4	0.44	6.4
P_3	0.13*	6.4	0.45	6.4
P_{3m}			0.45	6.4
$\overset{4}{\text{P}}-\overset{4}{\text{P}}-\text{O}-\overset{4}{\text{P}}-\overset{4}{\text{P}}$			0.41	6.8
$(-\overset{4}{\text{P}}-\overset{1}{\text{P}}-\text{O}-)_2$			0.41	6.8
P_{4m}			0.40	6.9

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