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Note

Gel chromatographic separations of monomeric oxo anions of phosphorus on Sephadex G-10 and Bio-Gel P-2

KIKUJIRO UJIMOTO, ISAO ANDO, TAKAKO YOSHIMURA*, KUMIKO SUZUKI and HIRONDO KURIHARA

Department of Chemistry, Faculty of Science, Fukuoka University, Nanakuma 11, Fukuoka (Japan)

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Many investigations have been reported concerning the separation of inorganic oxo anions of phosphorus by means of precipitation, solvent extraction and chromatographic techniques such as paper, thin-layer, ion exchange and gel chromatography and electrophoresis[1–5]. The monomeric oxo anions, *i.e.*, phosphinate, phosphonate and orthophosphate, are usually separated by anion-exchange chromatography because of its superior resolving power[6–13].

Gel chromatography, a method for separating solutes depending on their size, was successfully applied to the separation of monomeric, dimeric and trimeric oxo anions of phosphorus on a Sephadex G-25 column, regardless of the oxidation states of the phosphorus atoms[14]. It has been considered, however, that the separation of the monomeric oxo anions of phosphorus by gel chromatography is impossible, even when tightly cross-linked gels are employed.

We recently reported[15] that the distribution coefficient (K_d) of phosphinate, phosphonate and orthophosphate on Sephadex G-10 and G-15 columns varied significantly depending on pH of the eluent, and pointed out the possibility of separating these monomeric oxo anions by elutions at appropriate pH values.

As the result of the present investigation on the separation of these monomeric oxo anions by gel chromatography on Sephadex G-10, it was confirmed that orthophosphate was isolated from phosphinate and phosphonate by eluting with 0.1 *M* sodium chloride solution at pH 1.5, and phosphinate from phosphonate and orthophosphate at pH 10. The pH dependence of the K_d values of these oxo anions was also examined on a Bio-Gel P-2 column with 0.1 *M* sodium chloride solutions at various pH. With Bio-Gel P-2, the mixture of the three oxo anions was separated into its components by elution at pH 1.5.

EXPERIMENTAL

Sample solutions

All reagents used were of guaranteed reagent grade from Wako (Osaka, Japan) or Nakarai Chemicals (Kyoto, Japan), unless otherwise stated. Sodium phosphinate

* Ex-member of the staff of the Radioisotope Center, Fukuoka University, Nanakuma 11, Fukuoka, Japan.

(abbreviated to P^1) and sodium phosphonate (P^3) were recrystallized once from water. Potassium dihydrogen orthophosphate (P^5) was used without further purification.

The concentrations of stock solutions were 0.1 *M* for P^1 and 0.2 *M* for P^3 and P^5. Sample solutions containing P^1, P^3 and P^5 at various concentrations (see Table I) were prepared by mixing the stock solutions in appropriate proportions and stored at room temperature.

Sample solutions of P^1, P^3 or P^5 at a concentration of $2 \cdot 10^{-3}$ *M* were used to investigate the pH dependence of the K_d values on Bio-Gel P-2 (Bio-Rad Labs., Richmond, Calif., U.S.A.). Blue Dextran 2000 (Pharmacia, Uppsala, Sweden; 0.25%) and tritiated water (Radiochemical Centre, Amersham, Great Britain) were employed as standard materials with $K_d = 0$ and 1, respectively.

Eluents

The eluent used was 0.1 *M* sodium chloride solution at pH 1.5 or 10 adjusted with hydrochloric acid or sodium hydroxide solution. The same eluent, but at various pH values, was also used to obtain the pH profiles of the K_d values of P^1, P^3 and P^5 on Bio-Gel P-2. The procedure of pH adjustment was reported previously[15].

Columns and elution procedure

The dimensions of the Sephadex G-10 (dry particle size 40–120 μm) column were 93.5 × 1.5 cm. The column preparation and elution procedure were as reported previously[15]. The temperature was kept at 20 ± 0.5° by circulating thermostated

TABLE I

PHOSPHORUS AMOUNTS AND RECOVERIES IN THE SEPARATION OF MONOMERIC OXO ANIONS OF PHOSPHORUS ON SEPHADEX G-10

Sample No.	Amount taken (μg P)			pH 1.5				pH 10			
				$(P^1 + P^3)$		P^5		P^1		$(P^3 + P^5)$	
	P^1	P^3	P^5	Found (μg P)	Recovery (%)	Found (μg P)	Recovery (%)	Found (μg P)	Recovery (%)	Found (μg P)	Recovery (%)
1	32.4	32.1	30.9	62.7	97.2	32.4	105	32.8	101	58.4	92.7
				61.2	94.9	32.4	105	29.5	91.0	68.5	109
2	162	161	155	311	96.3	150	96.8	179	110	302	95.6
				310	96.0	165	106	157	96.9	331	105
3	324	321	309	651	101	297	96.1	336	104	587	93.2
				629	97.5	314	102	319	98.5	630	100
4	1619	1607	1547	3172	98.3	1482	95.8	1416	87.5	3177	101
				3138	97.3	1524	98.5	1326	81.9	3359	106
5	50.0	52.8	508	99.1	96.4	509	100	56.1	112	565	101
				101	98.2	508	100	46.8	93.6	580	103
6	50.0	518	50.8	576	101	55.5	109	53.7	107	566	99.5
				559	98.4	49.2	96.9	47.0	94.0	580	102
7	500	63.0	50.8	560	99.5	52.8	104	495	99.0	102	89.6
				551	97.9	52.1	103	508	102	110	96.7

Sample concentration: 1, 2, 3 and 4, $1 \cdot 10^{-3}$, $5 \cdot 10^{-3}$, $1 \cdot 10^{-2}$ and $5 \cdot 10^{-2}$ *M*, respectively, each of P^1, P^3 and P^5; 5, $1.6 \cdot 10^{-3}$ *M* of P^1 and P^3, $1.6 \cdot 10^{-3}$ *M* of P^5; 6, $1.6 \cdot 10^{-3}$ *M* of P^1 and P^5, $1.6 \cdot 10^{-2}$ *M* of P^3; 7, $1.6 \cdot 10^{-2}$ *M* of P^1, $1.6 \cdot 10^{-3}$ *M* of P^3 and P^5.

water through the column jacket. A 1-cm³ volume of the sample solution was chromatographed in each run and eluted at a constant flow-rate of 60 cm³/h.

The dimensions of the Bio-Gel P-2 (100–200 mesh) column were 93 × 1.5 cm. The column preparation and elution procedure were the same as for Sephadex G-10. The elutions were carried out at room temperature and at a flow-rate of 40 cm³/h.

Colorimetric determination of phosphorus concentration and calculation of K_d value

The phosphorus concentrations of the stock solutions and of the effluent collected in each fraction were determined colorimetrically by the use of a molybdenum(V)–molybdenum(VI) reagent[16], which was prepared according to the method of Hosokawa and Oshima[17].

The procedure for obtaining the K_d values of the oxo anions of phosphorus was reported previously[15].

RESULTS AND DISCUSSION

Separation of oxo anions of phosphorus on Sephadex G-10

Figs. 1 and 2 show typical elution diagrams on the Sephadex G-10 column at pH 1.5 and 10. The sample solution in Fig. 1 contained P^1, P^3 and P^5, each at the concentration of $5 \cdot 10^{-3}$ *M*. In Fig. 2, the concentration of P^1 ($1.6 \cdot 10^{-2}$ *M*) was ten-

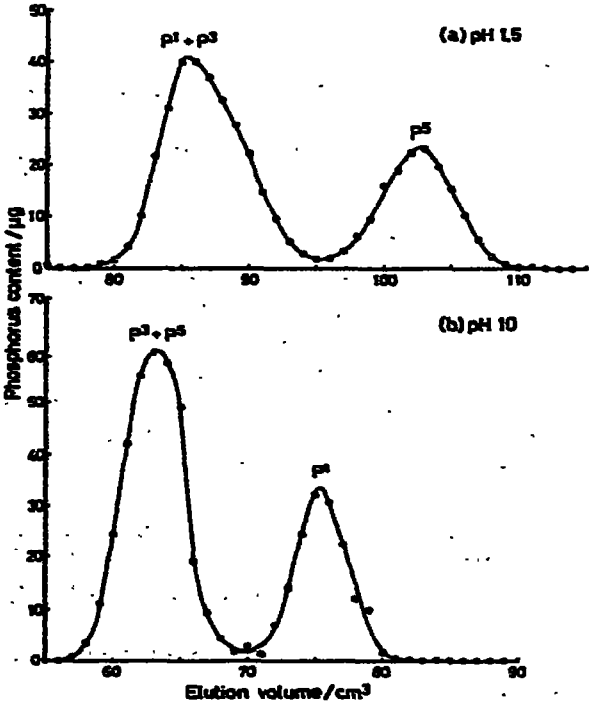

Fig. 1. Typical elution diagrams on a Sephadex G-10 column at pH 1.5 and pH 10. Sample concentration: $5 \cdot 10^{-3}$ *M* for each of P^1, P^3, and P^5. Column: 93.5 × 1.5 cm. Eluent: 0.1 *M* NaCl solution at pH 1.5 or 10.

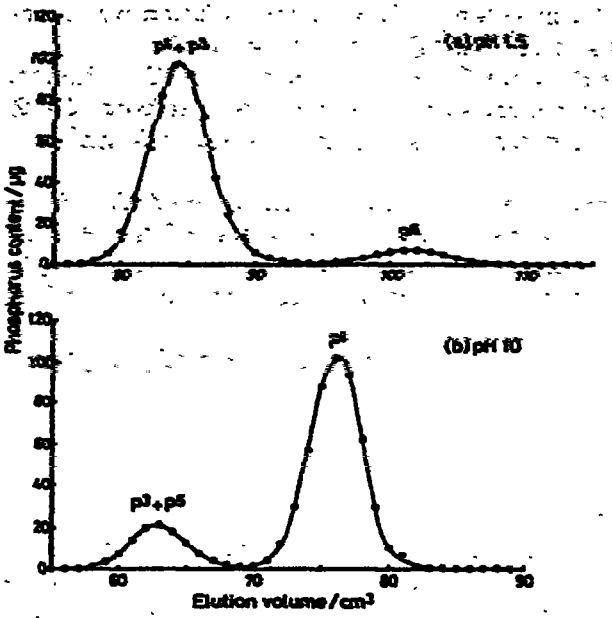

Fig. 2. Typical elution diagrams on a Sephadex G-10 column at pH 1.5 and 10. Sample concentrations: $1.6 \cdot 10^{-2}$ *M* for P^1, $1.6 \cdot 10^{-3}$ *M* for P^3 and P^5. Other details as in Fig. 1.

sold larger than that of P^3 or P^5. In both cases, it is obvious that P^5 is almost completely feparated from P^1 and P^3 at pH 1.5, and P^1 from P^3 and P^5 at pH 10, as expected from the pH dependence of the K_d values[15].

In Table I are summarized the phosphorus amounts, the percentage recoveries of P^5, the sum of P^1 and P^3 at pH 1.5, the recoveries of P^1 and the sum of P^3 and P^5 at pH 10, respectively.

In a preliminary gel chromatographic run of P^1 stock solution at pH 10, P^1 was shown to be contaminated with 2.2% P^3. Therefore, the amounts taken of P^1 and P^3 were corrected on the basis of this result. The average recoveries (and the standard deviations) were as follows: 97.9% (1.8%) for the sum of P^1 and P^3, 101.3% (4.2%) for P^5 at pH 1.5; and 98.5% (8.5%) for P^1, 99.6% (5.5%) for the sum of P^3 and P^5 at pH 10. Besides experimental error, the deviation of the recoveries is probably attributable to the gradual oxidation of P^1 to P^3 in aqueous solution. Since the extent of oxidation was not evaluated in each run, the amounts taken of P^1 and P^3 in Table I are not corrected for this oxidation.

In Table II are given the amounts of P^3 and its recovery, which were obtained indirectly by subtracting the amount of P^1 at pH 10 from that of P^3 and P^1 at pH 1.5, or the amount of P^5 at pH 1.5 from that of P^3 and P^5 at pH 10. The average recovery of P^3 was 98.4% (10.1%).

It is concluded that P^1 and P^5 were separated satisfactorily from the mixtures of P^1, P^3 and P^5 by this chromatographic method.

Separation of oxo anions of phosphorus on Bio-Gel P-2

Fig. 3 shows the pH dependence of the K_d values of P^1, P^3 and P^5 obtained on

TABLE II

PHOSPHORUS AMOUNTS AND RECOVERIES OF P^3 ON SEPHADEX G-10
Sample concentrations as in Table I.

Sample No.	pH 1.5		pH 10		$(P^3)_{calc.}$			
	$(P^1+P^3)_{exp.}$ (μg P)	$(P^5)_{exp.}$ (μg P)	$(P^1)_{exp.}$ (μg P)	$(P^3+P^5)_{exp.}$ (μg P)	$(P^1+P^3)_{exp.}-(P^5)_{exp.}$ (μg P)	Recovery (%)	$(P^3+P^5)_{exp.}-(P^5)_{exp.}$ (μg P)	Recovery (%)
1	61.95	32.4	31.15	63.45	30.8	96.0	31.05	96.7
2	310.5	157.5	168	316.5	142.5	88.5	159	98.8
3	640	305.5	327.5	608.5	312.5	97.4	303	94.4
4	3155	1503	1371	3268	1784	111	1765	110
5	100	508.5	51.5	572.5	48.5	91.9	64.0	121
6	567.5	52.4	50.4	573	517	99.8	521	101
7	555.5	52.5	501.5	106	54.0	85.7	53.5	84.9

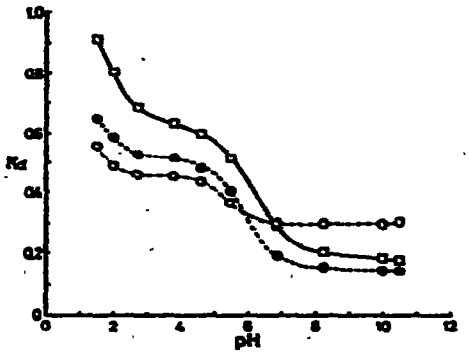

Fig. 3. pH dependence of K_d values of P^1, P^3 and P^5 on a Bio-Gel P-2 column (93 × 1.5 cm). Eluent: 0.1 *M* NaCl solutions at various pH values. ○, P^1; ●, P^3; □, P^5.

the Bio-Gel P-2 column with 0.1 *M* sodium chloride solutions at various pH as the eluent. As with Sephadex G-10 and G-15, the K_d values of the oxo anions of phosphorus vary significantly depending on the pH of the eluent, and the pH profiles are characteristic of the corresponding oxo anions. This behaviour on the Sephadex G gels was interpreted principally in terms of the different degrees of dissociation of the oxo acids depending on the pH of the eluent, as reported previously[15]. However, the pH profiles in Fig. 3 differ markedly from those obtained on Sephadex G-10 and G-15. The pH profile of P^1 has an additional inflexion point around pH 5, and the ΔK_d values for P^3 and P^5 are approximately twice those obtained on Sephadex G-10. These facts may be attributable to the properties of the gel matrix, which are dependent on the pH of the eluent.

Fig. 4 shows the pH dependences of the V_0, V_t and $V_t - V_0$ values on the Bio-Gel P-2 column, where V_0 is the void volume, V_t the total volume excluding the volume of the gel matrix and $V_t - V_0$ the internal volume of the gel phase. Blue Dextran 2000, a standard material with $K_d = 0$, had a tendency to adsorb on the gel, especially at pH 4–6. On the other hand, the elution peak of tritiated water, a standard with $K_d = 1$, was apt to shift to smaller elution volumes and to broaden at pH 3–5.

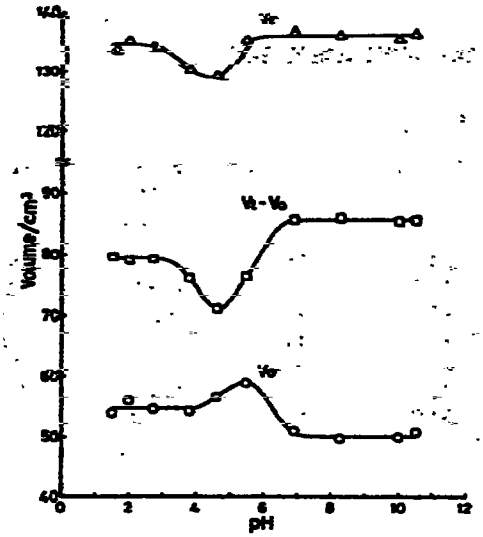

Fig. 4. pH dependences of V_0, V_t and $V_t - V_0$ values on a Bio-Gel P-2 column (93 × 1.5 cm). Eluent: 0.1 *M* NaCl solutions at various pH values.

Hence, the socalled "apparent" internal volume of the gel phase, $V_t - V_0$, had a minimum at pH 5. (The $V_t - V_0$ value on the Sephadex G-10 column was almost constant.) These trends are probably attributable to the protonation/deprotonation of the amide groups of the gel matrix. Therefore, the degree of Coulombic interaction between the solute anion and the gel matrix varies according to the electrostatic condition of the gel which in turn depends on pH. We have previously pointed out[18] that electrostatic interaction is one of the most important factors controlling the gel chromatographic behaviour of small inorganic ions on Sephadex G-10. In addition to the distribution of dissociative species of the oxo acids[15], the pH profiles of P^1, P^3 and P^5 would be affected by the variations in the $V_t - V_0$ value and also in the electrostatic interaction between the anions and the gel matrix, which are dependent on the pH of the eluent.

The pH profiles of the K_d values of P^1, P^3 and P^5 in Fig. 3 suggest that mixtures of these anions will be separated into their components by elution on the Bio-Gel P-2 column at pH <2.5. Fig. 5 shows the elution diagram of a mixture at pH 1.5. In contrast to the Sephadex G-10 column (Figs. 1 and 2), the oxo anions of phosphorus were separated almost completely in one chromatographic run, although the elution curve of P^1 overlapped partly with that of P^3. The separation mechanism on Bio-Gel P-2, including several kinds of side effects, is possibly different from that on Sephadex G-10.

Table III lists the phosphorus amounts and the percentage recoveries of P^1, P^3 and P^5. The average recovery is 99.85 ± 1.43%, much better than those on Sephadex G-10. The excellent accuracy of the recoveries is probably due to the fact that the chromatographic runs were carried out within 2 days of the preparation of the sample solution.

In conclusion, the monomeric oxo anions of phosphorus could be separated quantitatively by the gel chromatographic technique on Sephadex G-10 and Bio-Gel

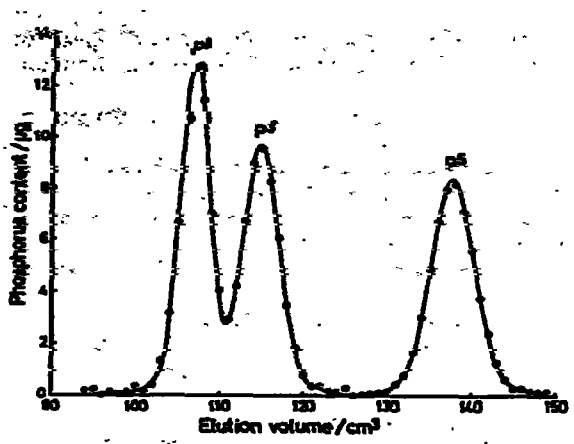

Fig. 5. A typical elution diagram on a Bio-Gel P-2 column at pH 1.5. Sample concentration: $2 \cdot 10^{-3}$ *M* for each of P^1, P^3 and P^5. Column: 93 × 1.5 cm. Eluent: 0.1 *M* NaCl solution at pH 1.5.

TABLE III

PHOSPHORUS AMOUNTS AND RECOVERIES IN THE SEPARATION OF MONOMERIC OXO ANIONS OF PHOSPHORUS ON BIO-GEL P-2

Eluent: 0.1 *M* NaCl solution at pH 1.5. Sample concentration, $2 \cdot 10^{-3}$ *M*.

Sample	*Amount*		
	Taken (μg P)	*Found (μg P)*	*Recovery (%)*
P^1	63.65	63.13	99.1
		63.82	100.3
P^3	57.23	56.98	99.6
		55.97	97.8
P^5	59.04	60.28	102.1
		59.18	100.2

P-2 columns. This method will be useful for sample solutions to which the anion exchange method is inapplicable because of the coexistence of salts at high concentrations.

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