Separation and Quantification for Various Phosphorus Oxoacids by Isotachophoresis

Takao YAGI,† Kayoko KOJIMA,† Hiroyuki NARIAI, and Itaru MOTOOKA*
Department of Chemistry, Faculty of General Education, Kobe University, Nada-ku, Kobe 657
†*Analytical Application Laboratory, Shimadzu Corporation, Nakagyo-ku, Kyoto 604*
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The analytical procedure for the separation and quantification of various phosphorus oxoacids was investigated by the capillary type isotachophoresis. The various phosphorus oxoacids, such as linear-, cyclic-, and lower phosphorus oxoacids, were separated at pH 5.5 with a 0.01 mol dm^{-3} hydrochloric acid–histidine–0.1% triton X-100 mixture as the leading electrolyte and a 0.01 mol dm^{-3} hexanoic acid as the terminating electrolyte. The PU value increased in the order of $P_{3m}<P_{4m}<P_3<P_2<\overset{1}{P}<\overset{3}{P}<P_1$. The calibration curves for the various phosphorus oxoacids were linear in the range of 0.5—3.5 μg as phosphorus oxoacids. Coefficients of variation for the measured phosphorus oxoacids were less than 2% except for P_3. Separation times were approximately 15 min. This procedure was applied to the ground phosphates.

Phosphorus oxoacids and their salts have an important role as fertilizers, detergents, watersofteners *etc.* As they have many varieties, paper- and ion exchange chromatography have been used for the separation and quantification of them. A disadvantage of these method is that they are laborious and time consuming.

Recently, more rapid and less laborious methods were developed by the use of a high liquid chromatography and an automatic phosphate analyzer.[1-4]

In the present paper, a procedure for the separation and quantification for chain-, cyclic-, and lower phosphorus oxoacids by isotachophoresis is described to remove the above disadvantage. It is applied to the ground phosphates.

Mikkers, *et al.*[5-7] have also attempted an isotachophoresis of phosphates, but they have only analyzed chain phosphates.

Experimental

Samples. Reagent-grade sodium phosphinate monohydrate ($NaPH_2O_2\cdot H_2O$; $\overset{1}{P}$) was used. Sodium phosphonate (Na_2PHO_3; $\overset{3}{P}$) and sodium dihydrogenorthophosphate (NaH_2PO_4; $\overset{5}{P}$ or P_1) were obtained by heating Wako's reagents to dehydrate water of crystallization. Tetrasodium pyrophosphate ($Na_4P_2O_7$; P_2), pentasodium triphosphate ($Na_5P_3O_{10}$; P_3) and trisodium *cyclo*-triphosphate ($Na_3P_3O_9$; P_{3m}) were prepared by heating sodium orthophosphate, purified by recrystallization, and dehydrated water of crystallization. Tetrasodium *cyclo*-tetraphosphate ($Na_4P_4O_{12}$; P_{4m}) was prepared according to the method of Bell *et al.*[8]

All phosphorus oxoanions were standardized by paper chromatography. The ground samples were obtained by grinding $Na_2H_2P_2O_7$ and $Na_5P_3O_{10}$, respectively, for the specified lengths of time in an AGA-type Ishikawa's mill with an agate mortor.

Apparatus and Procedure. A Shimadzu Capillary Tube Isotachophoretic Analyzer IP-2A was used. The isotachophoretic tubes for the separation composed of a main capillary column (0.5 mm i.d., 100 mm in length) and a pre-column (1.0 mm i.d., 100 mm in length). The conditions of the isotachophoretic analysis are given in Table 1. After the electric driving current of 200 μA was applied for 12 min to shorten the analytical time, that of 100 μA was done. The potential gradient detector was used. Paper chromatography was performed as reported previously.[9]

Table 1. Operational system for separation of phosphorus oxoacids

	Electrolyte	
	Leading	Terminating
Anion	0.01 mol dm^{-3} Cl^-	Hexanoate$^-$
Counterion	Histidine$^+$	H^+
pH	4.5—6.0	3.4
Additive	0.1% Triton X-100	
Solvent	Water	Water
Capillary tube	1.0×100 mm+0.5×100 mm	
Current	200 μA (12 min)→100 μA	
Detector	PGD	
Temperature	25 °C	
Chart speed	40 mm min^{-1}	

Results and Discussion

The separation of the various phosphorus oxoanions is affected by the pH of leading solution. The relationship between the pH of the leading solution and the *PU* value, which is an indicator in qualitative analysis, is shown in Fig. 1. When the *PU* values of oxoanions are close, a mixed zone of them is formed. It can be seen from Fig. 1 that the separation at pH 4.5, 5.5, and 6.0 is unfavourable, but good separation is obtained in the range of pH 5.3—5.7. Therefore, seven varieties of the phosphorus oxoacids were separated at pH 5.5.

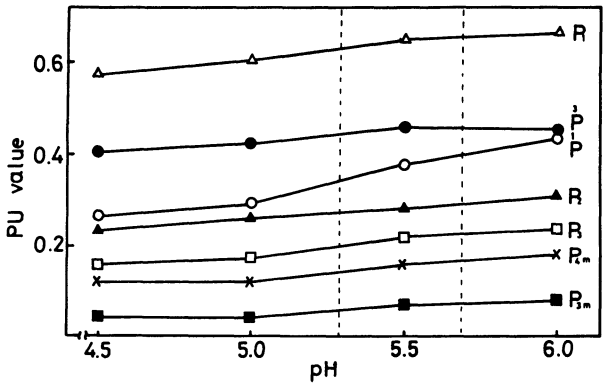

Fig. 1. Effect of pH of leading electrolyte on *PU* value.

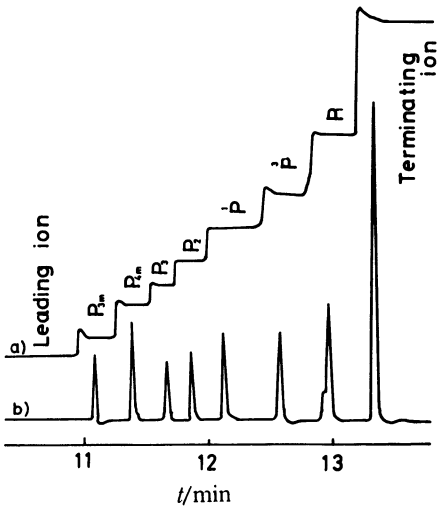

Fig. 2. Isotachopherogram of various phosphorus oxoacids.
a): Potential gradient, b): differential potential gradient.

Figure 2 shows the isotachopherogram of the various phosphorus oxoacids. An order of the *PU* value is $P_{3m}<P_{4m}<P_3<P_2<{}^{1}P<{}^{3}P<P_1$. These results indicate that the electrophoretic mobility of the chain phosphate ion increased with an increase in the number of phosphorus atoms, that of the cyclic phosphate ion decreased with an increase in the number of phosphorus atoms, and that of the lower phosphorus oxoanion with different oxidation numbers decreased with an increase of oxidation numbers.

TABLE 2. REPRODUCIBILITY AND MOLAR RESPONSE FOR VARIOUS PHOSPHORUS OXOACIDS

	PU value		Zone length		Molar response[c]
	$\bar{X}^{a)}(n=8)$	$CV^{b)}$ %	$\bar{X}^{a)}(n=8)$ mm	$CV^{b)}$ %	10^8 mm mol^{-1}
${}^{1}P$	0.394	1.02	36.8	1.09	5.57(0.95)
${}^{3}P$	0.498	0.80	29.3	1.02	5.28(0.90)
${}^{5}P(P_1)$	0.661	0.76	34.1	1.76	5.85(1.00)
P_2	0.305	2.95	19.4	1.55	7.38(1.26)
P_3	0.233	2.58	15.2	5.26	7.79(1.33)
P_{3m}	0.074	1.35	24.2	0.83	10.57(1.81)
P_{4m}	0.170	2.35	22.1	0.90	12.85(2.20)

a) Average value. b) Coefficient of variation ($CV=\sigma/\bar{X}\times 100$). c) Value in parentheses is relative molar response (as $P_1=1.00$).

Table 2 shows the reproducibilities of the *PU* value and the zone length, and molar response. All coefficients of variation (*CV*%) of the *PU* value were less than 3% and those of the zone length were less than 2% except for P_3. A high *CV* value (5.25%) of P_3 is due to only one abnormal data. If this abnormal data is removed, the *CV* value of P_3 is 2.22%. The molar response for the various phosphorus oxoacids in Table 2 was calculated from the zone length on the isotachopherogram and the amount of injected phosphorus oxoacids. The value in parentheses represents the relative molar response which is the value converted as that of P_1 is 1.00. It can be seen from these results that the relative molar response of both chain- and cyclic phosphates increased with an increase in the number of phosphorus atoms of the phosphates, that of the cyclic phosphates is higher value than that of the chain phosphates, and that of phosphorus oxoacids with different oxidation numbers doesn't depend on the oxidation numbers.

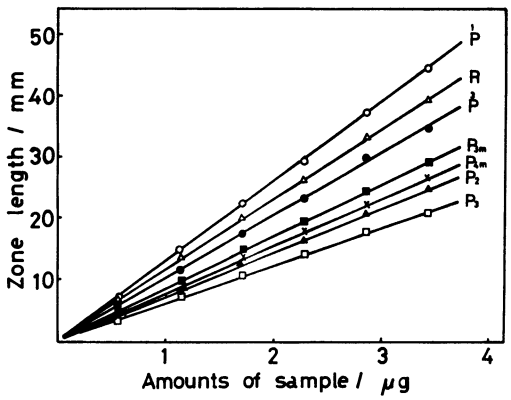

Fig. 3. Calibration curves of various phosphorus oxoacids.
Chart speed: 40 mm min^{-1}.

Figure 3 shows the calibration curves for seven varieties of phosphorus oxoacids. These phosphorus oxoacids were injected simultaneously. Linear relationship were obtained over the range of 0.5—3.5 μg of sodium salts of phosphorus oxoacids, but the calibration curve was nonlinear above 3.5 μg because the phosphorus oxoanions with adjacent *PU* values formed the mixed zone. When a single phosphorus oxoacid was injected, a linear relationship was obtained over a wide range of the amount of phosphorus oxoacids, 0.5—30.0 μg.

As the linear region of the calibration curve depends on the volume of the capillary tube, it is preferable to select an adequate capillary tube to obtain good results.

Application

The procedure mentioned above was applied to the separation and quantification of the ground phosphates.

The P–O–P linkages of the chain phosphates, such as pyro- and triphosphate, were severed to shorter chain

TABLE 3. ANALYTICAL RESULTS OF GROUND PYROPHOSPHATE

Grinding time/h	IP[a]		PC[b]	
	P_1(%)	P_2(%)	P_1(%)	P_2(%)
6	4.2	95.8	5.3	94.7
24	8.6	91.4	7.1	92.9
48	11.0	89.0	12.7	87.3
96	13.9	86.1	16.8	83.2
168	25.3	74.7	24.4	75.6

a) Isotachophoresis. b) Paper chromatography.

Table 4. Analytical results of ground triphosphate

Grinding time/h	IP[a]			PC[b]		
	P_1(%)	P_2(%)	P_3(%)	P_1(%)	P_2(%)	P_3(%)
6	6.5	26.0	67.5	8.7	26.2	65.1
48	10.4	46.3	43.3	11.1	46.2	42.7
72	12.2	60.0	27.8	14.5	57.4	28.1
168	17.4	72.7	9.9	19.1	72.7	8.2

a) Isotachophoresis. b) Paper chromatography.

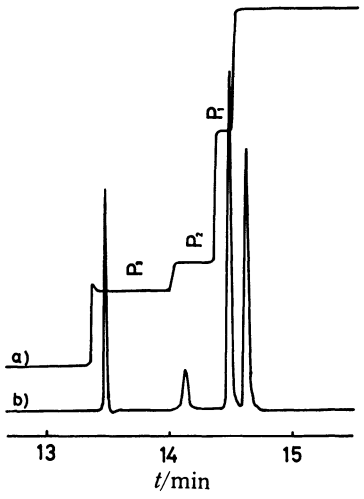

Fig. 4. Isotachopherogram of ground triphosphate.
a): Potential gradient, b): differential potential gradient.

phosphates due to grinding.[10] Therefore, the ground samples contained the different chain-length phosphates were obtained by grinding for different times.

Figure 4 is the representative isotachopherogram of the ground triphosphate. The results of the isotachophoresis of these ground samples have been summarized in Tables 3 and 4. These results are comparable with those of paper chromatography.

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