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SEPARATION AND QUANTIFICATION OF VARIOUS PHOSPHORUS OXOACIDS CONTAINING TWO PHOSPHORUS ATOMS BY ISOTACHOPHORESIS

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SUMMARY

The separation and determination of various phosphorus oxoacids containing two phosphorus atoms, such as $^{5}P–O–^{5}P$, $^{3}P–O–^{5}P$ and $^{3}P–O–^{3}P$ salts, by capillary isotachophoresis was investigated. The potential unit (PU) values, which are indicators in quantitative analysis, increased in the order $^{3}P–O–^{3}P < ^{3}P–O–^{5}P < ^{5}P–O–^{5}P$. The calibration graphs for these oxoacids were linear in the range $0–10^{-7}$ mole as phosphorus oxoacids. Separation times were approximately 20 min.

The procedure was applied to the analysis of some crude products and to the study of the hydrolytic degradation of some condensed phosphates. The amount of each phosphorus oxoacid could be determined rapidly and easily, and the results were in good agreement with those obtained by ion-exchange chromatography.

INTRODUCTION

Phosphorus oxoacids and their salts are widely used in food products and processing, detergents and cleaning compounds, etc. For their separation and determination, paper, gel and ion-exchange chromatography have been used. These methods lack speed and simplicity.

In this work, the separation and determination of some phosphorus oxoacids containing two phosphorus atoms, such as $^{5}P–O–^{5}P$, $^{3}P–O–^{5}P$ and $^{3}P–O–^{3}P$, by isotachophoresis was investigated and was applied to the analysis of some crude products and hydrolytic degradation products of $^{3}P–O–^{5}P$ and $^{3}P–O–^{3}P$.

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EXPERIMENTAL

Reagents

Unless otherwise stated, guaranteed reagents from Wako (Osaka, Japan) were used without further purification.

Disodium phosphonate pentahydrate ($Na_2PHO_3 \cdot 5H_2O$; $\overset{3}{P}$), sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$; $\overset{5}{P}$) and tetrasodium diphosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$; $\overset{5}{P}$–O–$\overset{5}{P}$) were purified by repeated recrystallization. Disodium diphosphonate ($Na_2P_2H_2O_5$; $\overset{3}{P}$–O–$\overset{3}{P}$) and trisodium isohypophosphate tetrahydrate ($Na_3P_2HO_6 \cdot 4H_2O$; $\overset{3}{P}$–O–$\overset{5}{P}$) were prepared according to the method given in the literature[1–5].

Isotachophoresis

A Shimadzu, IP-2A Capillary Tube Isotachophoretic Analyzer was used. The isotachophoretic tubes for the separation consisted of a main capillary column (100 mm × 0.5 mm I.D.) and a pre-column (100 mm × 1.0 mm I.D.).

The operational system of the isotachophoretic analysis is given in Table I. The analytical procedure used was as described previously[6].

TABLE I

OPERATIONAL SYSTEM FOR SEPARATION OF PHOSPHORUS OXOACIDS

Parameter	*Electrolyte*	
	Leading	*Terminating*
Anion	Cl^-	Hexanoate$^-$
Concentration	0.01 *M*	0.01 *M*
Counter ion	Histidine$^+$	H^+
pH	5.5	3.4
Additive	0.1% Triton X-100	
Solvent	Water	Water
Capillary tube	100 × 1.0 mm I.D. + 100 × 0.5 mm I.D.	
Current	200 μA (12 min) → 100 μA	
Detector	Potential gradient detector	
Temperature	20°C	
Chart speed	20 or 40 mm min^{-1}	

Anion-exchange chromatography

The gradient elution technique was employed to separate phosphorus oxoacids by anion-exchange chromatography. The elution conditions were as follows: column, 65 × 1.5 cm I.D., Bio-Rad AG 1-X8 (100–200 mesh); mixing bottle, 0.08 *M* sodium

chloride–0.005 *M* EDTA-Na_2 mixed solution, 750 ml; reservoir, 0.2 *M* sodium chloride–0.005 *M* EDTA-Na_2 mixed solution; fraction size, 10 g; sample volume, 1 ml.

The pH of the eluting solution was adjusted to 7.0 with dilute sodium hydroxide solution.

Colorimetric determination and identification of phosphorus oxoacids

The colorimetric determination was carried out according to the orthophosphoric heteropoly-blue method, by means of which phosphorus as oxidation states 3 and 5 can be determined simultaneously[7–9].

Hydrolytic degradation of $^{3}P–O–^{5}P$ *and* $^{3}P–O–^{3}P$

Hydrolytic degradation of $^{3}P–O–^{5}P$ and $^{3}P–O–^{3}P$ was started with the addition of hydrochloric acid. The pH of reaction mixtures was 2.0. After fixed time intervals, samples were removed with a 2-ml pipette into a test-tube, instantly neutralized with sodium hydroxide solution and cooled in an ice-bath to stop hydrolytic degradation.

RESULTS AND DISCUSSION

The separation of the phosphorus oxoacids is affected by the pH of the leading solution. Figs. 1 and 2 show the relationship between the pH of the leading solution and the PU values of five phosphorus oxoacids. The decreasing order of PU* values is $^{5}P > {}^{3}P > {}^{5}P–O–{}^{5}P > {}^{3}P–O–{}^{5}P > {}^{3}P–O–{}^{3}P$. With L-glutamic acid as the terminating electrolyte, the PU values of the phosphorus oxoacids differ sufficiently except at pH

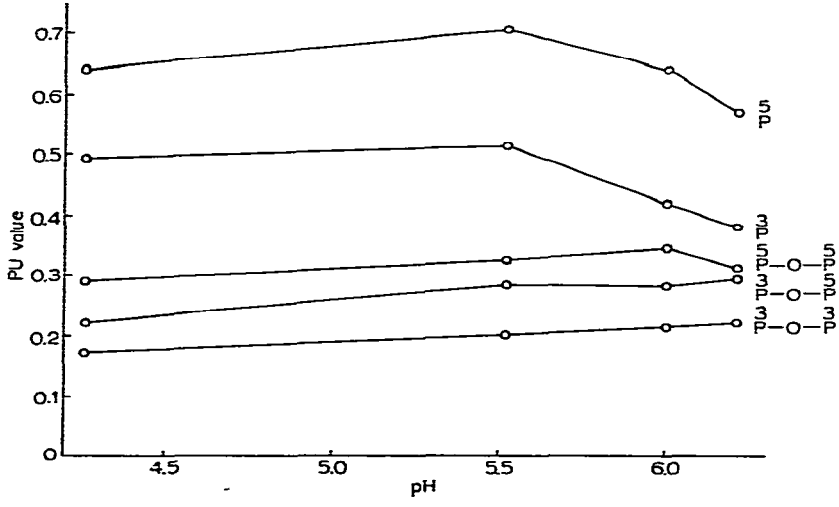

Fig. 1. Effect of pH of leading electrolyte on PU values. Leading electrolyte: L-histidine. Terminating electrolyte: L-glutamic acid.

* The PU (potential unit value) represents the ratio of the potential gradient difference of the sample ion to that of the leading ion and of the terminating ion to that of the leading ion ($P_S - P_L/P_T - P_L$).

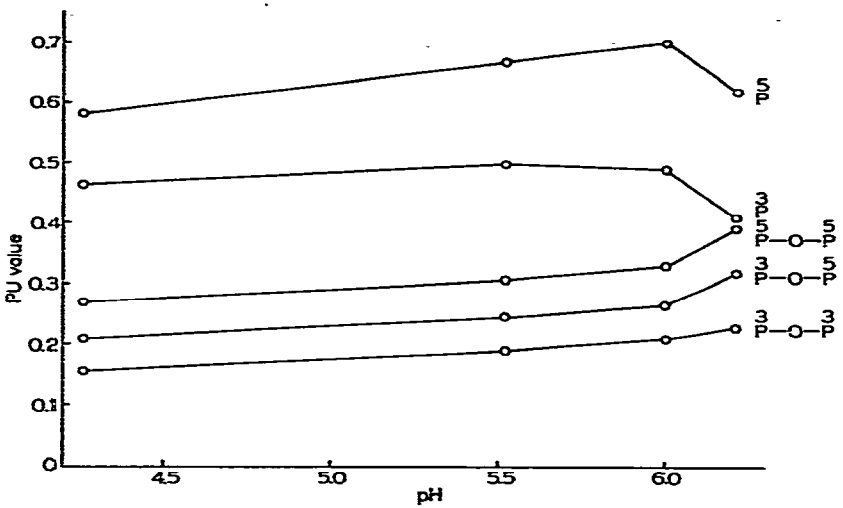

Fig. 2. Effect of pH of leading electrolyte on PU values. Leading electrolyte: L-histidine. Terminating electrolyte: hexanoic acid.

6.2, where the values for $^{5}P–O–^{5}P$ and $^{3}P–O–^{5}P$ are so close that their separation is difficult.

With hexanoic acid as the terminating electrolyte, the PU values also differ sufficiently, except at pH 6.2, where ^{3}P and $^{5}P–O–^{5}P$ are too close to separate. Hence good separations are achieved at pH values below 6.0 with both the terminating electrolytes. In this work, a pH of 5.5 was chosen with histidine as the counter ion and hexanoic acid as the terminating electrolyte.

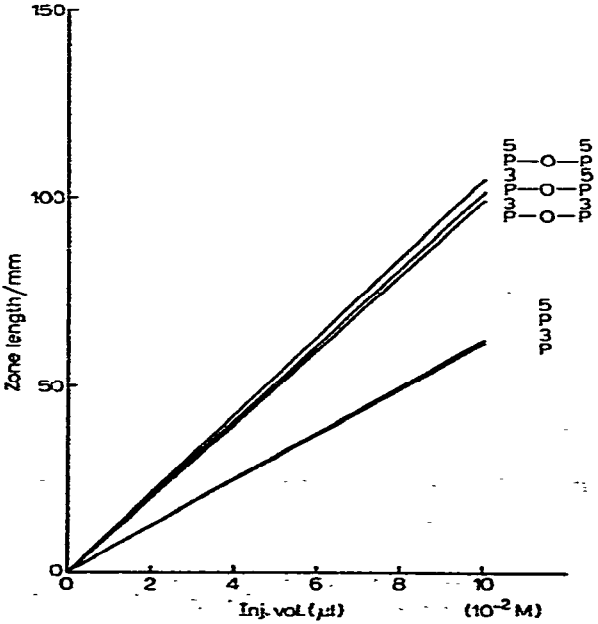

Fig. 3. Calibration graphs of the different phosphates.

Fig. 3 shows the calibration graphs for five varieties of phosphorus oxoacids. Linear relationships were obtained over the range of $0–10^{-7}$ mole of phosphorus oxoacids.

The slopes of the calibration graphs for $\overset{5}{P}–O–\overset{5}{P}$, $\overset{5}{P}–O–\overset{3}{P}$ and $\overset{3}{P}–O–\overset{3}{P}$, and for $\overset{5}{P}$ and $\overset{3}{P}$, are approximately equal.

From the relationship between zone length and the amount of the phosphorus oxoacid species, the zone length per phosphorus atom is almost the same for each. This finding shows that the volumes of $\overset{5}{P}–O–\overset{5}{P}$, $\overset{5}{P}–O–\overset{3}{P}$ and $\overset{3}{P}–O–\overset{3}{P}$, and those of $\overset{5}{P}$ and $\overset{3}{P}$, in the capillary tube during electrophoresis are almost equal.

Fig. 4 shows a typical isotachopherogram of a mixed sample of $\overset{5}{P}–O–\overset{5}{P}$, $\overset{5}{P}–O–\overset{3}{P}$ and $\overset{3}{P}–O–\overset{3}{P}$. The three phosphorus oxoacids were successfully separated, their PU values increasing in the order $\overset{3}{P}–O–\overset{3}{P} < \overset{3}{P}–O–\overset{5}{P} < \overset{5}{P}–O–\overset{5}{P}$. Although $\overset{3}{P}$ and $\overset{5}{P}$ were included as contaminants, they did not interfere in the separation of the three phosphorus oxoacids because their PU values were greater. The injection volume of the samples (10^{-2} *M*) had to be less than 2 μl because with more than 4 μl a mixed zone was formed.

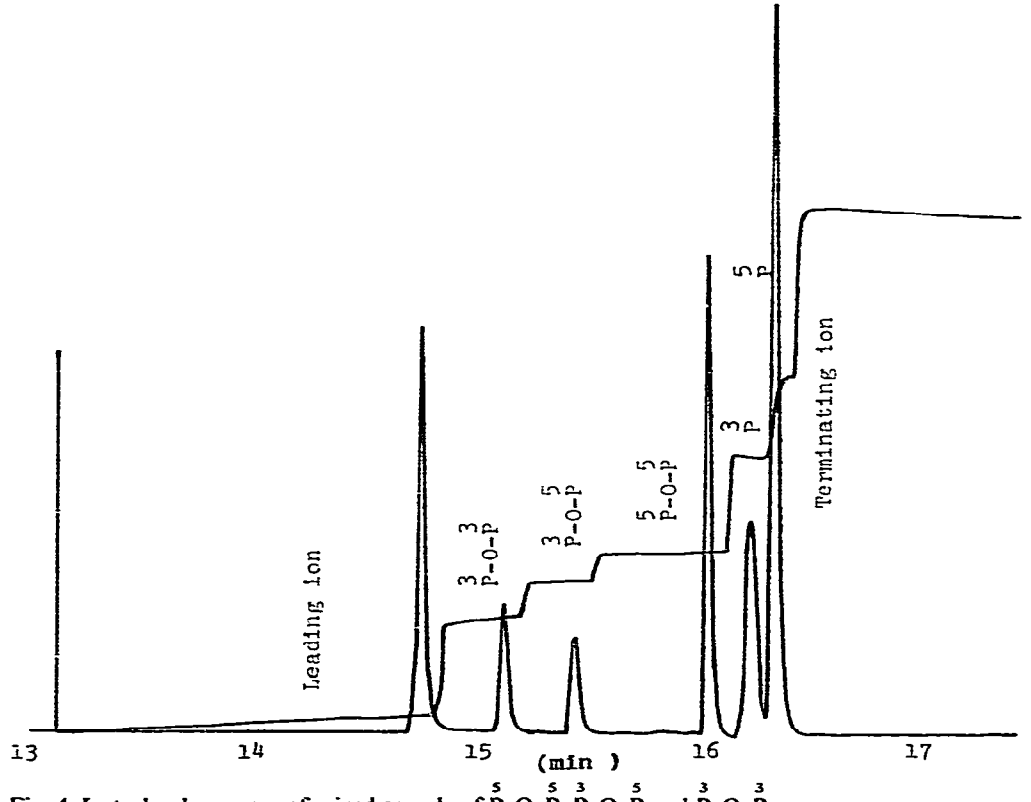

Fig. 4. Isotachopherogram of mixed sample of $\overset{5}{P}–O–\overset{5}{P}$, $\overset{5}{P}–O–\overset{3}{P}$ and $\overset{3}{P}–O–\overset{3}{P}$.

Application

The above procedure was applied to the analysis of some crude products and to the study of the hydrolysis of some condensed phosphates, *e.g.*, $^{3}P\text{–}O\text{–}^{5}P$ and $^{3}P\text{–}O\text{–}^{3}P$.

Analysis of crude products. As the salts of $^{3}P\text{–}O\text{–}^{5}P$ and $^{3}P\text{–}O\text{–}^{3}P$ were synthesized, they are always contaminated by some impurities. Figs. 5 and 6 show typical iso-

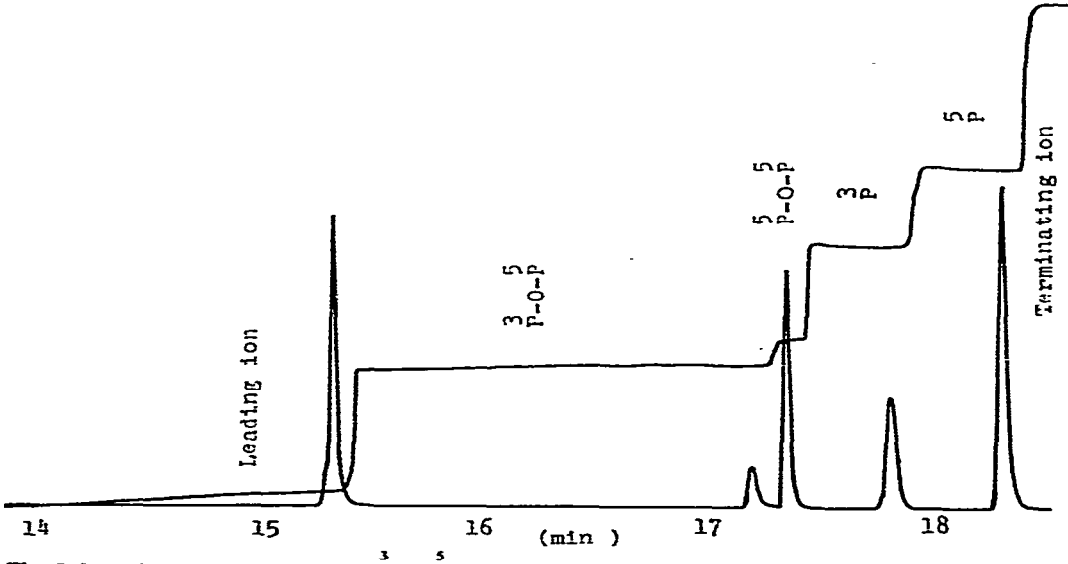

Fig. 5. Isotachopherogram of crude $^{3}P\text{–}O\text{–}^{5}P$.

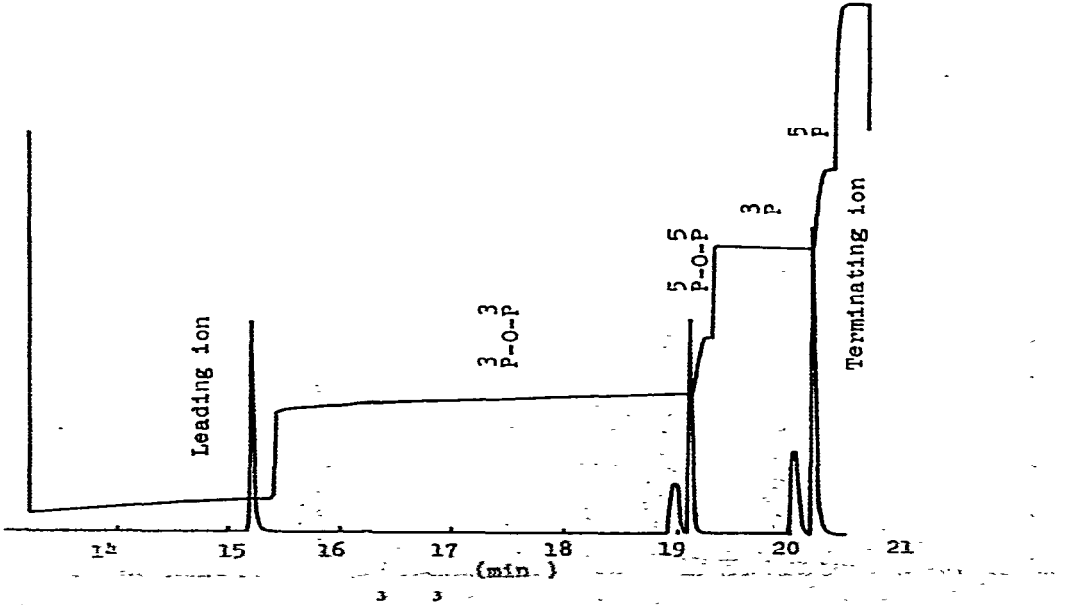

Fig. 6. Isotachopherogram of crude $^{3}P\text{–}O\text{–}^{3}P$.

tachopherograms of the crude $^{3}P\text{–}O\text{–}^{5}P$ and $^{3}P\text{–}O\text{–}^{3}P$. Table II compares the results for some crude products obtained by isotachophoresis (IP) and by ion-exchange chromatography (IEC). The amounts of each phosphorus oxoanion were determined from the zone length. IP and IEC gave comparable results, but the former method was far superior in speed of analysis and ease of handling.

TABLE II

COMPARISON OF IP AND IEC RESULTS

Sample	^{5}P (%)		^{3}P (%)		$^{5}P\text{–}O\text{–}^{5}P$ (%)		$^{3}P\text{–}O\text{–}^{5}P$ (%)		$^{3}P\text{–}O\text{–}^{3}P$ (%)	
	IP	*IEC*	*IP*	*IEC*	*IP*	*IEC*	*IP*	*IEC*	*IP*	*IEC*
$^{3}P\text{–}O\text{–}^{3}P$	2.4	3.1	15.9	16.6	2.2	1.7			79.5	78.6
$^{3}P\text{–}O\text{–}^{3}P$	1.1	0.5	12.4	14.8			6.6	4.8	79.8	79.8
$^{3}P\text{–}O\text{–}^{5}P$	14.6	14.7	13.7	13.8	3.1	2.7	68.7	68.8		
$^{3}P\text{–}O\text{–}^{5}P$	3.8	3.3	3.9	3.6			92.3	93.1		

Hydrolysis of $^{3}P\text{–}O\text{–}^{5}P$ and $^{3}P\text{–}O\text{–}^{3}P$. Hydrolytic degradation products were analysed by IP, and the results were in good agreement with those obtained by IEC. The plots of log (zone length) *versus* time for $^{3}P\text{–}O\text{–}^{5}P$ and $^{3}P\text{–}O\text{–}^{3}P$ were straight lines, indicating that the hydrolytic degradation of these phosphorus oxoanions obeys first-order kinetics. The first-order rate constants were determined from the values of the half-life period: for $^{3}P\text{–}O\text{–}^{5}P$, $k_{20} = 0.30 \cdot 10^{-2}$, $k_{30} = 0.75 \cdot 10^{-2}$ and $k_{40} = 1.78 \cdot 10^{-2}$ min^{-1}; for $^{3}P\text{–}O\text{–}^{3}P$, $k'_{20} = 0.54 \cdot 10^{-2}$, $k'_{30} = 1.54 \cdot 10^{-2}$ and $k'_{40} = 3.37 \cdot 10^{-2}$ min^{-1}. The hydrolysis of $^{3}P\text{–}O\text{–}^{3}P$ proceeded more rapidly than that of $^{3}P\text{–}O\text{–}^{5}P$. Values of the activation energies (E_a) of 16 kcal mol^{-1} for both $^{3}P\text{–}O\text{–}^{5}P$ and $^{3}P\text{–}O\text{–}^{3}P$ were determined from Arrhenius plots.

The values of k, k' and E_a agreed well with the literature values[10,11], and these quantities can therefore be determined rapidly and easily by IP.

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