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Note

Analyses of some phosphates by isotachophoresis with an internal standard

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There are many kinds of phosphates: linear and cyclic phosphates with different numbers of phosphorus atoms, and some with different oxidation numbers[1]. The isotachophoretic analysis (the mutual separation and determination) of some typical phosphates by the use of the absolute calibration-curve method has been reported, and gives good results[2–4].

In this work, some phosphates, P_1, P_2, P_3, P_{3m} and P_{4m}, were determined by isotachophoresis, with EDTA, oxalate and acetate anions as internal standards.

EXPERIMENTAL

Reagents

The disodium salt of EDTA ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$), sodium oxalate ($Na_2C_2O_4$), sodium acetate ($CH_3COONa \cdot H_2O$) and sodium orthophosphate ($NaH_2PO_4 \cdot 2H_2O$, P_1) were reagent grade commercial materials, used without further purification. Sodium pyrophosphate ($Na_4P_2P_7 \cdot 10H_2O$, P_2), sodium triphosphate ($Na_5P_3O_{10} \cdot 6H_2O$, P_3) and sodium cyclo-triphosphate ($Na_3P_3O_9 \cdot 6H_2O$, P_{3m}) were prepared by heating sodium orthophosphate at 250, 550 and 300°C, respectively, and then purified by recrystallization. Sodium cyclo-tetraphosphate ($Na_4P_4O_{12} \cdot 4H_2O$, P_{4m}) was prepared according to the method of Bell *et al.*[5]. Sodium diphosphonate ($Na_2P_2H_2O_5$, $\overset{3}{P}\text{–O–}\overset{3}{P}$) was obtained by heating sodium phosphonate at 160°C under reduced pressure and purified by several recrystallizations. All anions of phosphorus oxoacids were standardized by paper chromatography and isotachophoresis.

Sample for isotachophoresis

A 5-ml volume of each internal standard solution ($1.25 \cdot 10^{-2}$ mol/l), EDTA, sodium oxalate or sodium acetate, was placed in a 25-ml volumetric flask, various phosphates ($2.5 \cdot 10^{-2}$ mol/l phosphorus) were added such that the ratio of phos-

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phorus to the internal standard (P/I.S.) was 1/4, 1/3, 1/2, 1, 2, 3 and 4, respectively, and then the solution was diluted to the mark in water. The final concentration of the internal standard was $2.5 \cdot 10^{-3}$ mol/l. A 5-μl volume of the sample solution obtained was injected into the isotachophoretic analyzer. The system is the same as that previously used[3], except for the use of 0.01 mol/l hexanoate, pH 6.9, as the terminating anion.

Apparatus

A Shimadzu capillary tube isotachophoretic analyzer IP-2A equipped with isotachopac I-ElB was used.

RESULTS AND DISCUSSION

A typical isotachopherogram of the mixtures including five phosphate anions, P_1, P_2, P_3, P_{3m} and P_{4m}, and EDTA used as an internal standard is shown in Fig. 1. The six anions were clearly separated from each other because their potential unit (PU) values, which are indicators for qualitative analysis, differ sufficiently. A plot of the ratio of the zone length of these phosphates to that of the internal standard *vs.* their concentration ratio was linear within the concentration ratio range 1/4–4.

In the cases of oxalate and acetate anions as the internal standards, also a good separation and determination was obtained, except that P_{3m} is not separated from the oxalate anion.

The coefficient of variation (%) of the quantitative analysis of P_1 is summarized in Table I. The numerical values obtained by this (internal standard) method were less than 3%, being inferior to those (below 2%) obtainable by the absolute calibration-curve method. However, a great advantage of the present method is that little attention needs to be paid to the injection of the sample into the analyzer with a microsyringe.

Next, the effect of the injected sample size on the calibration curve was exam-

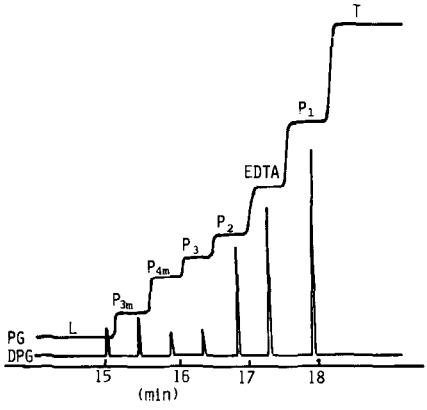

Fig. 1. Isotachopherogram of various phosphates and EDTA. L = Leading ion; T = terminating ion; PG = potential gradient; DPG = differential potential gradient.

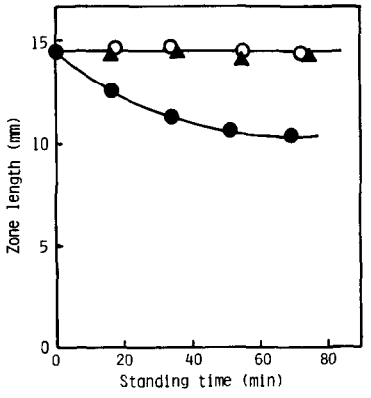

Fig. 2. Suppression of hydrolysis of the $\overset{3}{P}$–O–$\overset{3}{P}$ linkage (●) by EDTA (▲) and oxalate (○).

TABLE I

COEFFICIENT OF VARIATION (%) OF THE QUANTITATIVE ANALYSIS OF P_1

Ratio (P_1/*I.S.*)	*Internal standard method*			*Absolute calibration-curve method*
	EDTA	*Oxalate*	*Acetate*	
1	2.39	2.41	1.74	1.07
2	2.54	2.89	1.53	1.43
3	2.95	2.47	1.44	0.80
4	2.85	0.43	2.10	0.56
1/2	2.17	2.33	1.81	1.69
1/3	1.95	1.00	2.93	0.95
1/4	2.84	0.75	2.38	1.84

ined by changing the concentration of the sample and internal standard solution. The calibration curve was the same if the concentration of the sample and internal standard was halved, but deviated from linearity if the concentration was doubled. This result indicates that larger sample sizes give longer zone lengths which are undesirable for the quantitative analysis.

Application

The internal standard ions were employed also as masking agents for metal ions in order to prevent hydrolytic cleavage of the P–O–P linkage in phosphonate. (Note: phosphonate was also well separated and determined by this method.)

In general the anions of phosphorus oxoacids are subject to hydrolysis in the presence of metal ions. As EDTA and oxalate have masking ability[6], they act as both the internal standard and the masking agent.

Fig. 2 shows the change in the zone length of diphosphonate ($5 \cdot 10^{-3}$ mol/l $\overset{3}{\text{P}}\text{–O–}\overset{3}{\text{P}}$) in three situations: in the presence of (1) Fe^{3+} ($2 \cdot 10^{-5}$ mol/l), (2) Fe^{3+} and EDTA ($2 \cdot 10^{-3}$ mol/l) and (3) Fe^{3+} and oxalate ($2 \cdot 10^{-3}$ mol/l). In the presence of only Fe^{3+} the zone length decreased with increasing time because of hydrolysis of the $\overset{3}{\text{P}}\text{–O–}\overset{3}{\text{P}}$ linkage. On the other hand, when EDTA or oxalate is also present in solution, cleavage of this linkage is suppressed by their masking of the Fe^{3+}, resulting in no change in the zone length of $\overset{3}{\text{P}}\text{–O–}\overset{3}{\text{P}}$.

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