

Note

Anion-exchange chromatographic separation of phosphorus oxo-anions

TERUMASA NAKAMURA, TAKAYOSHI YANO, TOHRU NUNOKAWA and SHIGERU OHASHI

Department of Chemistry, Faculty of Science, Kyushu University 33, Hakozaki, Higashiku, Fukuoka 812 (Japan)

(Received May 23rd, 1978)

In our previous work¹, the anion-exchange chromatographic behaviour of various phosphorus oxo-anions was examined by use of Dowex 1-X8 resin with sodium chloride solution as the eluent, and some elution systems were proposed for the efficient separation of phosphorus oxo-anions by a single anion-exchange chromatographic run. However, it was demonstrated that the hydrolysis of P-O-P linkages in diphosphate anions takes place during anion-exchange chromatography with potassium chloride or sodium chloride solution as the eluent and that the presence of EDTA anions in the eluent resulted in the good separation of linear phosphates^{2,3}. It was therefore considered necessary to re-investigate the anion-exchange chromatographic separation of phosphorus oxo-anions with an eluent containing EDTA anions.

In this work we examined the possibilities of the separation of various phosphorus oxo-anions by a single anion-exchange chromatographic run with sodium chloride solution containing EDTA anions as the eluent.

EXPERIMENTAL

Reference substances for phosphorus oxo-anions

The following materials were used to determine the elution positions of individual phosphorus oxo-anions in anion-exchange chromatography: $\text{NaPH}_2\text{O}_2 \cdot \text{H}_2\text{O}$ [P(I)], $\text{Na}_2\text{PHO}_3 \cdot 5\text{H}_2\text{O}$ [P(III)], $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ [P(V)], $\text{Na}_2\text{H}_2\text{P}_2\text{O}_6 \cdot 6\text{H}_2\text{O}$ [P(IV)-P(IV)], $\text{Na}_2\text{P}_2\text{H}_2\text{O}_5$ [P(III)-O-P(III)], $\text{Na}_3\text{P}_2\text{HO}_6 \cdot 4\text{H}_2\text{O}$ [P(III)-O-P(V)], $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ [P(V)-O-P(V)], $\text{Na}_5\text{P}_3\text{O}_8 \cdot 14\text{H}_2\text{O}$ [P(IV)-P(III)-P(IV)], $\text{Na}_4\text{P}_3\text{HO}_8 \cdot \text{H}_2\text{O}$ [P(III)-O-P(IV)-P(IV)], $(\text{NH}_4)_5\text{P}_3\text{O}_9 \cdot x\text{H}_2\text{O}$ [P(V)-O-P(IV)-P(IV)], and $\text{Na}_{n+1}\text{P}_n\text{O}_{3n+1}$ with $\bar{n} = 4.5$.

Anion-exchange chromatography

Anion-exchange chromatography was carried out with a 65×1.3 cm I.D. column of Bio-Rad AG1-X8 (Cl^-) (100-200 mesh) and a multi-step gradient elution technique was employed¹. The eluent (containing 5 mM EDTA anions³) consisted in the mixing bottle of 0.02 M NaCl (750 ml) and in the reservoir of 0.12 M NaCl (fractions 1-150), 0.20 M NaCl (fractions 151-250), 0.35 M NaCl (fractions 251-350) and

1.50 M NaCl (fractions 351–400). The pH was *ca.* 7 and the flow-rate *ca.* 1 ml·min⁻¹.

The effluent was collected in 10-g fractions with an automatic fraction collector and the phosphorus content in each effluent fraction was determined colorimetrically with the formation of orthophosphoric and/or hypophosphoric heteropoly blue by use of a molybdenum(V)–molybdenum(VI) reagent⁴. The color development and the absorbance measurements were carried out by the procedures described in a previous paper¹.

RESULTS AND DISCUSSION

A typical anion-exchange chromatogram is shown in Fig. 1. In the first step of this chromatographic run, three kinds of monophosphorus oxo-anions, *viz.*, P(I), P(III) and P(V) anions, and two kinds of diphosphorus oxo-anions, *viz.*, P(IV)–P(IV) and P(III)–O–P(III) anions, were eluted, although the latter pair was poorly resolved and could be distinguished only by colorimetric analysis with the molybdenum(V)–molybdenum(VI) reagent and 1 M sodium hydrogen sulphite solution. Two kinds of diphosphorus oxo-anions, *viz.*, P(III)–O–P(V) and P(V)–O–P(V) anions, and triphosphorus P(IV)–P(III)–P(IV) anions were eluted in the second step, although two peaks for these diphosphorus P–O–P-anions partly overlapped. At the start of the

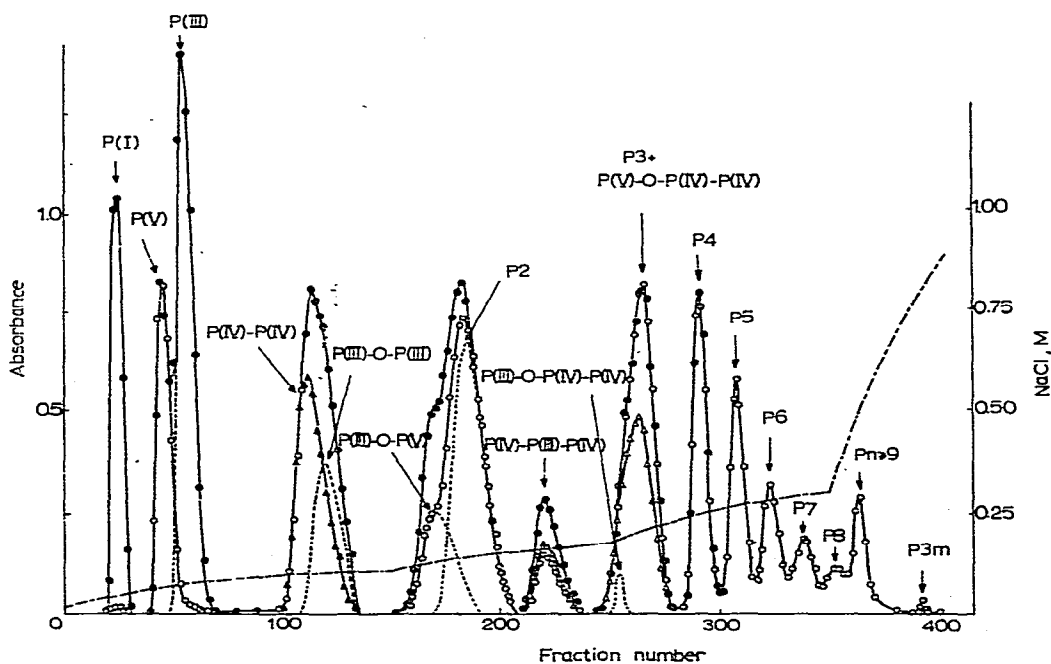


Fig. 1. Anion-exchange chromatogram of phosphorus oxo-anions. Diphosphate and higher oligophosphates are abbreviated to P2, P3, etc. Conditions for colour development and absorbance measurement: ○, Mo(V)–Mo(VI) reagent, $\lambda = 810$ nm; ●, Mo(V)–Mo(VI) reagent + 1 M NaHSO₃, $\lambda = 810$ nm; △, Mo(V)–Mo(VI) reagent, $\lambda = 610$ nm; ▲, Mo(V)–Mo(VI) reagent + 1 M NaHSO₃, $\lambda = 610$ nm. -----, calculated curve from the absorbance data; - · - · -, concentration of NaCl in the eluent.

third step, three kinds of triphosphorus oxo-anions, viz., P(III)-O-P(IV)-P(IV), P(V)-O-P(IV)-P(IV) and triphosphate anions, were eluted. The first occurred just before the completely overlapped peaks of the latter two triphosphorus oxo-anions. Tetra- to heptaphosphate anions were eluted successively in the later stages of the third step. The peak of octaphosphate anions appeared at the point of change to the final step and was followed by the peak of nona- and higher oligophosphate anions. A small peak of trimetaphosphate anions included in glassy sodium oligophosphate was eluted in the later stages of the final step.

In this chromatographic system, both chloride and EDTA anions are in competition with phosphorus oxo-anions for the ionic sites on the anion-exchange resin. With the concentration of EDTA anions fixed at 5 mM, some preliminary chromatographic runs were carried out with different sodium chloride concentrations in the eluents, but a substantial improvement in the resolution of the phosphorus oxo-anions compared with the results given in Fig. 1 was not obtained.

It is concluded that many kinds of phosphorus oxo-anions can be separated as well resolved peaks by multi-step exponential gradient elution, although some phosphorus oxo-anions are eluted very close to each other. At present, the system employed here is considered to be the most useful for the separation of a mixture of phosphorus oxo-anions with different oxidation states and structural complexities.

REFERENCES

- 1 M. Tominaga, T. Nakamura and S. Ohashi, *J. Inorg. Nucl. Chem.*, 34 (1972) 1409.
- 2 T. Fukuda, T. Nakamura and S. Ohashi, *J. Chromatogr.*, 128 (1976) 212.
- 3 T. Nakamura, T. Yano, A. Fujita and S. Ohashi, *J. Chromatogr.*, 130 (1977) 384.
- 4 N. Yoza, K. Ishibashi and S. Ohashi, *J. Chromatogr.*, 134 (1977) 497.