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Detection systems with a photodiode-array detector for flow-injection and high-performance liquid chromatographic determination of phosphinate, phosphonate and diphosphonate

Tetsuya Nakazato and Norimasa Yoza*

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

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ABSTRACT

Spectrophotometric detection systems for flow-injection analysis and high-performance liquid chromatography were designed for the determination of phosphinate, phosphonate, diphosphonate and isohypophosphate of lower oxidation numbers. Preoxidation and/or hydrolysis of these compounds to orthophosphate by peroxodisulphate in an oxidation reactor (140°C) were followed by a colour reaction with a molybdenum(VI) reagent in a second reactor. A photodiode-array detector (200–800 nm) was used to obtain the spectrophotometric characteristics of the coloured species. An unpredicted absorption spectrum was observed for the reaction product between phosphinate and molybdenum(VI). Both advantages and disadvantages of using an anion-exchange column (TSKgel SAX) for the separation of monomers and dimers, including orthophosphate and diphosphate (pyrophosphate), are discussed.

INTRODUCTION

As shown in Table I, various inorganic phosphorus compounds of different oxidation states are known. Orthophosphate and diphosphate (pyrophosphate) of oxidation number +5 are the most popular and important compounds in industrial applications and biological metabolism [1]. High-performance liquid chromatographic (HPLC) and flow-injection analysis (FIA) methods for these P^{V} compounds are well established [2]. Various chromogenic reagents have been successfully used to design detection systems for P^{V} compounds: an Mo^{VI} reagent [2], an Mo^{VI}–ascorbic acid reagent [4] and an Mo^{VI}–malachite green reagent [5]. All these reagents gave no direct colour reactions with P^{I} and P^{III} compounds (see Table I). Phosphinate and phosphonate have been determined using other detection systems for FIA [6] and HPLC [7–9].

Diphosphonate, a dimer of phosphonate of oxidation number +3, has recently been shown to act as an excellent phosphonylating agent. The versatile utility of diphosphonate has been demonstrated with a few examples of chemical syntheses [10,11], novel analogues of ADP and triphosphate. In order to investigate the kinetic and mechanistic aspects of the reactions of diphosphonate, the development of HPLC and FIA methods for the rapid and sensitive determination of phosphonate, diphosphonate and other oxo acids of lower oxidation states in Table I

* Corresponding author.

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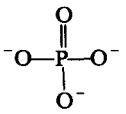
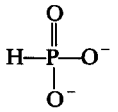
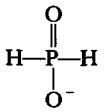
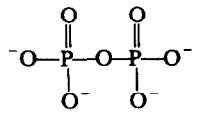
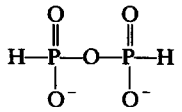
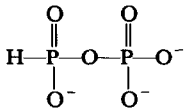
TABLE I

SYMBOLS AND STRUCTURAL CHARACTERISTICS OF PHOSPHORUS COMPOUNDS USED

Symbol[a]	Nomenclature[b]	Structure[c]	Oxidation state[d]
A	Orthophosphate		P^{V}
B	Phosphonate, phosphite		P^{III}
C	Phosphinate, hypophosphite		P^{I}
D	Diphosphate, pyrophosphate		$P^{V}P^{V}$
E	Diphosphonate, pyrophosphite		$P^{III}P^{III}$
F	Isohypophosphate		$P^{III}P^{V}$

[a] These are used in the figures throughout.
[b] Including trivial names.
[c] Indicate completely dissociated forms.
[d] Sometimes used as abbreviated notations in the text.

have been attempted in our laboratory [12,13]. As described above, molybdenum reagents for the detection of P^{V} compounds gave no colour reactions with the lower oxo acids, and therefore preoxidation of the lower oxo acids was needed for the detection of these compounds with molybdenum reagents. Sodium hydrogensulphite has been successfully used as an oxidant. However, the corrosive sulphite is harmful to laboratory instruments and may not be healthy for operators.

This work was initiated in an attempt to improve the laboratory and environmental aspects by using an alternative oxidant, peroxodisulphate [14,15], which has been examined for the oxidation and hydrolysis of phosphorus compounds in environmental samples [16,17]. The Mo^{VI} reagent used in this work was easy to prepare, stable on storage at room temperature and reacted rapidly with orthophosphate to form a molybdophosphate that could be detected at a wavelength of *ca.* 400 nm. A photodiode-array

detector in an FIA system provided absorption spectra over the range 200–800 nm for a coloured complex passing through a flow cell. The detector was useful to confirm the oxidation and/or hydrolysis of phosphorus compounds and to monitor an unpredicted reaction between phosphinate and molybdenum(VI) in the absence of the oxidant.

A separation column was used to achieve good separations of the monomers phosphinate, phosphonate and orthophosphate and of the dimers diphosphonate and pyrophosphate. Both the advantages and disadvantages of using an anion-exchange column (TSKgel SAX) for the separation of the monomers and dimers are discussed.

EXPERIMENTAL

Chemicals

Orthophosphate (KH_2PO_4), phosphonate ($Na_2PHO_3 \cdot 5H_2O$), phosphinate ($NaPH_2O_2 \cdot H_2O$) and pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) were of JIS-S grade (Kishida, Osaka, Japan). Diphosphonate ($Na_2P_2H_2O_5$) was prepared according to the literature [10]. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA).

Reagents and eluents

Eluents. Water was used as a carrier of injected samples for the FIA mode in Figs. 1 and 2. For the HPLC mode in Fig. 2, eluents for isocratic elution consisted of 0.02–0.10 *M* potassium sulphate and 0.1% (v/v) $Na_4EDTA(4Na)$ and/or Na_2EDTA.

Colour reagent. Sulphuric acid (0.3 *M*) containing 4.3 m*M* ammonium molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] or 0.03 *M* Mo^{VI}, which was prepared according to the literature [2], was used as a colour reagent. This Mo^{VI} reagent was stable for at least 6 months at room temperature.

Oxidant. A 0.5% potassium peroxodisulphate solution was used to oxidize monomeric lower oxo acids to orthophosphate for the FIA mode in Fig. 2. A 1.0% solution was used to oxidize all lower oxo acids to orthophosphate for the FIA and HPLC modes in Fig. 2. The solutions were stable for at least 3 months at room temperature.

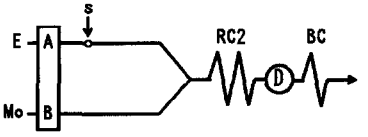


Fig. 1. Flow-injection system with Mo^{VI} reagent. E = water; Mo = Mo^{VI} reagent; pump A, 1.0 ml/min; B, 0.5 ml/min; S = sample injector (100 μl); RC2 = colour reaction coil (10 m × 0.5 mm I.D., PTFE, room temperature); D = spectrophotometric detector (200–800 nm); BC = back-pressure coil (3 m × 0.25 mm I.D., PTFE).

Equipment

The main components of the FIA system in Fig. 1 were pump A (Jasco 880 U) for the eluent, pump B (Kyowa Seimitsu KHU-W-52) for Mo^{VI} reagent, a sample injector (Rheodyne Model 7125), a reactor (RC2) made of a PTFE tubing and a spectrophotometric detector (Jasco Uvidec-100-IV) used to measure absorbance at 400 nm. A photodiode-array detector (Shimadzu SPDM6AS) was also used to measure absorption spectra at 200–800 nm.

The main components of the FIA–HPLC system in Fig. 2 were pump A (Jasco 880 PU) for the eluent and two-channel pumps B and C (Kyowa Seimitsu KHU-W-52) for the oxidant and the Mo^{VI} reagent. The two reactors (RC1 and RC2) were made of PTFE tubing and RC1 was held in a thermostated reaction bath (Sanuki R-3000C). The separation column was packed

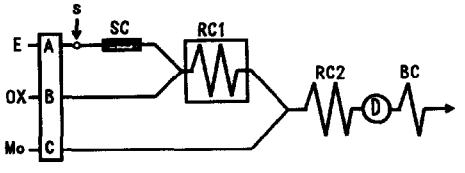


Fig. 2. FIA–HPLC system with an oxidant and Mo^{VI} reagent. E = water (FIA) or $K_2SO_4 + Na_4EDTA$ (HPLC); OX = $K_2S_2O_8$; Mo = Mo^{VI} reagent; pump A, 1.0 ml/min; B, 0.5 ml/min; C, 0.8 ml/min, except 0.5 ml/min in Fig. 5; S = sample injector (100 μl); SC = separation column (25 cm × 4 mm I.D., TSKgel SAX); RC1 = oxidation and hydrolysis reaction coil (10 m × 0.5 mm I.D., PTFE, 140°C); RC2 = colour reaction coil (10 m × 0.5 mm I.D., PTFE, room temperature); D = spectrophotometric detector; BC = back-pressure coil (3 m × 0.25 mm I.D., PTFE). The separation column (SC) is used for the HPLC mode and is demounted for the FIA mode.

with an anion exchanger (TSKgel SAX). The column was used for the HPLC mode and was demounted for FIA mode. The other components were similar to those in Fig. 1.

RESULTS AND DISCUSSION

Effect of temperature of colour reactor on FIA signal intensities and absorption spectra

As mentioned above, orthophosphate reacts with Mo^{VI} and Mo^{V}–Mo^{VI} reagents to form molybdophosphate (yellow) ($H_3PMo_{12}O_{40}$) and the so-called heteropoly blue, respectively [2]. The heteropoly blue method is more advantageous than the yellow molybdophosphate method with respect to sensitivity. We examined the detection of lower oxo acids with the combined use of the Mo^{V}–Mo^{VI} reagent and peroxodisulphate as an oxidant. It was found, however, that the Mo^{V}–Mo^{VI} reagent was deactivated by peroxodisulphate and was unable to detect not only the lower oxo acids, but also orthophosphate. The following experiments were carried out with the Mo^{VI} reagent. A two-channel FIA system (Fig. 1) without the use of peroxodisulphate and a three-channel FIA–HPLC system (Fig. 2) with the oxidant were employed.

Prior to the oxidation experiments with peroxodisulphate, the reactivities of orthophosphate, phosphonate and phosphinate with the Mo^{VI} reagent were studied by employing the FIA system in Fig. 1. The reactor (RC2) temperature

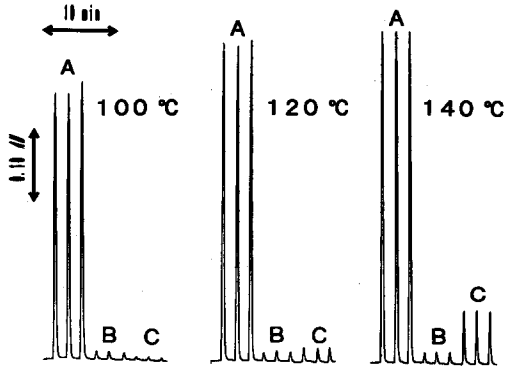


Fig. 3. Effect of colour reactor (RC2) temperature on FIA signal intensities of (A) orthophosphate, (B) phosphonate and (C) phosphinate. Manifold as in Fig. 1; measurement wavelength, 400 nm; sample, each 1×10^{-3} *M*.

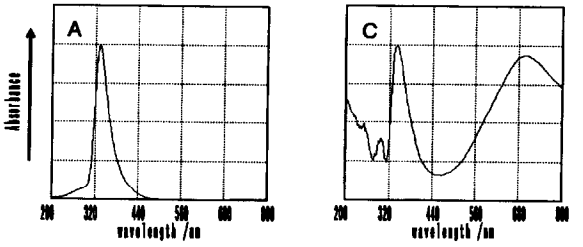


Fig. 4. Absorption spectra of coloured complexes measured by the photodiode-array detector. A and C correspond to signals A and C at 140°C in Fig. 3. The scale on the absorbance axis is arbitrary and relative to the highest maximum.

for the colour reaction was varied over the range 100–140°C. As expected, the peak intensities for the lower oxo acids were lower than those for orthophosphate at temperatures below 120°C (Fig. 3). The small signals for the lower oxo acids may be ascribed mainly to the impurity (orthophosphate) in commercial chemicals [18]. On the other hand, the appearance of the marked peak C for phosphinate at 140°C was unpredicted. The spectrum over the range 200–800 nm of the coloured species A and C at 140°C were recorded with a photodiode-array detector with a flow cell (8 μl) [19]. It is evident from Fig. 4 that the spectrum for phosphinate (C), with an additional absorption maximum at longer wavelength, is different from the typical spectrum of molybdophosphate (A), characteristic of orthophosphate and orthophosphate-producing compounds. Two possible speculations to be investigated in the future are that (1) phosphinate reacts with molybdenum(VI) at high temperature to form a phosphinate complex and (2) phosphinate acts as a reductant for molybdenum(VI) to form a mixed polymer of

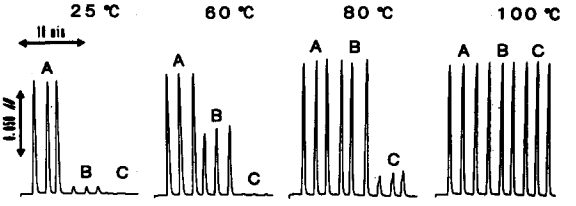


Fig. 5. Effect of oxidation reactor (RC1) temperature on FIA signals of (A) orthophosphate, (B) phosphonate and (C) phosphinate. Manifold, FIA mode in Fig. 2; oxidant, 0.5% $K_2S_2O_8$; measurement wavelength, 400 nm; sample, each 1×10^{-3} *M*.

molybdenum(V) and molybdenum(VI) or a reduced complex of molybdophosphate.

Effect of temperature of oxidation reactor on oxidation of lower oxo acids

The oxidative capability of peroxodisulphate for the lower oxo acids was examined by employing the three-channel FIA system in Fig. 2. Preoxidation of the lower oxo acids by peroxodisulphate was effected before the resulting orthophosphate was allowed to mix with the Mo^{VI} reagent. As shown in Fig. 5, the peak intensities of phosphonate and phosphinate, relative to that of orthophosphate, increases with increasing temperature of the oxidation reactor (RC1). Complete oxidation and quantitative colour formation were achieved at 100°C.

Similar experiments were carried out with the dimers pyrophosphate and diphosphonate, which needed to be prehydrolysed and/or preoxidized with peroxodisulphate. The two dimers, in addition to the monomers, were quantitatively detected at 140°C. Only incomplete colour formation was achieved for pyrophosphate at 100°C, probably owing to the rate-determining slow hydrolysis of pyrophosphate. At 140°C, the absorption spectra for phosphonate, diphosphonate and pyrophosphate, given by the photodiode-array detector, were identical with that in Fig. 4A, indicating that all species were completely converted into orthophosphate.

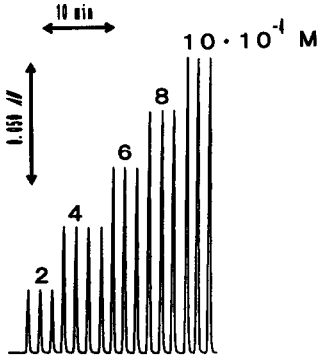


Fig. 6. FIA calibration of diphosphonate. Manifold, FIA mode in Fig. 2; RC1 temperature, 140°C; oxidant, 1.0% $K_2S_2O_8$; measurement wavelength, 400 nm; the concentration range is from 2×10^{-4} to 10×10^{-4} *M*. Each sample was injected in triplicate or quadruplicate.

The FIA calibration profile for diphosphonate is presented in Fig. 6. It shows good linearity (correlation coefficient ≥ 0.999), reproducibility (R.S.D. of measurement <1.0%) and detection limit (*ca.* 10^{-5} *M*).

HPLC separation of monomers

In the previous sections we have mentioned the effectiveness of peroxodisulphate as the oxidant in the FIA mode (Fig. 2). We now describe the application of FIA as a detection system in the HPLC separation of phosphorus compounds. An anion-exchange separation column was fitted and a mixed solution of EDTA and potassium sulphate was used as the eluent (Fig. 2). The pH values of the eluents could be adjusted by varying the ratio of Na_4EDTA and Na_2EDTA, maintaining the total EDTA concentration (0.1%, w/w) constant.

Figs. 7 and 8 show the HPLC profiles for a mixed solution of orthophosphate, phosphonate and phosphinate obtained by varying either the sulphate concentration (Fig. 7) or pH (Fig. 8). The three species could be detected quantitative-

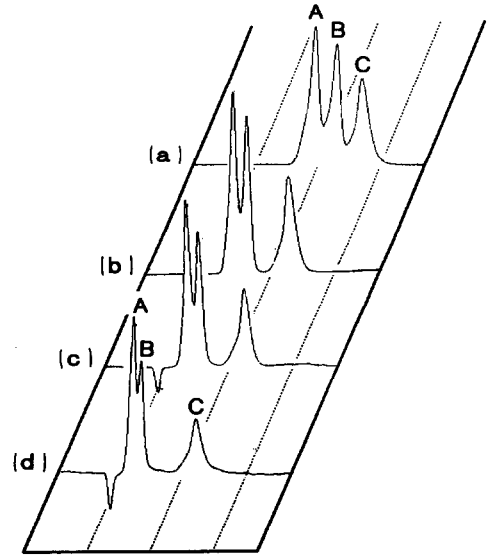


Fig. 7. Effect of potassium sulphate concentration on the separation of (A) orthophosphate, (B) phosphonate and (C) phosphinate. Manifold, HPLC mode in Fig. 2; eluent, 0.1% (w/w) Na_4EDTA + (a) 0.02, (b) 0.05, (c) 0.07 and (d) 0.10 *M* K_2SO_4; separation column, TSKgel SAX; measurement wavelength, 400 nm; sample, each 1×10^{-3} *M*.

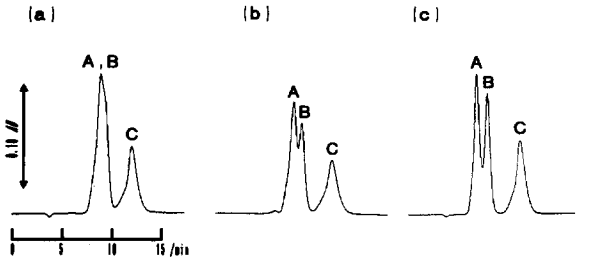
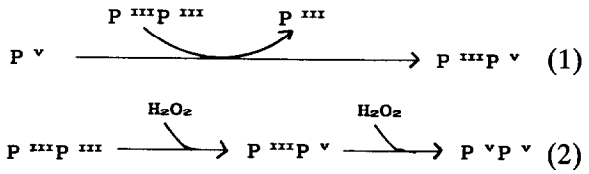


Fig. 8. Effect of pH on the separation of (A) orthophosphate, (B) phosphonate and (C) phosphinate. Manifold, HPLC mode in Fig. 2; eluent, 0.05 *M* K_2SO_4 + 0.1% EDTA; pH, (a) 5, (b) 7 and (c) 10; separation column, TSKgel SAX; measurement wavelength, 400 nm; sample, each 1×10^{-3} *M*.

ly. The separation of phosphinate from other species was satisfactory, but only incomplete resolution between orthophosphate and phosphonate could be achieved in spite of the variations in the eluent composition. The TSKgel SAX with the trimethylammonium ion as an anion-exchange group may not be a good choice for the separation of the monomers. We obtained a preliminary result that TSKgel IC-Anion-PW with the diethylmethylammonium ion as an anion-exchange group gives complete resolution of the three monomers whose retention times increase in the order phosphinate < orthophosphate < phosphonate. Detailed results will be reported elsewhere.

HPLC separation of dimers

It has been reported [10,11] that diphosphonate, $P^{III}P^{III}$, acts as a phosphonylating agent (eqn. 1) and is oxidizable by hydrogen peroxide (eqn. 2) to give isohypophosphate, $P^{III}P^{V}$, and pyrophosphate, $P^{V}P^{V}$. This section deals with the HPLC analysis of the phosphorus compounds involved in eqns. 1 and 2.



An HPLC profile for an authentic mixture of P^{V}, P^{III}, $P^{V}P^{V}$ and $P^{III}P^{III}$ is shown in Fig. 9. The two dimers were well separated from one another and from the monomers, but the resolution of P^{III} and P^{V} was incomplete, as was expected from the results in Figs. 7 and 8.

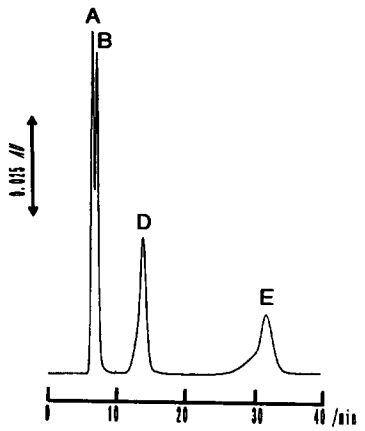


Fig. 9. HPLC profile for a mixed solution of (A) orthophosphate, (B) phosphonate, (D) pyrophosphate and (E) diphosphonate. Manifold, HPLC mode in Fig. 2; eluent, 0.11 *M* K_2SO_4 + 0.02% Na_4EDTA; separation column, TSKgel SAX; measurement wavelength, 400 nm; sample components, each 1×10^{-3} *M*.

The reaction products of P^{V} and $P^{III}P^{III}$ in eqn. 1 were analysed under the same conditions as in Fig. 9. As shown in Fig. 10, a peak F for $P^{III}P^{V}$ was observed around the retention time of $P^{V}P^{V}$ (Fig. 9). The unsymmetrical peaks such as D, E and F in Figs. 9 and 10 tended to be observed when the same column was used continuously for as long as 6 months. A similar

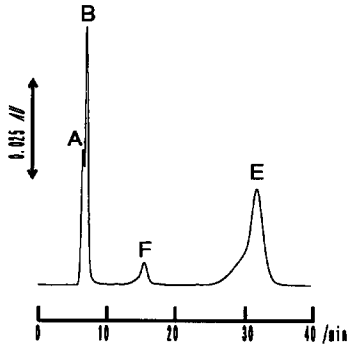


Fig. 10. Analysis of the reaction products of orthophosphate and diphosphonate. A = Orthophosphate; B = phosphonate; E = diphosphonate; F = isohypophosphate. Initial concentrations, 0.1 *M* orthophosphate and 0.3 *M* diphosphonate; incubation time, 3.8 h; incubation temperature, 50°C; manifold, HPLC mode in Fig. 2; separation column, TSKgel SAX; eluent, 0.11 *M* K_2SO_4 + 0.02% Na_4EDTA; measurement wavelength, 400 nm.

experiment on the HPLC separation of $P^{III}P^{V}$ and $P^{V}P^{V}$ was reported in a previous paper [11]. As only P^{V} units were monitored with Mo^{V}–Mo^{VI} reagent in the absence of an oxidant, three peaks of P^{V}, $P^{III}P^{V}$ and $P^{V}P^{V}$ were recorded, but the peaks for P^{III} and $P^{III}P^{III}$ could not be confirmed. On the other hand, the present method permitted the detection of all phosphorus compounds (Table I) involving any structural units of P^{V}, P^{III} and/or P^{I}.

The HPLC method also showed good quantitative capabilities with respect to linearity, reproducibility and detection limit, comparable to those in the FIA method.

CONCLUSIONS

The FIA detection system described is based on the preoxidation of lower oxo acids of phosphorus by peroxodisulphate and the coloration of the resultant orthophosphate with Mo(VI) reagent. A photodiode-array detector was advantageous not only for quantitative purposes but also for the spectral characterization of the coloured reaction products. The HPLC system, *i.e.*, the combination of the FIA system and the anion-exchange separation column, was confirmed to be powerful for the quantitative analysis of mixed samples of lower oxo acids of phosphorus.

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