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FLOW INJECTION SYSTEM AS A POST-COLUMN REACTION DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PHOSPHINATE, PHOSPHONATE AND ORTHOPHOSPHATE

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SUMMARY

A flow injection system was developed for the rapid flow analysis of lower oxo acids of phosphorus, such as phosphinate and phosphonate. A sodium hydrogen sulphite solution was used as an oxidizing agent for phosphinate and phosphonate, and a strongly acidic solution containing molybdenum(V) and molybdenum(VI) was used as a colour-forming reagent for the resultant orthophosphate. Lower oxo acids of phosphorus can be determined at a sampling rate of 60 samples per h with a relative standard deviation of less than 1 %. The flow injection system was found to be useful as a post-column reaction detector for high-performance liquid chromatography of lower oxo acids of phosphorus and orthophosphate.

INTRODUCTION

Flow injection analysis (FIA) is a simple and convenient approach to rapid chemical analysis by continuous flow[1]. In previous papers[2,3] we have reported a high-pressure flow injection system and its successful application to the rapid determination of inorganic ortho- and polyphosphates. Two kinds of molybdenum reagents were found to be useful: a mixture of molybdenum(V) and molybdenum(VI)[2], and molybdenum(VI) coupled to ascorbic acid[3]. Both reagents reacted with orthophosphate, P(V), to produce the so-called heteropoly blue complex, P(V)–Mo(V)–Mo(VI):

$$\text{P(V)} \xrightarrow{\text{Mo(V)–Mo(VI)}} \text{P(V)–Mo(V)–Mo(VI)} \quad (1)$$

$$\text{P(V)} \xrightarrow{\text{Mo(VI)}} \text{P(V)–Mo(VI)} \xrightarrow{\text{Ascorbic acid}} \text{P(V)–Mo(V)–Mo(VI)} \quad (2)$$

The high-pressure flow injection system employed was constructed so as to make possible the simultaneous hydrolysis of polyphosphates and the colour reaction of the resultant orthophosphate with the molybdenum reagents.

However, lower oxo acids of phosphorus such as phosphinate, P(I), and phosphonate, P(III), do not react directly with the reagents as in eqns. 1 and 2. A suitable

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oxidizing agent is required to convert these acids into orthophosphate which reacts with the molybdenum reagents.

It is known[4] that lower oxo acids of phosphorus are quantitatively oxidized in a solution of sodium hydrogen sulphite and molybdenum(V)–molybdenum(VI) reagent:

$$\text{P(I), P(III)} \xrightarrow{\text{NaHSO}_3} \text{P(V)} \xrightarrow{\text{Mo(V)–Mo(VI)}} \text{P(V)–Mo(V)–Mo(VI)} \qquad (3)$$

Here, sodium hydrogen sulphite acts as an oxidizing agent for the lower oxo acids. This principle has extentively been employed in our laboratory for the determination, by a manual (batchwise) procedure, not only of orthophosphate but also of phosphinate and phosphonate[5,6].

This work was undertaken to develop a flow injection system by which phosphinate, phosphonate and orthophosphate can be detected using the above reagents. Threee pumping channels were used to introduce an oxidizing agent, a sample solution and a molybdenum reagent, respectively. Rapid analysis of lower oxo acids of phosphorus and of orthophosphate can be achieved within 3 min, with satisfactory reproducibility (R.S.D. less than 1%). Only orthophosphate can selectively be detected in the absence of the oxidizing agent. This fact permits the differential analysis of lower oxo acids of phosphorus and orthophosphate.

This flow injection system was also found to be useful as a post-column reaction detector for high-performance liquid chromatography (HPLC) of phosphinate, phosphonate and orthophosphate.

EXPERIMENTAL

Reagents

Unless otherwise stated, guaranteed reagents from Wako (Osaka, Japan) and Kishida (Osaka, Japan) were used throughout.

A stock solution of the molybdenum(V)–molybdenum(VI) reagent for the determination of phosphorus compounds was prepared by the method described previously[2]. The reagent solution for the flow injection analysis was prepared by diluting 200 ml of this stock solution with distilled water to 1 l. A 1 *M* sodium hydrogen sulphite solution was prepared daily by dissolving $NaHSO_3$ in distilled water.

The eluent for the chromatographic separation of lower oxo acids of phosphorus and orthophosphate comprised 0.10 *M* potassium chloride, 0.05% Na_4EDTA and 0.05% Na_2EDTA, where EDTA = ethylenediaminetetraacetate.

Samples

Sample solutions of phosphinate, phosphonate and orthophosphate were prepared by dissolving sodium phosphinate monohydrate ($NaPH_2O_2 \cdot H_2O$), disodium phosphonate pentahydrate ($Na_2PHO_3 \cdot 5H_2O$) and potassium dihydrogen orthophosphate (KH_2PO_4), respectively, in distilled water.

Apparatus and procedures

Flow injection system. A flow injection system for the determination of lower oxo acids of phosphorus is shown in Fig. 1. The molybdenum(V)–molybdenum(VI)

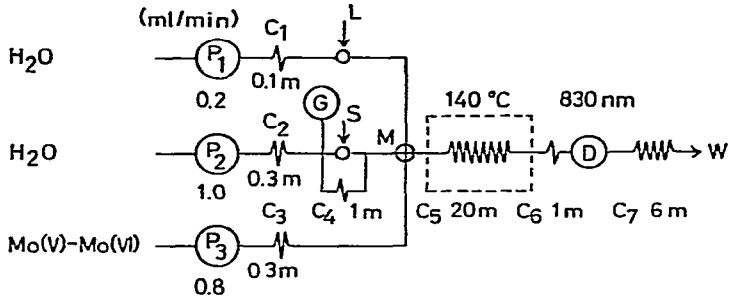

Fig. 1. Flow injection system for phosphinate, phosphonate and orthophosphate. P_1, P_2 and P_3 = Reciprocating pumps; G = pressure gauge; M = four-way connector; D = detector; W = waste; S = sample injection; L = sulphite injection; C_1, C_2 and C_3 = precoils (Technicon Part No. 116-0536-13); C_4 = bypass coil (PTFE, 0.3 mm I.D., 2.0 mm I.D.); C_5 = reaction coil (PTFE, 0.5mm I.D., 1.5 mm O.D.); C_6 = cooling coil (PTFE, 0.5 mm I.D., 1.5 mm O.D.); C_7 =back-pressure coil (PTFE, 0.3 mm I.D., 2.0 mm O.D.). The dotted line shows the part of the manifold immersed in the silicon oil -bath.

reagent was pumped into an analytical line at a constant rate of 0.8 ml/min with one channel of a two-channel reciprocating pump (Kyowa KHU-W-52). The other channel of the pump was used to introduce the sulphite solution into a water stream (0.2 ml/min) via a four-way loop-valve injector (Kyowa KMM 4V2). The function of the valve is described later. The sample solution (55 μl) was introduced with a loop-valve sample injector (Seishin VMU-6) into a water stream pumped with another reciprocating pump (Kyowa KWU 90H) at a constant rate of 1.0 ml/min. A bypass coil was employed to eliminate injection shock. The pulse produced by the reciprocating pumps was effectively damped by an elastic precoil placed immediately after the pump.

Sulphite, sample and molybdenum(V)–molybdenum(VI) reagent were mixed at a four-way connector (M, Kyowa K4P-U). The mixed solution was carried through a 20-m reaction coil, during which time the lower oxo acids of phosphorus were oxidized to orthophosphate and the resultant orthophosphate was allowed to react with the molybdenum(V)–molybdenum(VI) reagent to form a heteropoly blue complex. The absorption of this blue complex at 830 nm was monitored with a flow-through cell (volume 8 μl, path 8 mm) attached to a spectrophotometer (Hitachi 200-10). A long narrow coil was located at the exit of the cell to give a back pressure of about 6 kg/cm^2 as indicated on a pressure gauge (Kyowa KPG 50N). Under such a high pressure the reaction coil can be heated up to 150°C in a silicon oil-bath (Thomas T-201) without detector noise caused by gas bubbling. A 1-m cooling coil was used between the reaction coil and the detector.

HPLC system. A Hitachi Liquid Chromatograph 635 was used for the separation of phosphinate, phosphonate and orthophosphate (Fig. 2). The sample solution (100 μl) was introduced via a loop-valve sampler (Hitachi 635-5101) onto a separation column and chromatographed at a flow-rate of 1.0 ml/min by means of a reciprocating pump (Hitachi 635-5002) at a pressure of 150 kg/cm^2. The separation column (500 × 2.6 mm I.D.) was packed with an anion exchanger (Toyo Soda, TSK-GEL, IEX-220 SA).

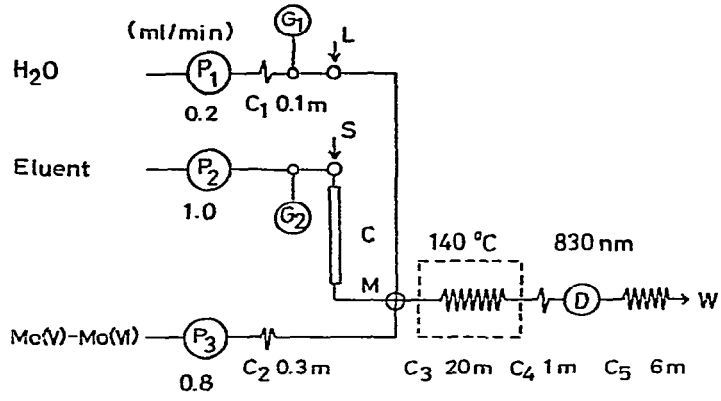

Fig. 2. HPLC system with the flow injection system as the detector. P_1, P_2 and P_3 = Reciprocating pumps; G_1 and G_2 = pressure gauges; S = sample injection; L = sulphite injection; C = column; M = four-way connector; C_1 and C_2 = precoils; C_3 = reaction coil; C_4 = cooling coil; C_5 = back-pressure coil; D = detector; W = waste.

RESULTS AND DISCUSSION

Flow injection analysis

As shown in Fig. 1 the flow injection system for phosphinate, phosphonate and orthophosphate has three pumping channels for the introduction of an oxidizing agent, a sample and a molybdenum(V)–molybdenum(VI) reagent. The sample solution was not introduced into the molybdenum reagent stream[2,3], but into a water stream. One of the advantages of this method is that a "solvent peak", which appeared when a water sample was directly injected into the molybdenum reagent[3], can be eliminated. Another advantage is that the flow injection system is easily interfaced with a chromatographic column as a post-column reaction detector without changing the total flow-rate or the residence time of the sample zone in the reaction coil.

As the sulphite solution tended to corrode stainless steel and to disturb the flow-rate of the reciprocating pump, it was introduced with the loop-valve injector shown in Fig. 3, to avoid contact of the reagent with the pump. This injector has two sampling loops: T_1 with a large volume of about 9.5 ml, and T_2. During the passage

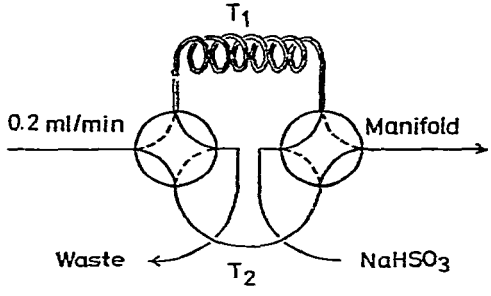

Fig. 3. Loop-valve injector. T_1 = Large volume loop (PTFE, 3 m × 2.0 mm I.D., 3.0 mm O.D.); T_2 = small volume loop (PTFE, 8 cm × 0.5 mm I.D., 1.5 mm O.D.).

of the carrier stream through T_2, T_1 is filled with sulphite solution via a hypodermic syringe. The valve is then switched to introduce the oxidizing agent into an analytical line. The carrier solution is pumped at the low rate of 0.2 ml/min in order to extend the period of supplying the oxidizing agent. The efficiency of the injector was examined by filling T_1 with an aqueous solution of methyl orange (0.005%), in place of the oxidizing agent. The absorption of the methyl orange solution was monitored at 470 nm. Five minutes after injection the signal had become steady (Fig. 4), and remained so for about 40 min, with sharp ascending and descending edges. The determination of phosphinate and phosphonate must be completed within this steady state.

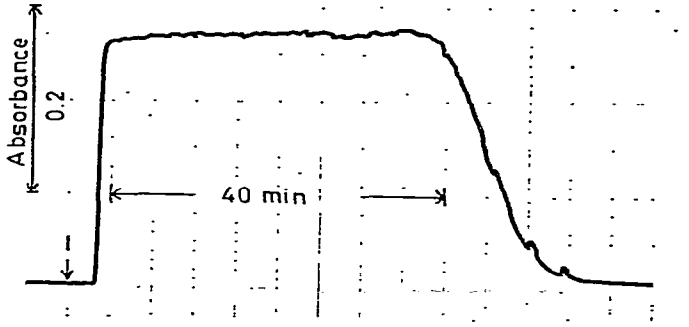

Fig. 4. Steady state signal obtained when a methyl orange solution in the large volume tubing (T_1, Fig. 3) was introduced into the analytical line. I = Injection point.

A slight drift of the steady state is observed (Fig. 4). Therefore it is necessary to find a concentration range in which the slight variation in sulphite concentration does not affect the detection peak. Fig. 5 shows the effect of the concentration of the sulphite solution on the peak heights of phosphinate, phosphonate and orthophos-

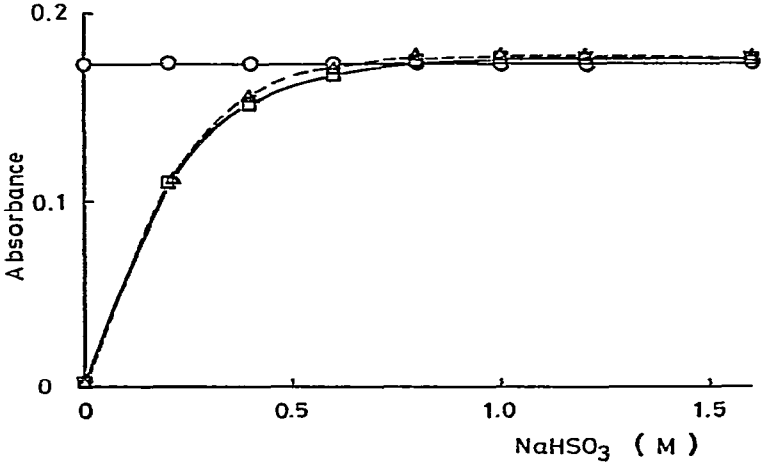

Fig. 5. Effect of the sodium hydrogen sulphite concentration on the peak heights of phosphinate (□—□), phosphonate (△—△) and orthophosphate (○—○). Sample: each $1.0 \cdot 10^{-4}$ *M*, 55 µl. The temperature of the heating bath was kept constant at 140°C.

phate. The peak heights for phosphinate and phosphonate increased with the concentration of sodium hydrogen sulphite. At concentrations higher than 0.8 *M* the oxidation was almost quantitative. Hence all further experiments were done with a 1.0 *M* sulphite solution.

The method for the introduction of sulphite solution may be regarded as a type of merging zone method in which both sample and reagent are injected simultaneously as narrow spikes, by the use of a multi-injection valve[1,7–9]. The great advantage of this method is that consumption of expensive reagents is minimized. However, as has already been pointed out, the drawback of this approach is that a baseline signal appears if the injected reagent alone is sensed by the flow-through detector. In fact a remarkable baseline signal was obtained when a sulphite solution was injected into the carrier stream as a narrow spike using a small volume loop (Fig. 6A). Such a background peak becomes very troublesome in the detection of phosphorus compounds at very low concentration. On the other hand, when a sulphite solution was injected using a large volume loop, a constant background level was obtained (Fig. 6B). The long steady state of the reagent provided by this modified method is very significant, especially when the system is used as a post-column reaction detector for HPLC.

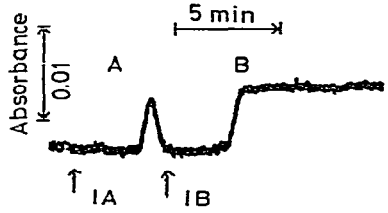

Fig. 6. Signals obtained by injecting sulphite solution using the small volume loop T_2 (A) and the large volume loop T_1 (B). IA and IB = Injection points of peaks A and B, respectively.

The rate of both the oxidation of phosphinate and phosphonate and the colour reaction of the resultant orthophosphate will depend on the temperature of the reaction bath. As shown in Fig. 7 the peak height for each sample increased with temperature. It is evident that the colour reaction of orthophosphate precedes those of phosphinate and phosphonate, which suggests that the oxidation of lower oxo acids of phosphorus is the rate-determining step in the overall process. More than 90% of the lower oxo acids was oxidized at 120°C, and oxidation was almost quantitative at 140°C. At temperatures higher than 150°C gas bubbles caused detector noise. Therefore all further experiments were done with the reaction bath at 140°C.

Fig. 8 shows concentration profiles for phosphinate, phosphonate and orthophosphate. In spite of the relatively slow travel of the sample zone in the reaction coil (about 2.5 min) peak broadening was not significant and the peaks were well resolved at a sampling rate of 60 samples per h. The relative peak heights of phosphinate, phosphonate and orthophosphate in Fig. 8 varied in accordance with their relative concentrations: 1.05, 1.00 and 1.00. This result indicates that the lower oxo acids are completely oxidized and detected. No baseline signal appeared when a water sample was injected. The calibration curves for the three compounds show good linearity,

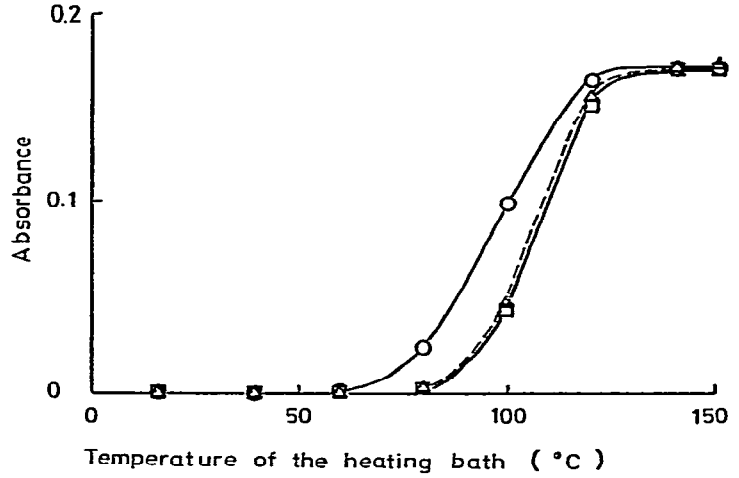

Fig. 7. Temperature dependence of the colour development of phosphinate (□—□), phosphonate (△—△) and orthophosphate (○—○). Sample: each $1.0 \cdot 10^{-4}$ *M*, 55 µl.

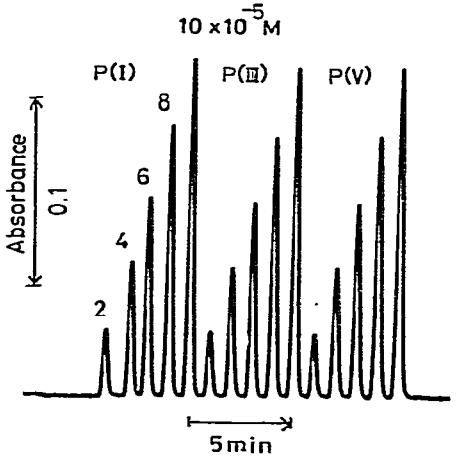

Fig. 8. Concentration profiles for phosphinate, P(I), phosphonate, P(III), and orthophosphate, P(V). The concentration of each sample increases from left to right, *i.e.*, from $2 \cdot 10^{-5}$ to $10 \cdot 10^{-5}$ *M*.

the relative standard deviation being less than 1%. It should be noted that only orthophosphate responds selectively in the absence of sulphite, while total phosphorus can be detected in the presence of sulphite.

Liquid chromatographic analysis

The flow injection system in Fig. 1 is very useful for the determination of total oxo acids of phosphorus and for the selective detection of orthophosphate in the presence of phosphinate and phosphonate, but does not allow differential analysis of each phosphinate, phosphonate and orthophosphate. Prior separation is therefore needed for this purpose. A chromatographic method employing an air-segmented flow system and a hypochlorite solution as oxidizing agent has successfully been

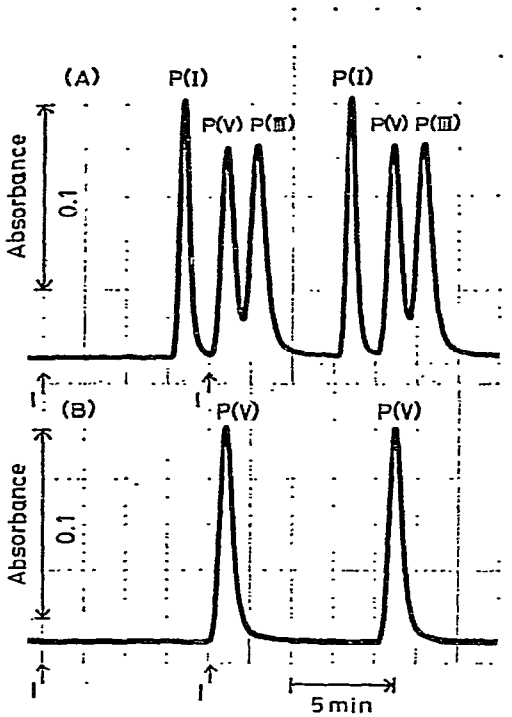

Fig. 9. High-performance anion-exchange chromatographic profiles for phosphinate, P(I), phosphonate, P(III), and orthophosphate, P(V), monitored in the presence (A) and in the absence (B) of sulphite solution. Each sample ($1.0 \cdot 10^{-4}$ *M*) was injected in duplicate. I = Injection point.

applied to the analysis of lower oxo acids of phosphorus[10]. However, the application of an unsegmented flow system has not yet been reported. In the present work, the manifold for the flow injection analysis (Fig. 1) was connected with a separation column (Fig. 2) to examine its efficiency as a post-column reaction detector for HPLC of phosphinate, phosphonate and orthophosphate.

Fig. 9 shows the elution profiles for a mixture of phosphinate, phosphonate and orthophosphate, with (A) and without (B) oxidizing agent, respectively. It is evident that orthophosphate can selectively be detected in the absence of oxidizing agent. On the other hand, phosphinate and phosphonate are oxidized and detected only in the presence of the oxidizing agent. This behaviour is very favourable for the determination of the chemical compositions of lower oxo acids of phosphorus with P(III) and P(V) units.

Although further improvement in the eluent composition is required to permit the complete separation of orthophosphate and phosphonate, the above results suggest that the flow injection system will play an effective rôle in accelerating the development of the HPLC of inorganic oxo acids of phosphorus.

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REFERENCES

1 J. Ruzicka and E. H. Hansen, *Anal. Chim. Acta*, 114 (1980) 19.
2 Y. Hirai, N. Yoza and S. Ohashi, *Anal. Chim. Acta*, 115 (1980) 269.
3 Y. Hirai, N. Yoza and S. Ohashi, *Chem. Lett.*, (1980) 499.
4 N. Yoza and S. Ohashi, *Bull. Chem. Soc. Jap.*, 37 (1963) 37.
5 K. Ujimoto, T. Nakamura, H. Asada, N. Yoza, Y. Takashima and S. Ohashi, *J. Inorg. Nucl. Chem.*, 32 (1970) 3177.
6 N. Yoza, K. Ishibashi and S. Ohashi, *J. Chromatogr.*, 134 (1978) 497.
7 H. Bergamin Filho, E. A. G. Zagatto, F. J. Krug and B. F. Reis, *Anal. Chim. Acta*, 101 (1979) 17.
8 E. A. G. Zagatto, F. J. Krug, H. Bergamin Filho, S. S. Jørgensen and B. F. Reis, *Anal. Chim. Acta*, 104 (1979) 279.
9 J. Mindegaard, *Anal. Chim. Acta*, 104 (1979) 185.
10 F. H. Pollard, G. Nickless, D. E. Rogers and M. T. Rothwell, *J. Chromatogr.*, 17 (1965) 157.