Journal of Chromatography, 318 (1985) 319–324
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,227

SIMULTANEOUS DETERMINATION OF PHOSPHATE AND PHOSPHONATE BY FLOW INJECTION ANALYSIS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A SERIES DETECTION SYSTEM

YOSHINOBU BABA*, NORIMASA YOZA and SHIGERU OHASHI
Department of Chemistry, Faculty of Science, Kyushu University, Hakozaki, Higashiku, Fukuoka 812 (Japan)
(Received September 13th, 1984)

SUMMARY

Two detectors were located in series for the simultaneous determination of phosphate and phosphonate. A molybdenum(V)–molybdenum(VI) reagent was used to detect phosphorus(V) at the first detector and phosphorus(III) at the second detector, following oxidation by a sulphite solution. Phosphate and phosphonate can be determined simultaneously at a total maximum sampling rate of 80 samples per hour, with a relative standard deviation of less than 1.5%. This series detection system, linked to a liquid chromatograph, was found to be useful for both the separation and the identification of various oxo acids with P^{V} and P^{III} units.

INTRODUCTION

Simultaneous determination of a number of components in a mixture by flow injection analysis (FIA) of a single sample injection is an interesting problem[1–4]. In a previous paper[5], we reported the design of a flow injection system with parallel detection, and its application to the simultaneous determination of phosphate and phosphonate. The parallel detection system was constructed by arranging two identical detection systems symmetrically. The sample solution was injected and split into two parts, one of which was introduced into each detection system. The system was designed to be used as a "high-pressure flow injection system", based on the formation of heteropoly blue complex[6,7]. The important features of the parallel system were the single sample injection and the use of a conventional FIA method with two detectors in parallel.

The work reported here was undertaken to design a series detection FIA system and to apply it to the simultaneous determination of phosphate and phosphonate. This system is different from the parallel detection FIA system in two respects: (1) the flow is not split after the injection, and (2) the two detectors are in series not in parallel. The potential of this system as a post-column reaction detector in high-performance liquid chromatography was also shown, not only for the separation of phosphorus(V) compounds and phosphorus(III) compounds, but also for the identification of these compounds, by the differentiation of P^{V} and P^{III}.

0021-9673/85/$03.30

EXPERIMENTAL

Chemicals

Unless otherwise stated, all chemicals (Kishida, Osaka, Japan) were of guaranteed grade and were used without further purification. Reagents and samples were prepared according to the previous paper[5], except that the concentration of the sodium hydrogen sulphite solution was increased to 1.0 *M*.

Apparatus and procedures

The FIA manifold with a series detection system is shown in Fig. 1. The first detector (D_A) was for the detection of P^V units and the second one (D_B) was for total phosphorus (P^V and P^{III}). The sample solution (200 μl) was introduced with a loop-valve sample injector (S, Seishin VMU-6) into a water stream pumped with a reciprocating pump (P_1, Seishin PSU-3.2W) at a flow-rate of 1.0 ml/min. The sample was mixed with the molybdenum(V)–molybdenum(VI) reagent pumped at a flow-rate of 0.5 ml/min with a second reciprocating pump (P_2, Seishin PSU-2.5W). The mixed solution was carried through a 10-m reaction coil (C_2), where polyphosphate was hydrolysed to orthophosphate and the latter reacted with the Mo^V–Mo^{VI} reagent to form a heteropoly blue complex. The absorbance of this blue complex at 830 nm was monitored with a flow cell (volume 8 μl, path 10 mm) attached to a spectrophotometer (D_A, Jasco, UVIDEC 100 IIW).

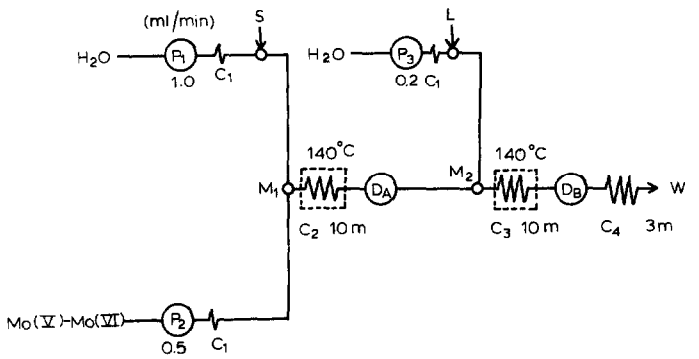

Fig. 1. FIA manifold with a series detection system. P_1, P_2 and P_3 = reciprocating pumps; M_1 and M_2 = three-way connectors; C_1 = precoil (Technicon Part No. 116-0536-13); C_2 and C_3 = reaction coils (PTFE, 0.5 mm I.D., 2 mm O.D.); C_4 = back-pressure coil (PTFE, 0.25 mm I.D., 2 mm O.D.); S = sample injector; L = sulphite injector; D_A and D_B = detectors; W = waste. The parts enclosed by dashed lines were immersed in a thermostatted silicone oil bath at 140°C.

The mixture of sample and reagent was then mixed with the sulphite solution introduced into a water stream via a four-way loop-valve injector (L, Kyowa KMM4V2). The mixed solution was passed through a second 10-m reaction coil (C_3), where the lower oxo acids of phosphorus were oxidised and the resultant orthophosphate reacted with the Mo^V–Mo^{VI} reagent to form heteropoly blue complex. The absorbance of the complex at 830 nm was monitored with a flow cell attached to an identical spectrophotometer (D_B).

A narrow coil (C_4) at the exit of the cell prevented detector noise caused by gas bubbling in the cell, even at temperatures as high as 140°C[6].

The HPLC system was as described previously[5].

RESULTS AND DISCUSSION

Design of series detection system

In the series detection FIA system (Fig. 1), the injected sample passes through the first confluence point (M_1), the first reaction coil (C_2), the first detector (D_A), the second confluence point (M_2), the second reaction coil (C_3) and the second detector (D_B). The sulphite solution is introduced at M_2.

Polymeric oxo acids of phosphorus were hydrolysed to monomeric orthophosphate and/or phosphonate in the first reaction coil. The formation of heteropoly blue complex by the reaction of resultant orthophosphate with Mo^{V}–Mo^{VI} reagent also took place in the first reaction coil. Phosphonate, which did not form heteropoly blue complex, was then oxidized with sulphite during passage through the second reaction coil[7–9] and the resultant orthophosphate was detected. It was confirmed that the introduction of sulphite did not affect the absorption characteristics of heteropoly blue complex produced in the first reaction coil. In this manner, it was possible to determine P^{V} and P^{III} simultaneously with a single sample injection.

Simultaneous determination of orthophosphate and phosphonate

As mentioned above, the sample passes through two reaction coils, so the peak detected by the second detector is expected to be broader than that detected by the first. We used sample solutions of phosphorus compounds to estimate the peak broadening and to examine the efficiency of the system, as follows.

A sample solution of orthophosphate was injected. As shown in Fig. 2, the FIA profiles showed differences with respect to the residence time [141 sec (A) and 251 sec (B)], the peak height [0.12 a.u. (A) and 0.089 a.u. (B)] and the peak width, σ_t [13 sec (A) and 15 sec (B)]. On the other hand, the ratio of the peak areas was confirmend to be 1:1. It should be noted that the peak width in Fig. 2B, expressed in seconds, must be multiplied by a factor of 1.13 (= 1.7/1.5) for the measurement of the peak area, because the flow-rate through the first detector (1.5 ml/min) was different from that through the second (1.7 ml/min).

If a sample solution containing orthophosphate and phosphonate is injected, only orthophosphate is expected to be detected in the absence of sulphite, whereas

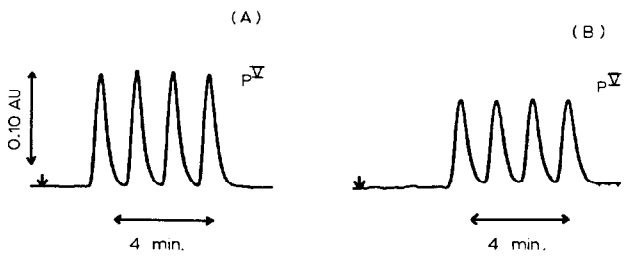

Fig. 2. FIA profiles for orthophosphate (P^{V}), in the absence (A) and in the presence of sulphite (B). The sample solution was injected in quadruplicate; the sample concentration was $2.0 \cdot 10^{-5}$ *M*.

both are expected to be detected in the presence of sulphite. Fig. 3 shows calibration profiles for a series of samples containing an equimolar mixture of orthophosphate and phosphonate. Either the total amount of orthophosphate and phosphonate (B) or orthophosphate only (A) can be determined. In accordance with expectation, the ratio of peak areas for each sample was confirmed to be 2:1. The maximum sampling rate, S_{max} ($= 3600/6\sigma_t$), corresponding to σ_t value of 15 sec, was 40 samples per hour, which means a total sampling rate of 80 samples per hour in the series detection system.

The calibration curves showed good linearity (correlation coefficient = 0.999) and the relative standard deviation of measurement was between 1.1 and 1.3%.

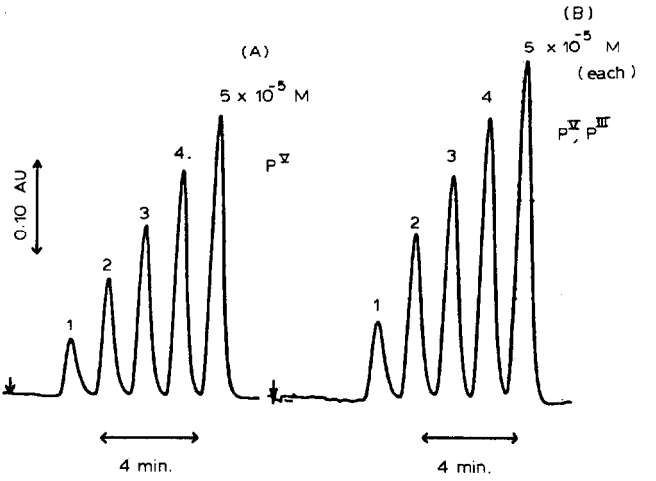

Fig. 3. Calibration profiles for an equimolar mixture of orthophosphate (P^V) and phosphonate (P^{III}), in the absence (A) and in the presence (B). The concentration of each sample increases from left to right, *i.e.*, from $1.0 \cdot 10^{-5}$ to $5.0 \cdot 10^{-5}$ *M*.

Liquid chromatographic detector

The series detection system was found to be very useful for the simultaneous determination of phosphate and phosphonate. A coupled system of FIA and HPLC, however, was expected to be more effective for the characterization of multicomponent samples[5–7,10–13]. Previously[5], we reported the on-line coupling of a chromatographic column to the parallel detection FIA system, which was applicable not only to the separation of phosphorus oxo acids but also to the identification of these compounds. We demonstrated the feasibility of the coupling HPLC and the series detection system by use of more complex phosphorus compounds. The sampling line at M_1 in Fig. 1 was replaced by the outlet of a separation column, so that we could examine the efficiency of the system as a post-column reaction detector for HPLC of various oxo acids of phosphorus.

Fig. 4 shows the elution profile of a mixture of phosphonate, orthophosphate, diphosphate and triphosphate, in the absence (A) and in the presence (B) of sulphite. It is evident that ortho-, di- and triphosphate can be detected in both detectors. On

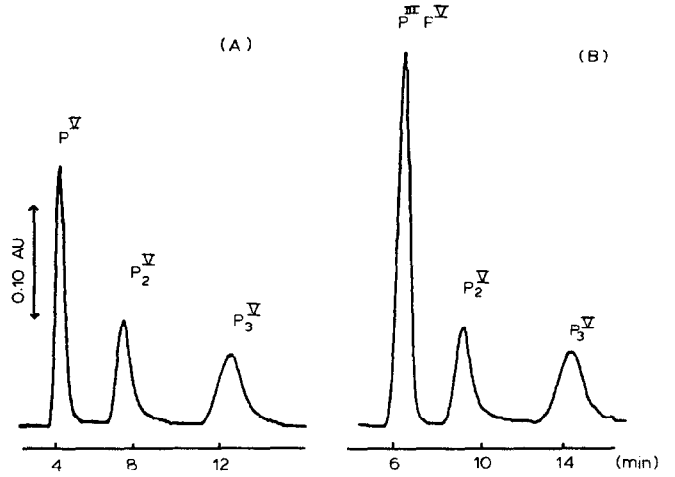

Fig. 4. HPLC profiles for phosphonate (P^{III}), orthophosphate (P^{V}), diphosphate (P_2^V) and triphosphate (P_3^V) monitored by the series detection FIA system, in the absence (A) and in the presence of sulphite (B). The concentration of each sample is $1.0 \cdot 10^{-4}$ *M* (as phosphorus).

the other hand, phosphonate is detected only in the presence of sulphite. The ratio of the peak areas for the first peaks in Fig. 4 A and B, corresponding to orthophosphate (A) and to orthophosphate and phosphonate (B), was confirmed to be 1:2, and the ratios for both the second and the third peaks were confirmed to be 1:1.

Fig. 5 shows the elution profile of a mixture of phosphonate (P^{III}) and its dimer (P^{III}–O–P^{III}). In the presence of sulphite two peaks were observed with reasonable areas. In the absence of sulphite no peak was observed.

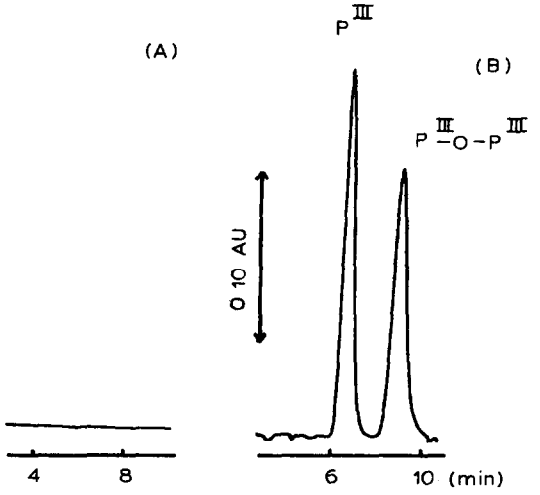

Fig. 5. HPLC profiles for phosphonate (P^{III}) and diphosphonate (P^{III}–O–P^{III}) monitored by the series detection FIA system, in the absence (A) and in the presence of sulphite (B). The concentration of each sample is $1.0 \cdot 10^{-4}$ *M* (as phosphorus).

Comparison of the series detection and the parallel detection FIA systems

The efficiency of the series detection system was comparable with that of the parallel detection FIA system[5] with respect to the sampling rate (S_{max}: 80 samples/h for series; 100 samples/h for parallel), the reproducibility (the relative standard deviation: 1.1–1.3% for series; 0.4–0.9% for parallel) and the sensitivity (apparent molar absorptivity corresponding to the slope of the calibration curve: 6000 M^{-1} cm^{-1} for series; 5000 M^{-1} cm^{-1} for parallel). An advantage of the series system over the parallel system is that the reliability of detection is improved by decreasing the pumping channels from seven (parallel) to three (series). A similar conclusion was reached when the two FIA systems were used as HPLC detectors.

REFERENCES

1 J. Ruzicka and E. H. Hansen, *Flow Injection Analysis*, Wiley, New York, 1981; (Japanese edition, N. Ishibashi and N. Yoza, Kagaku-Dojin, Kyoto, 1983).
2 K. K. Stewart, *Anal. Chem.*, 55 (1983) 931A.
3 J. Ruzicka, *Phil. Trans. Roy. Soc. Lond.*, A305 (1982) 645.
4 M. D. Luque de Castro and M. Valcarcel, *Analyst (London)*, 109 (1984) 413.
5 Y. Baba, N. Yoza and S. Ohashi, *J. Chromatogr.*, 295 (1984) 153.
6 Y. Hirai, N. Yoza and S. Ohashi, *Anal. Chim. Acta*, 115 (1980) 269.
7 Y. Hirai, N. Yoza and S. Ohashi, *J. Chromatogr.*, 206 (1981) 501.
8 *Gmelin's Handbuch, Schwefel B-II*, Verlag Chemie, Weinheim, 1960, p. 544.
9 N. Yoza and S. Ohashi, *Bull. Chem. Soc. Jap.*, 37 (1964) 37.
10 D. Betteridge, N. G. Coutney, T. J. Sly and D. G. Porter, *Analyst (London)*, 109 (1984) 91.
11 R. S. Brazell, R. W. Holmberg and J. H. Moneyhun, *J. Chromatogr.*, 290 (1984) 163.
12 N. Yoza, T. Shuto, Y. Baba, A. Tanaka and S. Ohashi, *J. Chromatogr.*, 298 (1984) 419.
13 H. Yoshida, S. Kito, M. Akimoto and T. Nakajima, *J. Chromatogr.*, 240 (1982) 493.