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SIMULTANEOUS DETERMINATION OF PHOSPHATE AND PHOSPHONATE BY FLOW INJECTION ANALYSIS WITH A PARALLEL DETECTION SYSTEM

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SUMMARY

A parallel detection flow injection system was designed for the simultaneous determination of phosphate and phosphonate. A molybdenum(V)–molybdenum(VI) reagent and a sulphite solution were used to differentiate colorimetrically between P^{III} and P^{V}. A total maximum sampling rate of 100 samples per h could be achieved with a relative standard deviation of less than 1%. The parallel detection system was useful as a post-column reaction detector for high-performance liquid chromatography of various oxo acids of P^{III} and P^{V}.

INTRODUCTION

In previous papers[1,2] we reported the design of a high-pressure flow injection analysis (FIA) system and its application to the rapid determination of various inorganic phosphorus compounds. For the determination of orthophosphate (P^{V}) and polyphosphates (P_n^{V}) of oxidation number V, the flow injection system was held at high pressure and the hydrolysis of polyphosphates and the colour reaction of the resultant orthophosphate with a molybdenum(V)–molybdenum(VI) reagent were achieved simultaneously in a reaction coil maintained at 140°C. On the other hand, if phosphonate (P^{III}) of oxidation number III and phosphinate (P^{I}) of oxidation number I were to be determined with the same FIA system, sodium hydrogensulphite as an oxidizing reagent[3,4] was introduced additionally to convert these phosphorus compounds into orthophosphate. The sequential operation of these two procedures, with and without the presence of sulphite, permitted the differential analysis of P^{III} and P^{V} compounds with a single spectrophotometric detection system[2].

In this work we attempted to achieve the simultaneous determination of P^{V} and P^{III} compounds by employing FIA with a dual-detection or two-channel detection system[5] in which the two procedures mentioned above can be achieved simultaneously with a single sample injection. There are two possible arrangements of detectors: in parallel and in series. This work deals with the design of a parallel detection system in which two identical detectors are arranged in parallel.

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EXPERIMENTAL

Reagents

Unless otherwise stated, all chemicals (Kishida, Osaka, Japan) were used without further purification.

A stock solution of the molybdenum(V)–molybdenum(VI) reagent for the determination of phosphorus compounds was prepared by the method described previously[1]. The reagent for the flow injection analysis was prepared by diluting 200 ml of this stock solution to 1 l in distilled water. A 0.5 *M* sodium hydrogensulphite solution was prepared daily by dissolving $NaHSO_3$ in distilled water.

The eluent consisted of 0.23 *M* potassium chloride and 0.1% (w/v) Na_4EDTA.

Samples

Disodium phosphonate ($Na_2PHO_3 \cdot 5H_2O$), disodium hydrogenorthophosphate ($Na_2HPO_4 \cdot 12H_2O$) and tetrasodium diphosphate ($Na_4P_2O_7 \cdot 10H_2O$) were guaranteed reagents. Pentasodium triphosphate ($Na_5P_3O_{10} \cdot 6H_2O$) was purified by repeated recrystallization. Disodium diphosphonate ($Na_2P_2H_2O_5$) was prepared according to the literature[6].

Flow injection system

A manifold with a parallel detection system is shown in Fig. 1. Two identical detectors were arranged in parallel. The lower analytical line was for the detection of only P^V units and the upper one was for the total phosphorus, P^V and P^{III}.

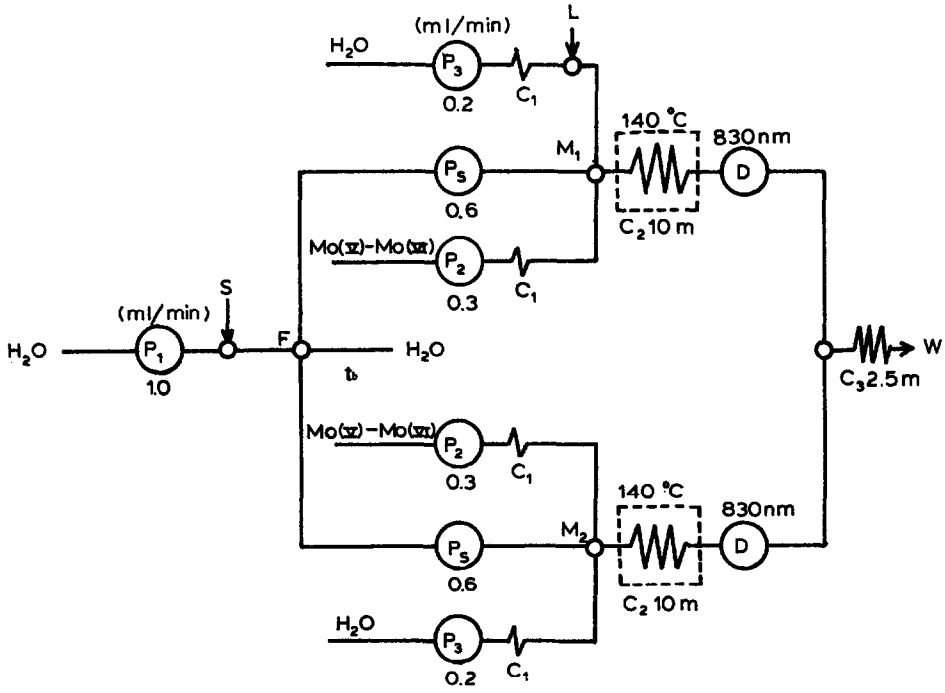

Fig. 1. Manifold with parallel detection system.

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, Kyowa KHU-W104) at a flow-rate of 1.0 ml/min. The carrier stream was then split into two parts at a four-way connector (F, Kyowa K4P-U) to be introduced into each analytical line at a flow-rate of 0.6 ml/min by means of a two-channel reciprocating pump (P_s, Seishin PSU-2.5W). In order to balance the flow-rate of liquid propelled by the pump (P_1) with that of liquid to be aspirated by the pump (P_s), water was supplied from a compensating reservoir through a branch tubing (t_b).

In the lower analytical line, the sample was mixed with the Mo(V)–Mo(VI) reagent pumped at a flow-rate of 0.3 ml/min with a reciprocating pump (P_2, Kyowa KHU-W52). The mixed solution was carried through a 10-m reaction coil, during which time polyphosphates were hydrolyzed to orthophosphate and the resultant orthophosphate was allowed to react with the Mo(V)–Mo(VI) reagent to form a heteropoly blue complex.

In the upper analytical line, the sample was mixed with the Mo(V)–Mo(VI) reagent and the sulphite solution. The sulphite solution was introduced into a water stream via a four-way loop-valve injector (L, Kyowa KMM4V2)[2]. The mixed solution was carried through a 10-m reaction coil, during which time the oxidation of lower oxo acids of phosphorus and the hydrolysis of polyphosphates were achieved simultaneously and the resultant orthophosphate was allowed to react with the Mo(V)–Mo(VI) reagent to form the heteropoly blue complex.

The absorption of the blue complex at 830 nm was monitored with flow-through cells (volume 8 μl, path 10 mm) attached to spectrophotometers (Jasco, Japan Spectroscopic Co., UVIDEC 100IIW) arranged in parallel. A narrow coil was located at the exit of the cells to give a back pressure of about 5 kg/cm^2. Under such a high pressure the reaction coil can be heated up to 150°C in a silicone oil-bath (Thomas T-201) without detector noise caused by gas bubbling.

HPLC system

A Jasco high-performance liquid chromatograph TRI-ROTAR was used. The sample solution (100 μl) was introduced onto the separation column (500 × 2.6 mm I.D.) packed with TSK-GEL IEX-220SA anion exchanger and chromatographed at a flow-rate of 1.0 ml/min and a pressure of about 190 kg/cm^2.

RESULTS AND DISCUSSION

Design of parallel detection system

The parallel detection FIA system in Fig. 1 was constructed by arranging two identical single detection systems symmetrically. As shown in previous papers[1,2], the single detection system was designed to be used as a "high-pressure flow injection system" by which experiments could be done even at temperatures as high as 140°C. The application of a back-pressure coil becomes very important at such high temperatures to avoid gas bubbling.

One important task in designing a parallel detection system is to split the injected sample or the sampling stream at point F under the restricted conditions that (1) the analytical line is maintained at a high pressure such as 5 kg/cm^2 and (2) the flow-rate for sample splitting can be selected arbitrarily in a continuous mode.

The use of a peristaltic pump was found to be unsuitable, although this type of pump has widely been used as a proportionating pump in AutoAnalyzer technology[7]. Relatively inexpensive reciprocating pumps, although more expensive than simple peristaltic pumps, were employed that were developed for post-column reactions in high-performance liquid chromatography (HPLC).

The injected sample passes through the splitting point (F), the splitting pump (P_s), the confluence point (M), the reaction coil and the detector (D) to the waste (W). The flow-rate for the sample splitting in each analytical line can be controlled continuously by each P_s. Water is supplied from a compensating reservoir (open beaker) through the brach tubing (t_b) in order to compensate for the difference between the flow-rate of the sampling stream (P_1) and the total flow-rate of the two splitting pumps. The advantage of this method is that the ratio of sample splitting can be selected arbitrarily without complicated handling. For convenience, the ratio of sample splitting was controlled at 1:1, with the splitting flow-rate of 0.6 ml/min.

Dispersion in the splitting pump

As mentioned above the sample zone was allowed to pass through the plungers in the splitting pump in Fig. 1. This approach may be unfavourable for the construction of FIA systems, because greater peak broadening is expected to occur in the pump[5]. This section deals with the estimation of the peak broadening.

It is known that the total peak variance, σ_t^2, in FIA has three contributions

$$\sigma_t^2 = \sigma_i^2 + \sigma_r^2 + \sigma_d^2 \quad (1)$$

where σ_i^2 is the variance of the injection, σ_r^2 that in the reactor and σ_d^2 that in the detector, respectively, which are expressed in seconds.

In a parallel detection system, another contribution, *i.e.*, the variance in the splitting pump, σ_p^2, is added to the total peak variance. Consequently, the total peak variance, $\sigma_t'^2$, in the parallel detection system is given by:

$$\sigma_t'^2 = \sigma_i^2 + \sigma_r^2 + \sigma_d^2 + \sigma_p^2 \quad (2)$$

According to eqns. 1 and 2, the variance in the splitting pump is:

$$\sigma_p^2 = \sigma_t'^2 - \sigma_t^2 \quad (3)$$

Fig. 2 shows the manifolds with a single detection system to measure dispersion in the splitting pump (P_s). Each manifold is the same as the analytical lines in the parallel detection system. The contrast between manifolds I and II is that the sample solution either passes through the pump (I) or does not (II). By injecting a suitable dye solution in a carrier stream it is possible to measure the total variances, $\sigma_t'^2$ (I) and σ_t^2 (II), in the manifolds. Therefore, σ_p can be estimated according to eqn. 3 and the maximum sampling rate which is related to the standard deviation of the peak, $S_{max.} = 3600/6\sigma_t'$ or $3600/6\sigma_t$ (samples per h), can also be estimated.

To estimate σ_p and $S_{max.}$, a single peak was recorded by injecting 20 μl of the dye solution (methyl orange, 0.015% w/v) and the peak width was measured at $0.61H$ where H is peak height. The values of σ_t' and σ_t were 14 and 11 sec, respectively. The

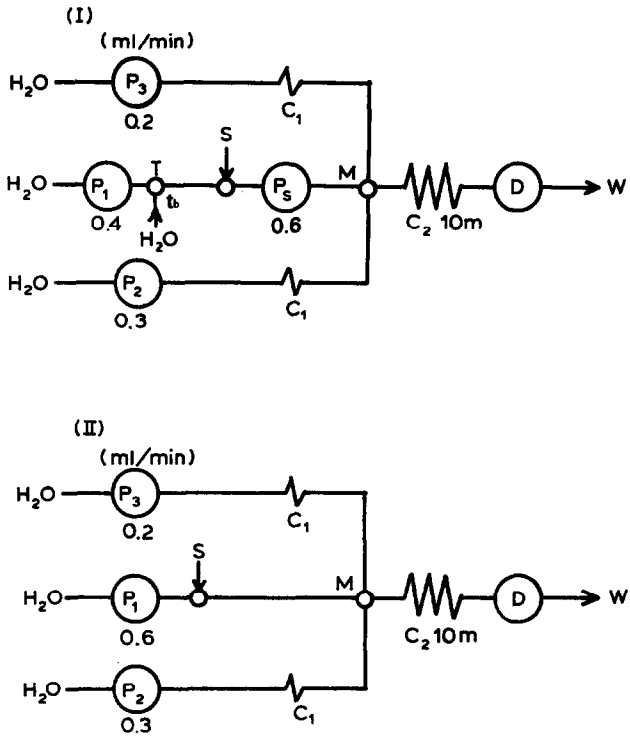

Fig. 2. Manifold with single detection system.

σ_p and $S_{max.}$ values in the manifold I were calculated to be 9 sec and 43 samples per h, respectively. The $S_{max.}$ value for the manifold I was 20% less than that for manifold II, 54 samples per h. This situation may be tolerable for practical purposes.

Simultaneous determination of orthophosphate and phosphonate

Since the dispersion of the sample zone in the splitting pump was confirmed to be less serious than expected, the performance of the parallel detection system was then examined by using sample solutions of phosphorus compounds.

A sample solution of orthophosphate was injected. Since the splitting ratio was adjusted to be 1:1 (each flow-rate 0.6 ml/min), orthophosphate in both analytical lines was expected to give identical FIA profiles due to the heteropoly blue complex produced by the reaction of orthophosphate with the molybdenum reagent. As shown in Fig. 3, the FIA profiles showed good similarity with respect to the residence time (184, 194 sec), the peak height (0.27, 0.29 a.u.), the peak width (σ_t' 12 sec) and the maximum sampling rate (50 samples per h).

If a sample solution containing orthophosphate and phosphonate is injected, both orthophosphate and phosphonate are expected to be detected in the presence of sulphite (upper analytical line in Fig. 1), while only orthophosphate is detected in the absence of sulphite (lower line). Fig. 4 shows calibration profiles for a series of samples containing equimolar mixtures of orthophosphate and phosphonate. The total amount of orthophosphate and phosphonate (A) and only orthophosphate (B)

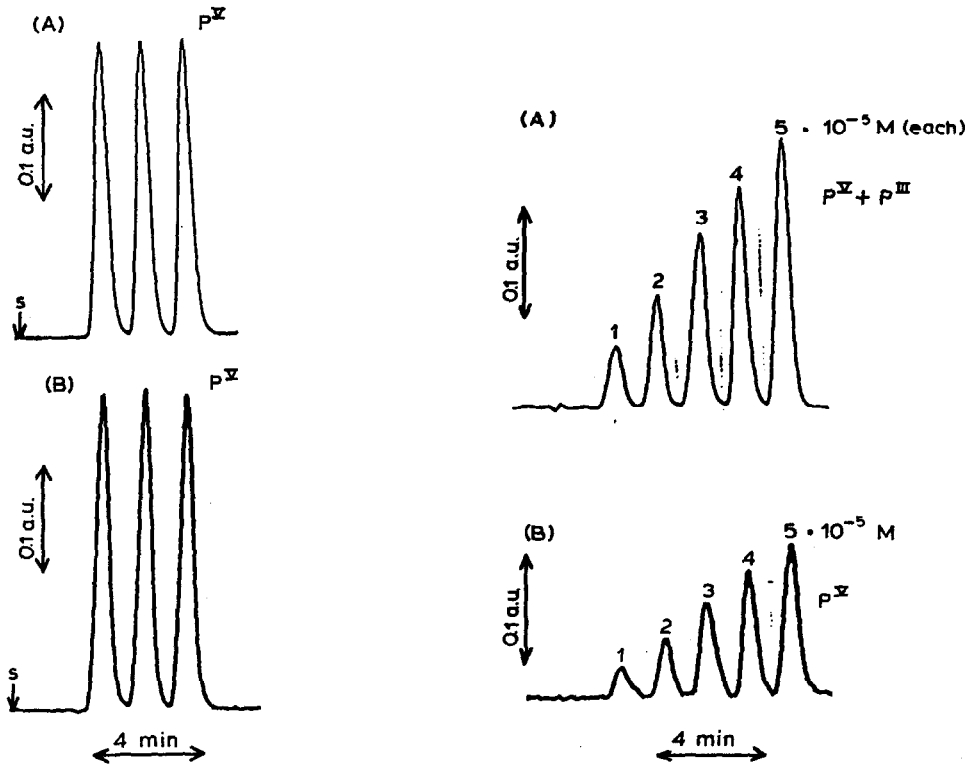

Fig. 3. FIA profiles for orthophosphate, in the presence of sulphite (A) and in the absence of sulphite (B). The sample solution was injected in triplicate. Sample concentration is $1.0 \cdot 10^{-4}$ *M*.

Fig. 4. Calibration profiles for a series of samples containing equimolar mixtures of orthophosphate and phosphonate. (A), Determination of total amount of orthophosphate and phosphonate; (B), determination of amount of orthophosphate only.

can be determined. As expected, the ratio of peak heights in Fig. 4A and B for each sample was confirmed to be 2:1. The maximum sampling rate, corresponding to a σ_t' value of 12 sec, was 50 samples per h, which means a total sampling rate of 100 samples per h in the parallel detection system.

The calibration curves showed good linearity (correlation coefficient = 0.999) and the relative standard deviation of measurement was between 0.4 and 0.9%.

Liquid chromatographic detector

The parallel detection system in Fig. 1 is very useful for the simultaneous determination of phosphate and phosphonate. A greater efficiency of this technique for the characterization of complex phosphorus compounds may be possible by combining the parallel detection technique with an HPLC technique. The on-line coupling of a chromatographic separation column with AutoAnalyzer II[8–10] or FIA[1,2] has been successfully applied to the analysis of polyphosphates or lower oxo acids of phosphorus. However, the application of a parallel detection system has not yet been reported. By replacing the sampling line in Fig. 1, the outlet of a separation column

was connected with the parallel flow injection system at the splitting point F to examine the efficiency of the system as a post-column reaction detector for HPLC of various oxo acids of phosphorus.

Fig. 5 shows the elution profiles for a mixture of phosphonate, orthophosphate, diphosphate and triphosphate, in the presence (A) and in the absence (B) of sulphite. It is evident that ortho-, di- and triphosphate can be detected in both analytical lines. On the other hand, phosphonate is detected only in the presence of sulphite.

A similar chromatographic experiment was carried out with the single detection system in Fig. 2 (II) as the detector under the same conditions (Fig. 6).

It is evident from Figs. 5 and 6 that the elution pattern from the parallel detection system is similar to that from the single detection system with respect to retention time, peak resolution and sensitivity of detection. The ratios of the peak widths of P^V, P_2^V and P_3^V in Fig. 5 to those in Fig. 6 were calculated to be 1.3, 1.1 and 1.0, respectively. The respective ratios of peak heights were calculated to be 0.79,

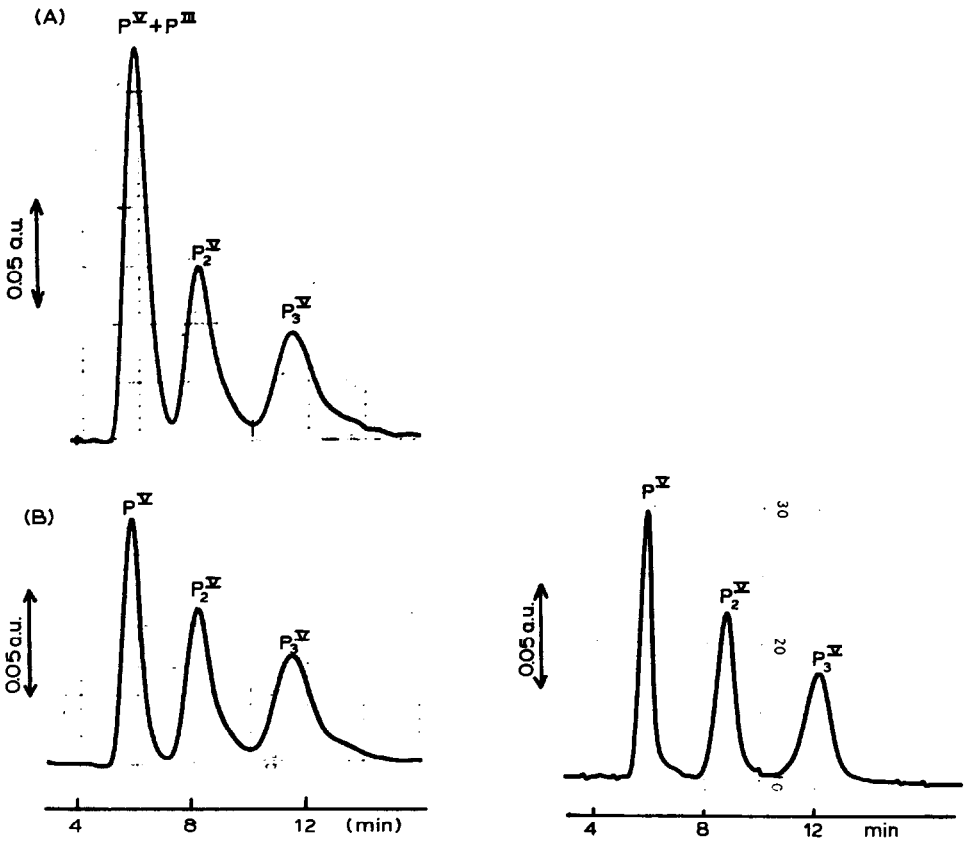

Fig. 5. Elution profiles for a mixture of phosphonate, orthophosphate, diphosphate and triphosphate, in the presence (A) and absence (B) of sulphite.

Fig. 6. Elution profile for a mixture of orthophosphate, diphosphate and triphosphate monitored by the single detection system. The sample concentration of each sample is $1.0 \cdot 10^{-4}$ *M*.

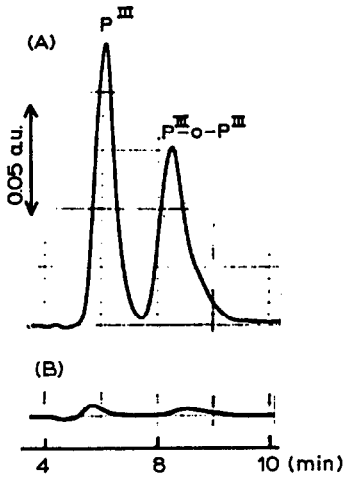

Fig. 7. HPLC profile for a mixture of phosphonate (P^{III}) and its dimer (P^{III}–O–P^{III}), in the presence (A) and absence (B) of sulphite.

0.92 and 0.98 for P^V, P_2^V and P_3^V. It was found that the dispersion in the splitting pump had little effect on the peak resolution and the sensitivity of detection when the parallel detection system was used as a post-column reaction detector.

Fig. 7 shows an HPLC profile for a mixture of phosphonate (P^{III}) and its dimer (P^{III}–O–P^{III}). In the presence of sulphite (A) two peaks were observed with reasonable areas. If the sample contains only P^{III} and P^{III}–O–P^{III}, no peak is expected to appear in the absence of sulphite (B). The small peaks (B) may be ascribed to phosphorus (V) contaminats, such as P^V and P^{III}–O–P^V.

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