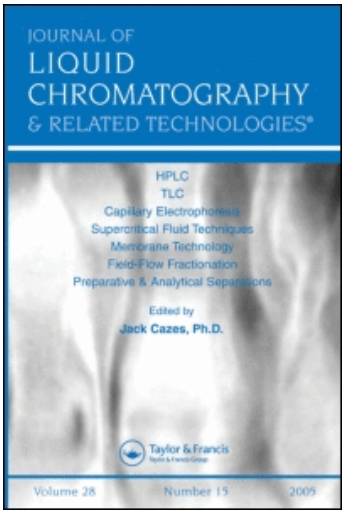

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A SPECTROPHOTOMETRIC DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF INORGANIC POLYPHOSPHATES

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ABSTRACT

A spectrophotometric detector for high-performance liquid chromatography of inorganic polyphosphates such as orthophosphate, diphosphate and triphosphate is described. This method is based on the employment of an air-segmented technique (AutoAnalyzer II, Technicon) as a detector, in combination with a post-column reaction using a molybdenum reagent.

INTRODUCTION

Rapid and automatic analysis of phosphorus compounds is increasingly becoming important in various fields such as biological, agricultural, industrial and environmental chemistry (1). One of the important requirements is to develop flow-through detectors that are useful for the achievement of high-performance liquid chromatographic separation of inorganic polyphosphates such as orthophosphate (P_1), diphosphate (pyrophosphate, P_2) and triphosphate (tripolyphosphate, P_3). Some attempts to monitor phosphorus compounds continuously have recently been reported based

on an atomic absorption method (2, 3), a flame emission method (4) and a spectrophotometric method (5, 6).

The employment of absorbance at 420 nm by the well-known phosphovanadomolybdate method has been shown to be a convenient way of monitoring polyphosphates in an effluent (5). However, there are many cases where the spectrophotometric determination of phosphorus must be carried out at longer wavelength to avoid the interference due to absorption at such short wavelength as 420 nm by foreign compounds that may often exist in environmental samples. This work was undertaken to examine the applicability of AutoAnalyzer II of Technicon (7-9) as a flow-through detector that permits the measurement of absorbance at 830 nm due to the blue complex formed by a post-column reaction of polyphosphates in an effluent with a molybdenum reagent. The advantages and limit of the application of the AutoAnalyzer II are described below.

MATERIALS

Orthophosphate, KH_2PO_4, diphosphate, $Na_4P_2O_7 \cdot 10\ H_2O$, ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, and other reagents for chromatographic separation and colorimetric detection were guaranteed reagents from Wako(Osaka, Japan). Triphosphate, $Na_5P_3O_{10} \cdot 6\ H_2O$, was prepared by the repeated purification of anhydrous triphosphate (Wako).

Unless otherwise stated, the compositions of the reagents designated in Fig. 1 are as follows.
Sulfuric acid; 9 M sulfuric acid.
Ascorbic acid; 0.1 M L-ascorbic acid containing 50 ml of acetone and 0.5 ml of Aerosol 22 (Technicon) per liter.
Mo reagent; 1 % (0.008 M) ammonium molybdate.
Surfactant; 0.5 ml Aerosol 22 per liter.

A mixed solution of 0.23 M KCl and 0.1 % EDTA (4Na) was used as an eluent in chromatographic experiments.

RESULTS AND DISCUSSION

A Chromatographic System

A flow sheet for the AutoAnalyzer II connected with a separation system (Hitachi Liquid Chromatograph 635) is shown in Fig. 1. A sample solution is injected at a point S via a variable loop sampler onto a separation column (C) and chromatographed at the flow rate of 1.0 ml/min by the employment of a reciprocating pump (P). The separation column (Ø 2.6 mm x 500 mm) is packed with an anion exchanger (TSK-GEL, IEX-220 SA). A portion of the effluent (0.6 ml/min) is introduced into the AutoAnalyzer II. Various kinds of reagent solutions are then introduced into the manifold of the AutoAnalyzer II to accelerate the hydrolysis of polyphosphates to orthophosphate in the first coil (98 °C) and to achieve the color reaction of the resultant orthophosphate with a molybdenum reagent and a reducing reagent (ascorbic acid) in the second coil (82 °C). A surfactant is also added to promote the smooth flowing of a stream. The flow rate for each reagent soltion is indicated in Fig. 1. It is a charasteristic feature of the AutoAnalyzer II that air-segmentation is used to prevent the carry-over of the sample and to assist in the mixing of the sample and reagents (7-9). The resampling, accompanied by the debubbling and wasting at a point W at the exit of the first coil, is needed to reduce the acid concentration in the second coil, because the rate of color development is greatly depressed at the high acid concentration. The absorbance of heteropoly blue complex at 830 nm is measured using a spectrophotometric detector with a flow-through cell (light path; 15 mm).

Effect of Acid Concentration on Color Development

As mentioned above the acid concentration in the first coil is desired to be as high as possible to accelerate the hydrolysis

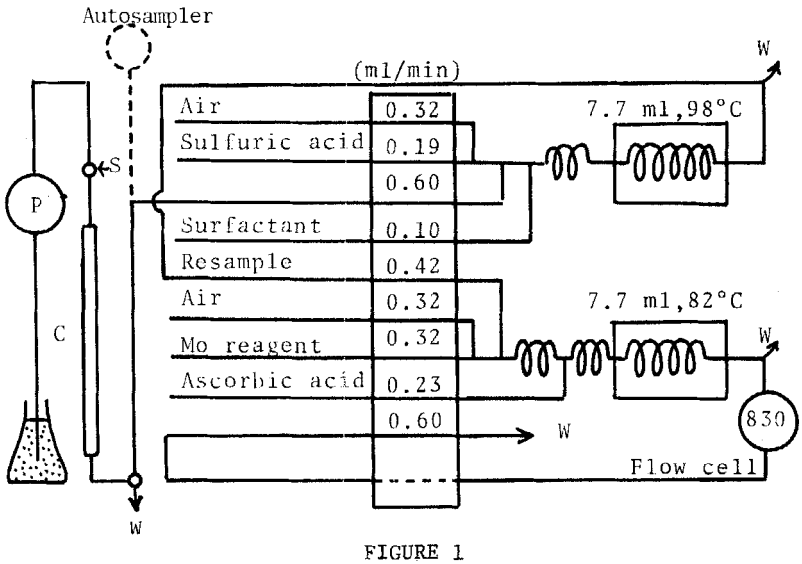

FIGURE 1

A chromatographic system using AutoAnalyzer II as a detector.

of polyphosphates to orthophosphate. On the other hand there is an appropriate range of acid concentration, i.e., 0.4-0.9 M H_2SO_4, that permits the rapid and quantitative color development in the second coil. The acid concentration in the first coil was varied to examine its effect on the hydrolysis of polyphosphates, keeping that in the second coil constant, 0.85 M H_2SO_4. In this experiment the AutoAnalyzer II in Fig. 1 was disconnected from the separation column and connected with an automatic sampler. Each sample solution of orthophosphate, diphosphate and triphosphate was introduced into the AutoAnalyzer II at the flow rate of 0.6 ml/min for 40 s and the absorbance at 830 nm was measured. In contrast to the less variation in the absorbance for orthophosphate, the absorbances for diphosphate and triphosphate vary greatly with the acid concentration (Fig. 2). This suggests that the hydrolysis of polyphosphates is the rate-determining step in the overall process of colorimetric detection of polyphosphates and about 95 % of polyphosphates can be hydrolyzed at the acid concentration of 1.95 M sulfuric acid in the first coil. Therefore, the experiments here-

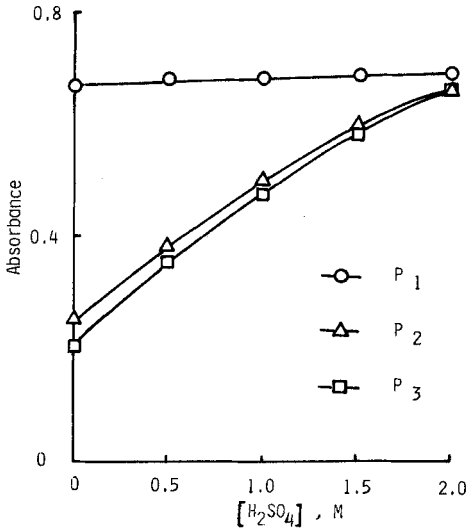

FIGURE 2

Effect of the acid concentration in the first coil on the peak heights of orthophosphate, diphosphate and triphosphate. The concentration of each sample; 1.0×10^{-4} M (as P).

after were carried out by measuring the absorbance at 830 nm under the condition that the acid concentrations in the first and second coils are adjusted to 1.95 and 0.85 M, respectively. A concentration profile is shown in Fig. 3 when various concentrations of orthophosphate, diphosphate and triphosphate are introduced at the flow rate of 0.6 ml/min for 40 s. A good linearity is shown between the peak height and the concentration of each sample. It is noted that the heteropoly blue complex can be detected more sensitively at 830 nm than at 660 nm, though the measurement at 660 nm has been widely employed (7).

Effect of Background Electrolytes on Color Development

Since potassium chloride and tetrasodium salt of ethylenediaminetetraacetic acid are used as eluting agents in chromatographic experiments it is necessary to examine the effect of these

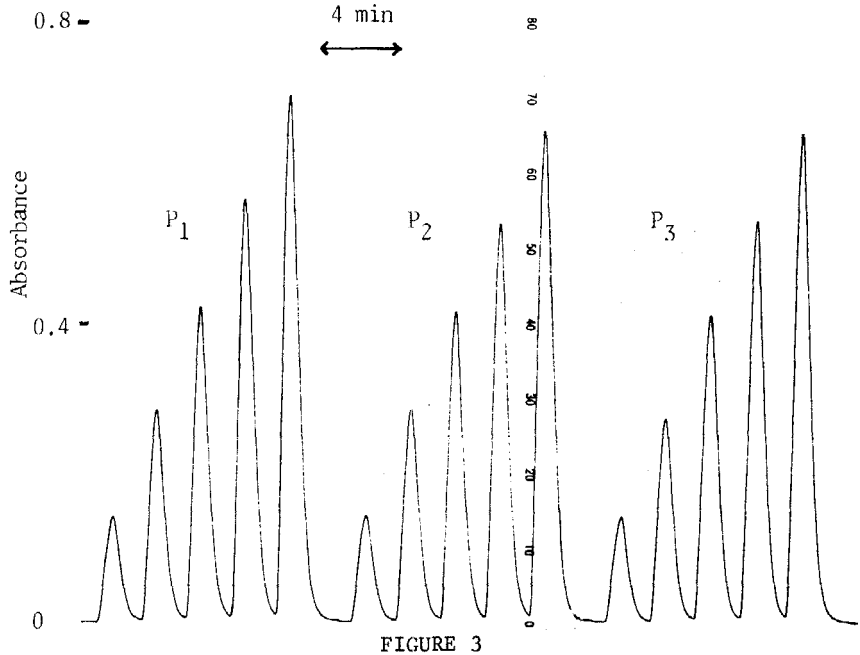

FIGURE 3

Representative concentration profiles for orthophosphate, diphosphate and triphosphate obtained by using an autosampler.

The concentration of each sample increases from left to right; 2×10^{-5}, 4×10^{-5}, 6×10^{-5}, 8×10^{-5} and 1×10^{-4} M (as P).

background electrolytes on the colorimetric detection of polyphosphates. It was confirmed that the presence of 0.23 M KCl and/or 0.1 % EDTA (4 Na) did not affect the colorimetric detection at 830 nm.

Elution Curves

In order to examine the applicability of the AutoAnalyzer II as a chromatographic detector it was connected with the separation column. An equimolar mixed solution of orthophosphate (P_1), diphosphate (P_2) and triphosphate (P_3) (100 µℓ) was injected

and chromatographed at the flow rate of 1 ml/min. An elution profile recorded at the chart speed of 12 min/inch is shown in Fig. 4. The same sample solution was injected twice successively with the time interval of about 24 min to confirm the reproducibility. It is evident from Fig. 4 that three peaks for orthophosphate, diphosphate and triphosphate are well resolved each other and the reproducibility is satisfactory to give a relative standard deviation of less than 1.0 %. Calibration curves for three phos-

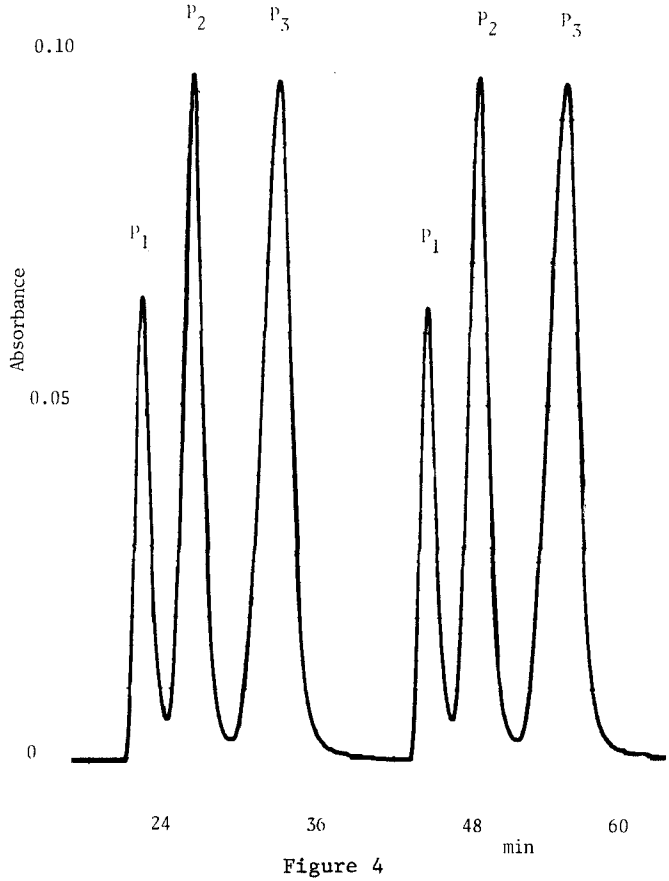

Figure 4

An elution profile for a mixed solution of orthophosphate, diphosphate and triphosphate.

The concentration of each sample component; 1.0×10^{-4} M

phates, based on the measurement of their peak heights, are shown in Fig. 5. Although these calibration curves tend to be somewhat concave it is not serious problem for the practical use in quantitative determination.

There are two points to be solved in the future. The first is the reduction of the residence time of the sample in the detector. Since the residence times of all samples in the detector are previously confirmed to be 18 min, the residence times of three peaks in the separation column are calculated from Fig. 4 to be in the range of 4-15 min. It is hoped that the residence time in the detector will be reduced to a level less than 5 min to minimize the band broadening in the detector. The second point is the quantitative analysis of polyphosphates at such low concentrations as 10^{-7} M. We have a preliminary result that the sensitivity of detection increases proportionally with the increase in sample volume and an equimolar mixture of each 10^{-6} M orthophos-

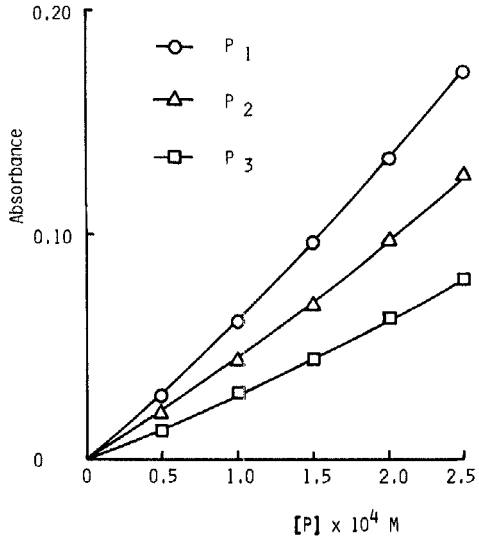

FIGURE 5

The heights of chromatographic peaks vs. the sample concentrations (as P) of orthophosphate, diphosphate and triphosphate.

phate, diphosphate and triphosphate can be quantitatively analyzed if the sample volume is increased from 100 μℓ to 2 ml.

ACKNOWLEDGMENTS

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