Fate Analysis of Phosphorus Compounds in Environmental Waters by Flow Injection Analysis and High-Performance Liquid Chromatography

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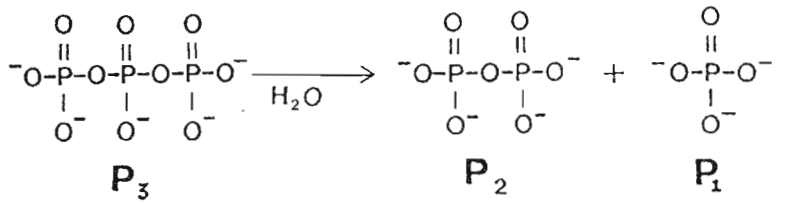
SUMMARY

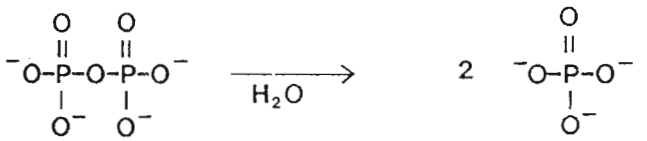
The concept of "fate analysis" of phosphorus compounds in aquatic media was explained with several examples ; a phosphorus compound is dissolved in a sample water to be examined and the convesion of its chemical form is monitored as a function of time to evaluate the quality - the chemical property and biological activity - of the water. The difference between the concepts of speciation and fate analysis was described. The interchangeable use of a high-pressure flow injection system as an HPLC detector was recommended to achieve such fate analysis of pyrophosphate and tripolyphosphate in deionized water and river water. The FIA system was improved with respect to the preparation method of a Mo(V)-Mo(VI) reagent and the use of a dry reactor with a heating aluminum block (140°C). Fate analysis in deionized water indicated the abnormally high catalytic activity of the deionized water for the hydrolysis of pyrophosphate and tripolyphosphate. The catalyst with pseudo-enzymatic function in the deionized water was deactivated by being autoclaved at 121 °C. Pyrophosphate and tripolyphosphate in river water were hydrolyzed more rapidly than one could expect from kinetic data in chemical hydrolysis , but more slowly than in the deionized water.

INTRODUCTION

In the first generation of environmental phosphorus chemistry with subjects of water pollution it was of primary importance to determine by classical methodologies, besides organic phosphorus, the total amounts of inorganic phosphates such as orthophosphate (P_1), pyrophosphate (diphosphate, P_2) and tripolyphosphate (triphosphate, P_3) in aquatic media.[1,2]



(1)



(2)

Inorganic polyphosphates are known to be hydrolyzed according to Eqs. 1 and 2, but are not very unstable with the half-lives of chemical hydrolysis of about 1 year for P_3 and 3 years for P_2 in a neutral medium at 30°C.[8] In the last decade it was emphasized with a key word " speciation " that chemical forms of phosphorus should be characterized to understand the mechanism of eutrophication, which accelerated the introduction of modern analytical techniques such as flow injection analysis (FIA)[3] and high-performance liquid chromatography (HPLC) into the field of phosphorus chemistry. During the course of our works of designing FIA and HPLC systems for the speciation of phosphorus compounds in lake waters, river waters and ground waters, we noticed that the relative amounts of P_2 and P_3 were negligibly small compared to that of P_1 in all natural waters tested, in spite of their great difference in the total amount of P_1, P_2 and P_3 or in the degree of water pollution. Our understanding was that P_2 and P_3 introduced from various waste sources might be hydrolyzed more rapidly than we expected from the kinetic data in

chemical hydrolysis so that one could observe only the distribution of P_1, P_2 and P_3 in or near the final state of conversion, the dynamic equilibrium state, from which no information might be available about chemical properties and biological activities of environmental waters.

An alternative approach to evaluate the qualities of environmental waters is based on the concept of " fate analysis " in which the conversion of chemical forms of phosphorus and its locational displacement are monitored as a function of time. For example,in the former case , P_3 is dissolved in a water sample to be examined and the change in chemical form of phosphorus ,probably with the greater contributions of enzymes[8-14] and microorganisms[1,2], is monitored at reasonable time-intervals. Fate analysis is able to observe the kinetic process of chemical and biological conversions of phosphorus in the state far from equilibrium and offer much quantitative information about chemical properties and biological activities of the medium, which could not be obtained by the speciation procedure. Fate analysis may not be conceptually new, but no successful experiments with phosphorus compounds have been reported because of the lack of analytical techniques to be used for rapid analysis.

This work was undertaken to design a high-pressure FIA system that could be interchangeably used as a high-performance liquid chromatographic detector. Basic principle of construction is similar to that shown in previous papers.[4,5] Two points were improved from the viewpoints of avoiding the use of fumy acids in preparing a molybdenum(V)-molybdenum(VI) reagent and of using a "dry" reactor with an aluminum block[6] in stead of a "wet" reactor with silicone oil as a heating medium. The Mo(V)-Mo(VI) reagent was easy to prepare and very stable at least for two months at normal temperature. The dry reactor as well as the wet reactor were effective to permit the reproducible detection of orthophosphate and polyphosphates at 140 °C with the Mo(v)-Mo(VI) reagent. Some applications with these systems are shown as to the fate analysis of P_2 and P_3 in various aquatic media; deionized water, autoclaved-deionized water and river water.

EXPERIMENTAL

The main components for FIA and HPLC systems were a reciprocating pump with four plungers (Jasco RP-4F), a spectrophotometer (Jasco, UVIDEC 100-IIW) and an HPLC system (Jasco, TRIROTAR). An ion-exchange separation column (4 mm ID x 25 cm, TSK-gel SAX, 10 μm) was employed. An eluent for the isocratic elution is shown in Fig.1. Deionized water was prepared by employing a commercially available apparatus (TOYO AQUARIUS GS-20N).

The Mo(V)-Mo(VI) reagent was prepared as follows; About 5.3 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}\ 4H_2O$, was dissolved in 900 ml of water, with the subsequent addition of 100 ml of sulfuric acid (about 18 M) to prepare a 0.03 M Mo(VI) solution (1.8 M H_2SO_4). To the Mo(VI) solution, 0.65 g of metallic zinc (sandy, free from As) was added. A part of the Mo(VI) was reduced to Mo(V) during the complete dissolution of the zinc with magnetic stirring. The total volume was finally adjusted with water to one liter. The resultant Mo(V)-Mo(VI) reagent was stable at least for two months at room temperature and ready for the determintion of orthophosphate , polyphosphates and some organic phosphorus by the so-called heteropoly blue method.

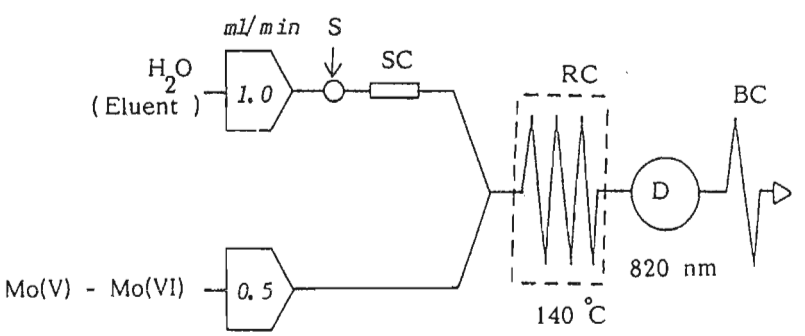


Fig. 1. An FIA system interchangeable as an HPLC detector.
< RC > reaction coil, 0.5 mm ID x 20 m PTFE; 140 °C;< BC > back-pressure coil, 0.25 mm ID x 1 m PTFE; < S > sample injector (0.1 ml loop); < D > spectrophotometric detector; < SC > anion exchange separation column, TSK-gel SAX, 4 mm ID x 25 cm ;
< Eluent > 0.23 M KCl + 0.003 M EDTA-2Na + 0.02 M NH_3.

RESULTS AND DISCUSSION

Figure 1. shows an FIA system with a separation column or, in other words, an HPLC system with an FIA system as a post-column reaction reactor. The acidic Mo(V)-Mo(VI) reagent not only accelerated the hydrolysis of polyphosphates to orthophosphate, but also reacted with the resultant orthophosphate to form a heteropoly blue complex, P(V)-Mo(V)-Mo(VI), with a spectrum (Fig. 2)similar to that developed with the original method.[7] The molar absorption coefficent of the blue complex at 822 nm (maximum) was 2.7 x $10^4 M^{-1} cm^{-1}$. The Mo(V)-Mo(VI) reagent was practically trnsparent at 820 nm.

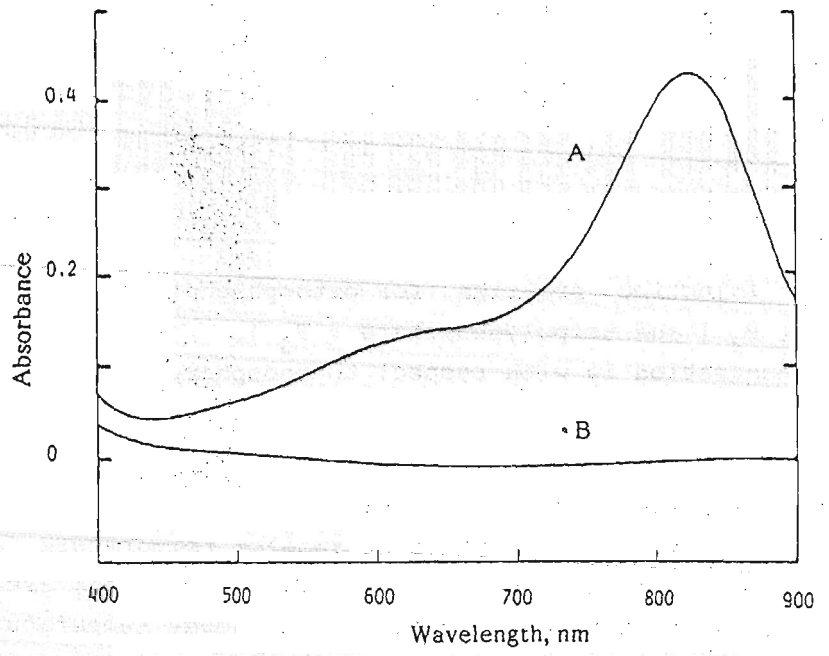


Fig. 2. Absorption spectra .
< A > P(V)-Mo(V)-Mo(VI) complex (P_1 = 0.016 mM);
< B > Mo(V)-Mo(VI) reagent; Reference, distilled water.

Unless otherwise stated, the reaction coil was maintained at 140 ℃ for routine analysis. Qunatitative and reproducible detection with the FIA system under such condition(displacing the separation column in Fig.1) is shown in Fig. 3 for P_1, P_2 and P_3.

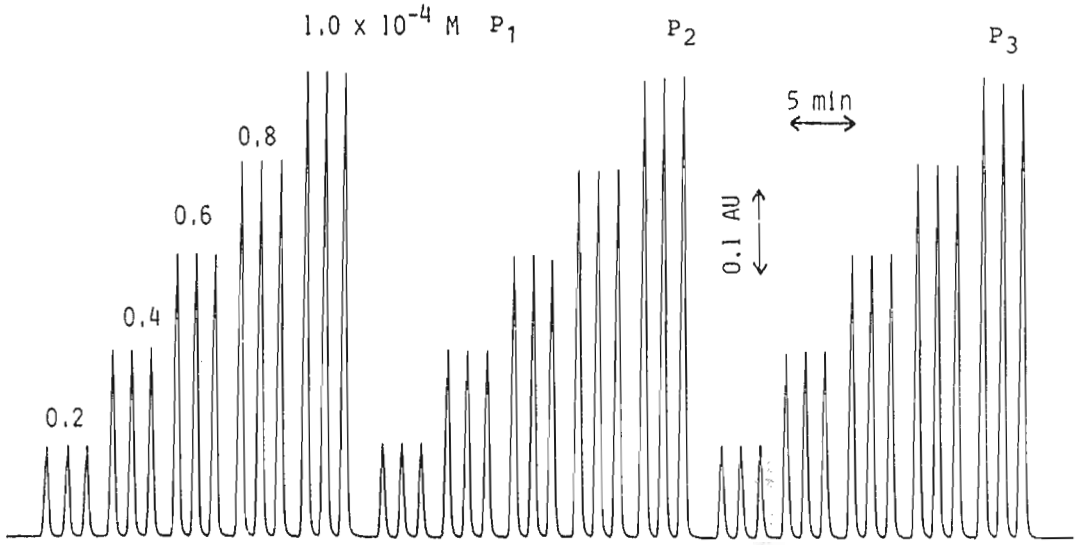


Fig. 3. Flow injection profiles for orthophsphate (P_1), pyrophosphate (P_2) and tripolyphosphate (P_3).
Each molar concentration is with respect to phosphorus.

With the separation column a equimolar mixture of P_1, P_2 and P_3 was separated to examine the effect of reactor temperature on the color development of three phosphates (Fig. 4). Peak areas were measured for quantitative evaluation . At lower temperature below 100 ℃ only small peak of orthophosphate was observed. The peak areas at 140 ℃ and 150 ℃ were confirmed to be 1:2:3 for P_1 P_2 and P_3, which indicated the quantitative detection of three species in the dry reactor as well as in the wet reactor. The reactor temperature should not be above 150 ℃ to avoid the probable break of the PTFE coil under such high back-pressure.

Figure 5 shows fate analysis of pyrophosphate and tripolyphosphate which are known chemically stable in a neutral

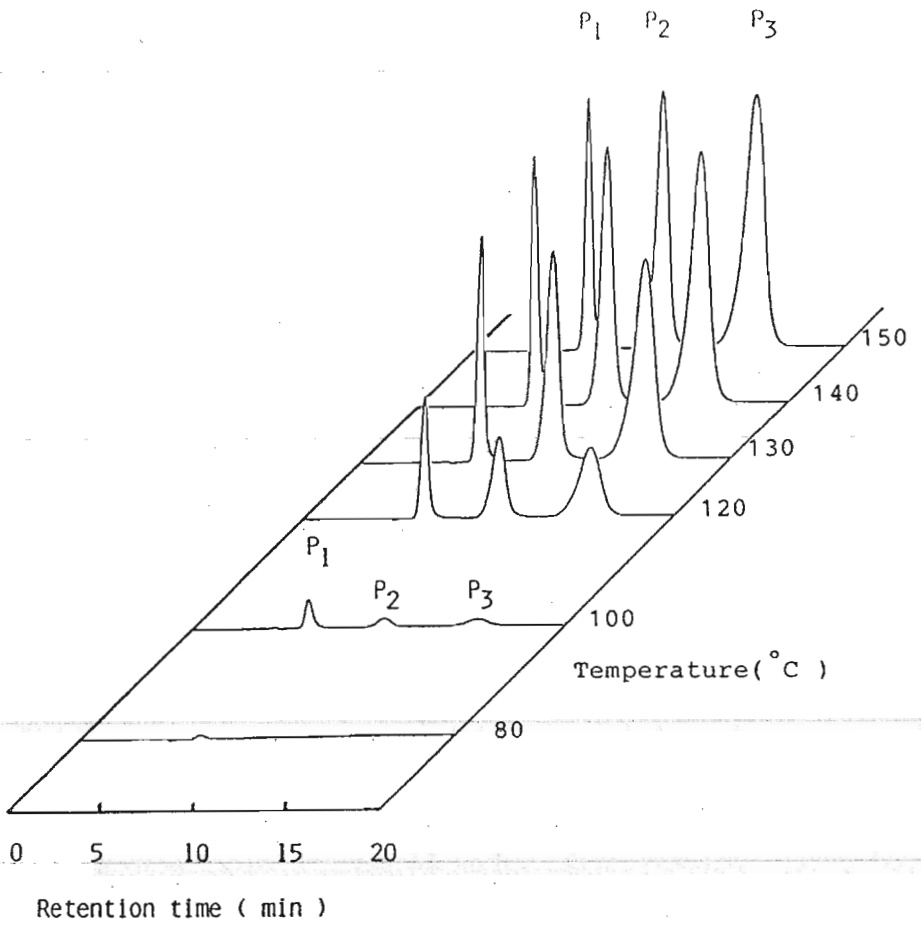


Fig.4. Effect of reactor temperature on the color development of orthophosphate (P_1), pyrophosphate (P_2) and tripolyphosphate (P_3).
An equimolar mixture (0.1 mM) of three phosphates was eluted.

medium at 30 ℃ with the half lives of about 3 years (P_2) and 1 year (P_3). It was surprising that both phosphorus compounds were hydrolyzed very rapidly (Figs. 5 A and 5B) , while these compounds were stable in deionzed water autoclaved at 121 ℃. (Figs. 5 C and D). Such abnormal catalytic activity in deionized water was found eight years ago by Hirai et al. in our laboratory with an FIA technique for the selective detection of orthophosphate in the presence of pyrophosphate, but its catalytic mechanism has not been disclosed, though the results in

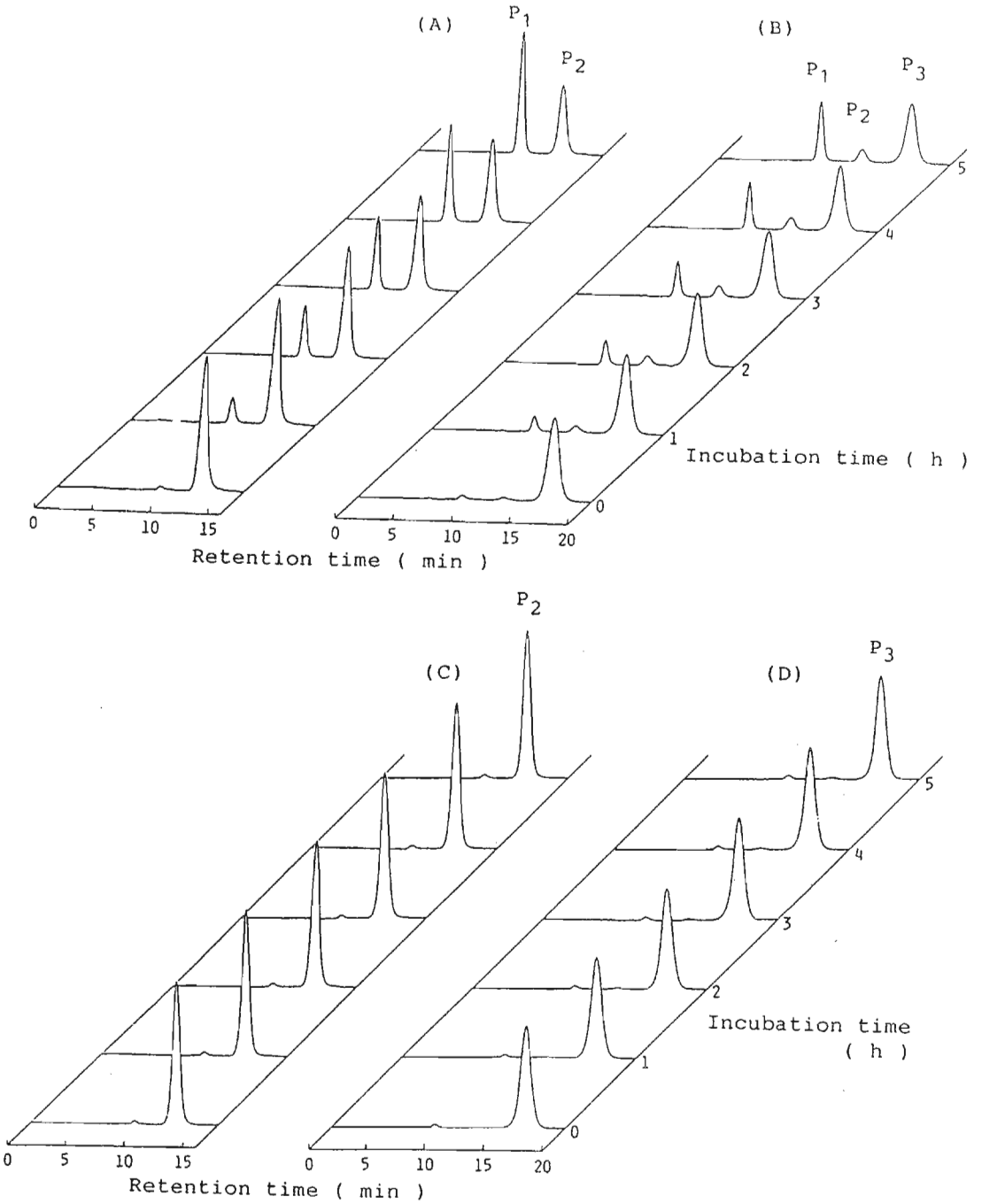


Fig. 5. Fate analysis of pyrophosphate and tripolyphosphate in deionized water and deionized-autoclaved water.
‹ A › 0.01 mM P_2 in deionized water; ‹ B › 0.01 mM P_3 in deionized water; ‹ C › 0.01 mM P_2 in deionized water autoclaved at 121 °C for 1 h; ‹ D › 0.01 mM P_3 in deionized water autoclaved at 121 °C for 1 h.

FIg. 5 suggest the presence of biofunctional substances such as enzymes that may be deactivated at 121 ℃. The uptake of phosphorus by microorganisms or the conversion of inorganic phosphorus to organic phosphorus in the deionized water is less likely , because all phosphorus are always recovered as inorganic orthophosphate, pyrophosphate and tripolyphosphate on the HPLC profiles.

Figure 6 shows fate analysis of pyrophosphate and triphosphate in a river water(Tataragawa, Fukuoka city). The phosphates were dissolved in the river water pretreated with a 0.45 μm membrane filter. It was confirmed by a separate experiment that the original river water contained no detectable amounts of pyrophosphate and tripolyphosphate. Orthophosphate was detected in the original water as can be seen in the HPLC profiles at the initial time of incubation in Fig. 6. Pyrophosphate and tripolyphosphate added were hydrolyzed more rapidly in the river water than we expected from the half-lives in chemical hydrolysis, but more slowly than in the deionized water.

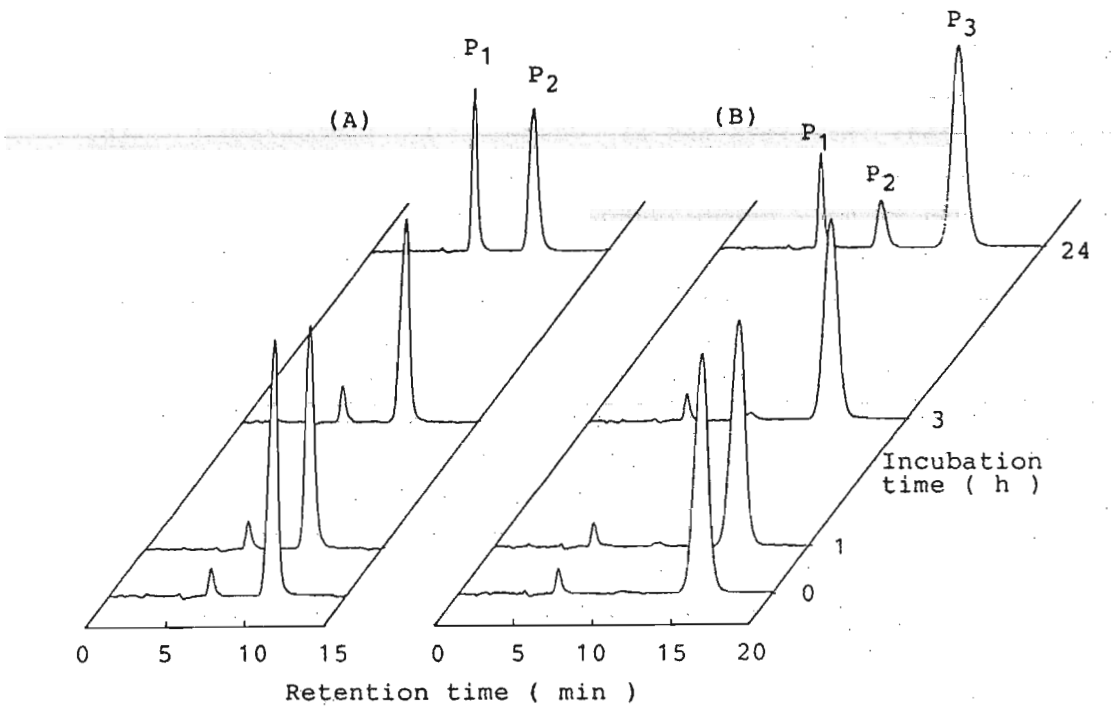


Fig. 6. Fate analysis of pyrophosphate and tripolyphosphate in river water (Tataragawa, Fukuoka).
< A > 0.01 mM P_2; < B > 0.01 mM P_3.

Detailed discussion about the contrbutions of chemical hydrolysis,[1,8] enzymatic hydrolysis[9-14] and bioavailability[1,2,10] in determining the fate of phosphorus compounds in aquatic media will be presented elsewhere in the near future.

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