

## On-site Analysis for Phosphorus and Nitrogen in Environmental Water Samples by Flow-injection Spectrophotometric Method

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On-site analytical methods for the determination of trace amounts of phosphorus and nitrogen in environmental water samples were investigated by using flow-injection (FI) technique. Detection reactions used for on-site chemical analysis for ortho-phosphate, nitrite and nitrate were examined. For economizing the reagents and cutting down waste arose along with chemical analysis to enhance zero emission, a micro-flow injection ( $\mu$ FI) technique, in which each flow rate was below one tenth of conventional FI systems and was  $50\mu\text{l min}^{-1}$ , was investigated fundamentally and applied to on-site analysis, and also a micro-reactor such as a miniature column for the reduction of nitrate to nitrite was developed. To realize the on-site spectrophotometric measurement, an easy-to-move and a one-box type chemical analyzer was newly developed. The analyzer is based on a  $\mu$ FI technique, works with a battery (12V) and can analyze samples rapidly on site with better precision and higher sensitivity than conventional FI systems. For the sensitive determination of phosphate, an ion association reaction of molybdophosphate with Malachite Green was used. Nitrate and nitrite were determined spectrophotometrically on the basis of a diazotization-coupling reaction, where nitrate was reduced to nitrite with a Cd/Cu miniature column prior to the reactions. A newly developed portable micro-flow analyzer was found to be useful for on-site determination of phosphorus as ortho-phosphate at ppb-levels, and nitrogen as nitrite and nitrate at ppb-levels in environmental samples.

**Keywords** On-site chemical analysis, micro-flow injection, phosphate, nitrite, nitrate

In recent years, fresh and sea waters, as well as air, have become increasingly polluted with an increase in human activities. For example, large quantities of phosphates have been used in detergents, and have become a cause of eutrophication of sea and lake, which has often resulted in red tides.

In general, trace amounts of metal ions in environmental water samples can be determined successfully by atomic spectroscopy such as atomic absorption spectrophotometry and inductively coupled plasma-atomic emission spectrometry, whereas nonmetallic substances, such as phosphate, nitrogen, boron and organic substances, are less sensitive to be determined by atomic spectroscopy.

In an environmental analysis, real-time and in-situ measurements of analytes are often very important to obtain accurate and rapid information on that situation. Analytical methods coupled with flow injection analysis (FIA) have proved to be very useful and versatile in many practical analyses, where the enhancement of sensitivity and precision are sometimes essentially important. By coupling an FI technique with some detection methods such as spectrophotometry and fluorophotometry, analytical procedures become very simple and rapid compared with batch-wise methods, and furthermore complicated and troublesome pretreatments, such as solvent extraction, gas diffusion separation, heating

and column reactions, are automatically carried out on line in a few minutes or less. Usually detection limit and precision are fairly well improved in FIA.

In this paper, the author examined the on-site spectrophotometric methods for phosphorus and nitrogen compounds in environmental water samples by using a  $\mu$ FI technique.

### Experimental

#### Apparatus

Schematic diagram of a FI system used in this

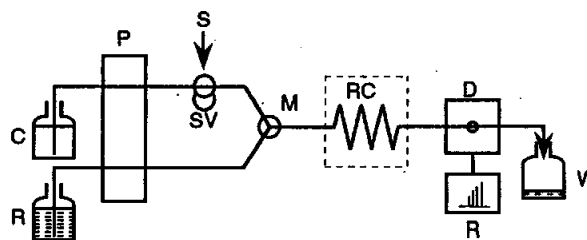


Fig.1 Flow diagram for micro-flow injection system

C: carrier; R: reagent solution;  
 P: pump (total flow rate:  $100\mu\text{l/min}$ );  
 S: sample injection; SV: sample volume ( $20\mu\text{l}$ );  
 M: mixing joint; RC: reaction coil ( $0.25\text{mm i.d.} \times 75\text{cm}$ );  
 D: detector; R: recorder; W: waste.

work is shown in Fig.1. In fundamental experiments, absorbances were measured with a Soma S-3250 visible detector with a 10-mm flow cell (2 $\mu$ l), absorbances or peaks were recorded with a Toa Dempa FBR-251A recorder, and a carrier and a reagent solution were propelled using a double plunger-type micropump (Sanuki Kogyo, DM2M-1016), which was altered to propel 2.5 or 5 $\mu$ l per one stroke of plunger. A sample solution was injected via a six-way injection valve with a variable loop into the carrier stream, which flows downstream and merges with a reagent stream. All flow lines were made of PTFE tubing (0.25mm i.d.). In the  $\mu$ FI system, a carrier and a reagent solution were propelled at each flow rate of 50  $\mu$ l min<sup>-1</sup> by a double-plunger micropump, 20  $\mu$ l of samples was injected and flowed in a 75 cm of 0.25 mm i.d. reaction tubing.

### Reagents

4-Nitrophenol was used as a model sample, and was dissolved in water to give a 10<sup>-3</sup> M stock solution, and borax solution (10<sup>-3</sup> M, pH 9.2) was used for a model reagent solution. A reagent solution used for the determination of phosphate was prepared to give a 0.11M molybdate, 1.26M sulfuric acid, 2x10<sup>-4</sup> M Malachite Green and 0.05% polyvinylalcohol. A reagent solution used for the determination of nitrite and nitrate was prepared to give a 1000ml solution containing 20g sulfanilamide, 0.5g N-(1-naphthyl) ethylenediamine and 25ml of concentrated hydrochloric acid. A copperized cadmium was prepared using powdered cadmium (Wako Pure Chem., 60~80 mesh) according to the procedure of the Japanese Industrial Standard (JIS K-0101-1991). Copperized cadmium was packed in a silica tube (0.53mm i.d. x 10cm).

### Strategy for on-site analysis of environmental samples

In this work, the methods for the on-site analysis of environmental samples were developed by taking the following points into consideration. (1) Analyte concentrations are low and sometimes in the ranges from sub-ppb to ppm: sensitive methods are necessary; (2) interferences from foreign substances are sometimes considerable: methods must be selective; (3) large number of samples must be treated: simple and rapid procedures are desirable; (4) speciation and total analysis are sometimes required: simple and rapid pretreatments are necessary; (5) time-dependent analysis are often required because analytes change in their chemical species or their concentrations by microorganisms and by chemical reactions: analyses must be carried out rapidly on site; (6) waste must be lowered as little as possible. As the method satisfying the six points mentioned above, flow injection spectrophotometric method based on micro-flow injection was adopted.

## Results and Discussion

### Fundamental investigation for the assembly of micro-flow injection system

In this study, fundamental investigation was carried out to assemble a  $\mu$ FI system. The specifications of micro flow were determined to be as follows; each flow rate was 50 $\mu$ l min<sup>-1</sup>, waste 100 $\mu$ l min<sup>-1</sup>, sampling rate one sample per min, the reproducibility of peaks less than 1%, and the detection limit of absorbance (S/N=3) about 10<sup>-4</sup> absorbance unit. To satisfy these specifications, fundamental experiments were carried out using the model sample, 4-nitrophenol (4-NP), and the borax solution (pH 9.2) as the reagent solution.

Pumping systems for propelling microliter flow were examined by using two syringe pumps for automatic titration, a peristaltic pump and two types of double-plunger pumps. Of these, double plunger pumps gave the most reproducible results and the

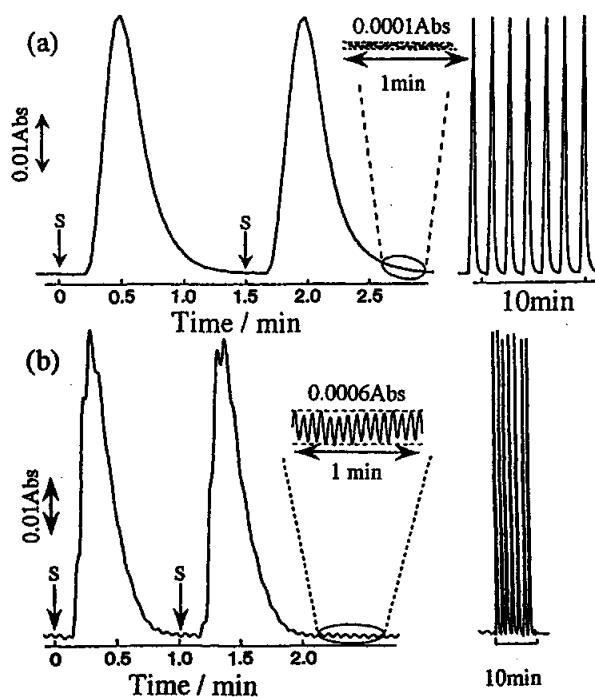


Fig.2 Pumping system (a: 2.5 $\mu$ l/stroke b: 5 $\mu$ l/stroke)

smallest noise of backgrounds. Figure 2 shows peak shapes and noise levels of the backgrounds obtained by using plunger-type pumps. It is found that a smaller volume per one stroke gave better reproducibility and less noise of backgrounds. Therefore further experiments were carried out by using the double plunger pump of 2.5 $\mu$ l per stroke.

Figure 3 shows the effect of the length of reaction tubing on peak shape; 50 cm of 0.25 mm i.d. PTFE tubing was enough for the complete mixing between 4-NP and the borax solution. Figure 4 shows the effect of sample volume on peak shape. By injecting more than 20 $\mu$ l of the sample, peak heights were almost identical; the peak reached the coefficient of

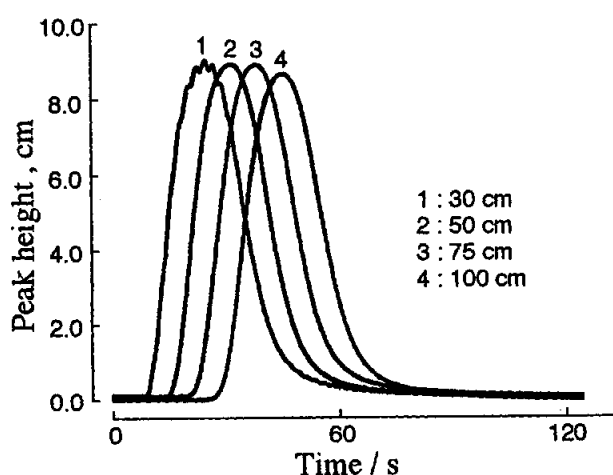


Fig.3 Effect of length of reaction tubing (0.25 mm i.d.)

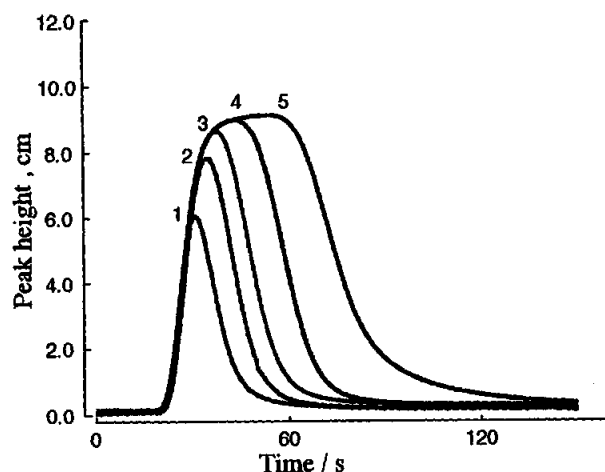


Fig.4 Effect of sample volume on peak shape

Sample:  $1 \times 10^{-5} \text{M}$  *p*-Nitrophenol.

Sample volume 1, 10  $\mu\text{l}$ ; 2, 15  $\mu\text{l}$ ; 3, 20  $\mu\text{l}$ ; 4, 25  $\mu\text{l}$ ;  
5, 35  $\mu\text{l}$

1.0. Considering the results of the state of dispersion fundamental experiments, a  $\mu\text{FI}$  system was assembled to satisfy the specifications mentioned above; the reaction tubing and sample volume were 0.25mm i.d. x 75cm and 20 $\mu\text{l}$ , respectively. Figure 5 shows the typical example of the calibration graph and the reproducibility using the model sample and the reagent.

On the basis of the results obtained in the fundamental experiments, the on-site  $\mu\text{FI}$  system was developed. The system was composed of a newly-designed double-plunger micropump, a sample injector, a reaction coil, a thermostated compartment and a detector, which were connected with 0.25mm i.d. PTFE tubing. A visible detector possessing a light-emitting diode (LED) was used. The system can work with battery and is easy-to-carry, and the data can be memorized in a IC card or micro-computer.

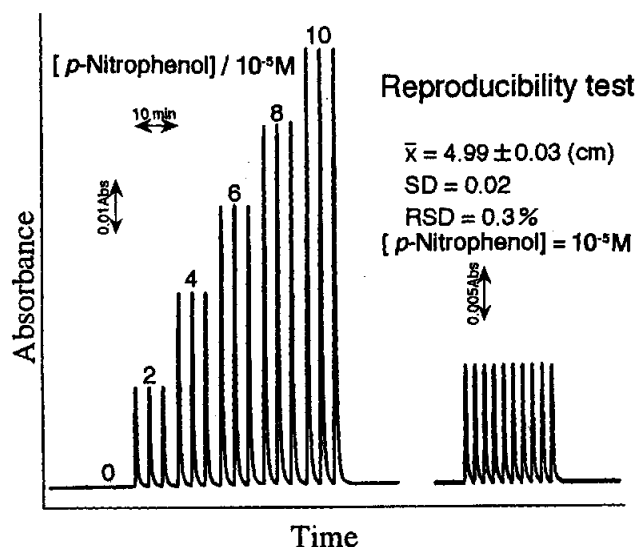


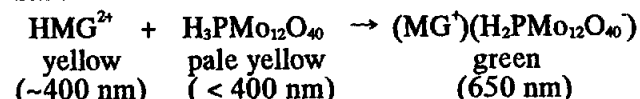
Fig.5 Linearity test of calibration graph and reproducibility using  $\mu\text{FI}$  system

$\bar{x}$ , mean peak height; SD, standard deviation; RSD, relative standard deviation.

#### Application of $\mu\text{FI}$ system to the determination of phosphate, nitrite and nitrate

##### Phosphate determination

Ion association reactions in aqueous media, which produce color change, have been used for the sensitive determination of phosphate in a batch-wise method<sup>1)</sup> and a flow-injection method<sup>2)</sup>, where the ion association of a heteropoly acid with a cationic triphenylmethane dye, Malachite Green, was examined. The coloration reaction is represented as follows:



The molar absorptivity of the colored ion associate is about  $9 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 650nm, which is about

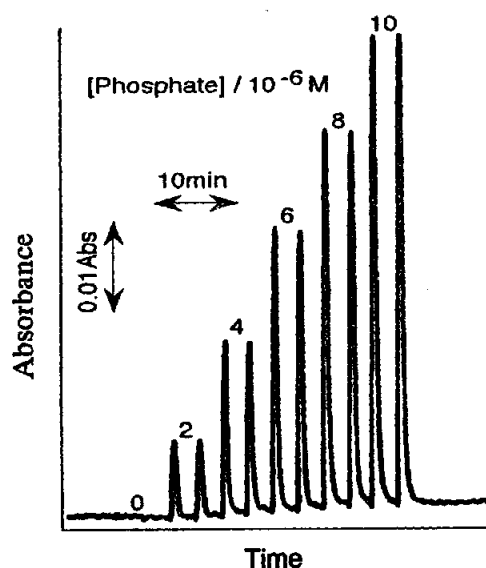


Fig.6 Flow signals for phosphate determination

10 times as large as molybdenum blue of phosphate. This reaction was applied to the on-site determination of phosphate in river water samples. Figure 6 shows the flow signals for phosphate determination, which were obtained using the  $\mu$ FI system with a 660nm LED, whose maximum wavelength is a little longer than the maximum absorption wavelength of the ion associate. The limit of detection (LOD) was about 1 ppb as phosphorus, which is almost the same as is obtained at 650nm.

#### Nitrite and nitrate determination

Nitrite has often been determined spectrophotometrically on the basis of a diazotization-coupling reaction with N-(1-naphthyl)ethylenediamine and sulfanilamide; the azo compound produced can be measured at 540nm. This color development reaction was investigated by using the  $\mu$ FI system with a 525nm LED, whose maximum wavelength is a little shorter than the maximum absorption

wavelength. The typical example of a calibration graph and the results for a river water sample are shown in Fig.7. The sensitivity was enough for the determination of nitrite in river and sea water samples: LOD was about 1ppb as  $\text{NO}_2^-$ -N. Nitrate was determined by using the same reagent solution as nitrite. As a carrier solution,  $10^{-3}$ M EDTA (pH 8.2) was used. A micro reactor (0.53mm i.d. x 10cm) packed with powdered (Cd/Cu) was installed in line after the injection valve. By using this reactor, more than 95% of nitrate was reduced to nitrite, though the sampling rate was about half of the nitrite determination. Figure 8 shows the flow signals for nitrate determination; the sensitivity was enough for the determination of nitrate in river and sea water samples: LOD was about 1ppb.

#### Conclusion

On-site analytical methods for the determination of phosphorus and nitrogen compounds in environmental samples were examined. New instrumentation based on flow injection analysis using micro-flow of a carrier and a reagent streams was investigated and applied to the on-site analysis of environmental samples.

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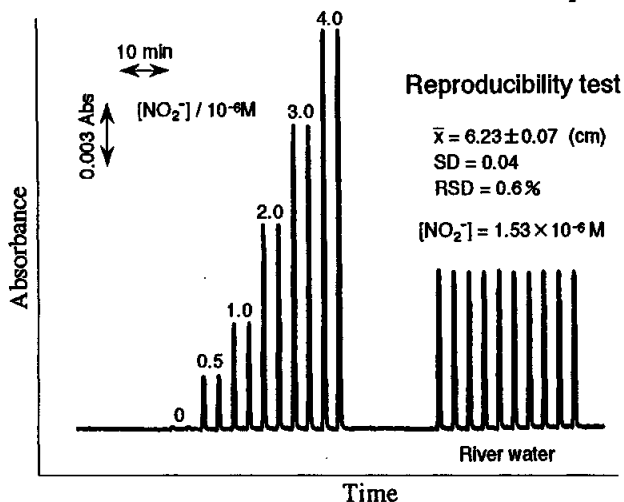


Fig.7 Flow signals for nitrite determination and for a practical sample (river water)

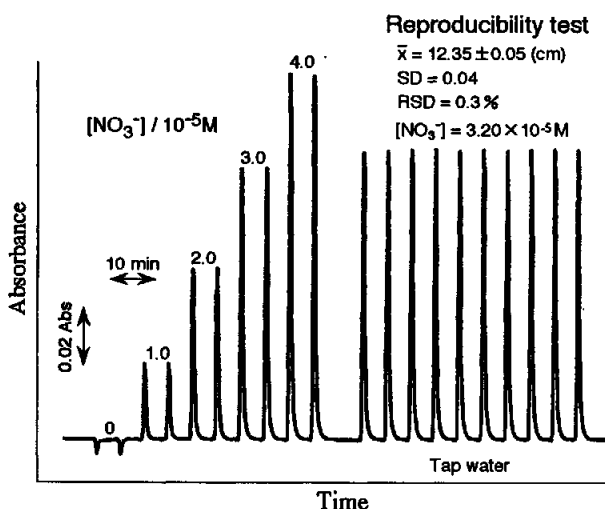


Fig.8 Flow signals for nitrate determination and for a practical sample (river water)

Column : (Particle Cd/Cu) 0.8mm i.d. x 10cm