

Chiang Mai J. Sci. 2014; 41(3) : 606-617 http://epg.science.cmu.ac.th/ejournal/ Contributed Paper

# Hydrodynamic Sequential Injection with Stopped-flow Procedure for Consecutive Determination of Phosphate and Silicate in Wastewater

Sarawut Somnam\*[a], Shoji Motomizu [b], Kate Grudpan [c,d] and Jaroon Jakmunee\*[c,e]

- [a] Department of Chemistry, Faculty of Science and Technology, Chiang Mai Rajabhat University, Chiang Mai 50300, Thailand.
- [b] Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan.
- [c] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
- [d] Center of Excellence for Innovation in Analytical Science and Technology, Chiang Mai University, Chiang Mai 50200, Thailand.
- [e] Center of Excellence for Innovation in Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

\*Author for correspondence; e-mail: sarawut\_som@cmru.ac.th; scijjkmn@chiangmai.ac.th

Received: 18 March 2013 Accepted: 13 June 2013

#### ABSTRACT

A hydrodynamic sequential injection system was proposed for the determination of phosphate and silicate in wastewater and fish pond water samples. It was based on the molybdenum blue method with spectrophotometric measurement at 710 nm. Stopped-flow procedure was applied in order to increase the reaction time. Two calibration graphs, one for the determination of phosphate concentration and another for phosphate plus silicate concentration were established in the range of  $0.1 - 4.0 \text{ mg L}^{-1}$  and  $1.1 - 12.0 \text{ mg L}^{-1}$ , respectively, with a detection limit ( $3\sigma$ ) of 80 µg P L<sup>-1</sup>and 90 µg Si L<sup>-1</sup>. Sample throughput was 20 h<sup>-1</sup> for consecutive determination of both analytes. Contents of phosphate and silicate found in wastewater samples by the developed method were in good agreement with those obtained by a batch spectrophotometric method.

Keywords: hydrodynamic sequential injection, stopped-flow, phosphate, silicate, wastewater

# **1. INTRODUCTION**

Phosphorus occurs in natural water and wastewater almost solely as phosphate that is an essential nutrient supporting the phytoplankton growth in aquatic environments resulting in the eutrophication phenomenon in the case of excess quantity [1-3]. Silicon can be present in natural water as suspended particles, colloidal particles (xSiO<sub>2</sub>.yH<sub>2</sub>O), as well as the monomeric or polymeric state of silicic acid [4-5]. Silicate in water is undesirable for industrial uses because of corrosion and difficulty of removing in equipment, particularly on high pressure steam turbine, and thus greatly affects turbine operating efficiency [6].

The determination of phosphate and silicate in water is useful for monitoring their effect to the environment. In this work, an automated hydrodynamic sequential injection (HSI) system developed further from hydrodynamic injection (HI) principle [7-8], was applied for consecutive determination of phosphate and silicate. Figure 1 illustrates the principle. When the carrier stream is halted, hydrodynamic pressure will take place in tubing. If the opened-channels are connected to the main tubing (position A), sample solution could be aspirated at this position with hydrodynamic pressure and inserts into the well-defined length of conduit. Therefore, the exact volume of solution could be calculated by volume  $= \pi r^2 L$ . Although the solution is aspirated with the longer time, the excess volume (more than the interval of L) will flow pass over the opened-channels at position A.



**Figure 1.** The HI principle, a) a fixed volume of sample is loaded through the openedchannels (A) with hydrodynamic pressure in a well-defined conduit, of length (L) and internal radius (r), while the carrier stream is stopped, b) the inserted sample is propelled along with the flow of carrier stream.

By the concept of HSI, reagent(s) and sample are sequentially introduced into the system while the carrier stream is stopped. The exact volumes of solutions are introduced without the use of injection device but employing HI principle. The concept of HSI is similar to that of sequential injection analysis (SIA). HSI offers many advantages similar to SIA such as loading precisely volume of solution, operating in automated mode and low chemical consumption. Moreover, HSI system is better than SIA in term of instrument cost because expensive high quality

pump, selection valve and computer are needed for SIA assembling but are not necessary in HSI. Although flow injection analysis (FIA) use simple and low cost devices, but high consumption is occurred unavoidably because of the continuous flow nature of the technique. Therefore, it may be mentioned that the novel analytical technique, HSI, can overcome some drawbacks of both FIA and SIA.

The common analytical methodology involves the reactions of both phosphate and silicate ions with molybdate in acidic medium, producing the yellow molybdophosphate and molybdosilicate [3]. They are further reduced by the reducing agent; such as stannous chloride [9] or ascorbic acid [10] to produce molybdenum blue compound. It is well-known that the reaction rate between molybdate and phosphate or silicate is slow. When apply this reaction to the flow-based analysis, stopping the flow temporarily after sample and reagent are mixed together can increase the reaction time, and thus enhance the sensitivity. Moreover by stopping the flow, the reaction time can be prolonged without increasing the length of the reaction coil, thus avoiding an increase of dispersion [11].

In this work, an automated stoppedflow HSI system was developed for the determination of phosphate and silicate based on molybdenum blue reaction. With the advantages of HSI concept, the two analytes can be determined consecutively in one system, with using of simple and cost-effective instrument, low chemical consumption and high degrees of automation.

#### 2. MATERIALS AND METHODS

# 2.1 Reagents and Chemicals

Analytical reagent grade of chemicals were prepared using throughout the ultrapurified water from Elix 3/ Milli-Q element system (Nihon Millipore, Japan) and stored in polyethylene bottles.

A stock solution of 1,000 mg P L<sup>-1</sup> was obtained by dissolving 0.4412 g potassium dihydrogen phosphate (Wako Pure Chemicals, Japan) in 100 mL of water. Working standard solutions were prepared daily by diluting the stock standard solutions to desired concentration. The working standard solutions of silicate were diluted from stock standard solution (Na<sub>2</sub>SiO<sub>3</sub> in 0.2 M Na<sub>2</sub>CO<sub>3</sub>, 1 mL = 1000  $\mu$ g Si, Wako Pure Chemicals, Japan) to desired concentration.

Chromogenic reagents, molydate solution, consisted of two types. For determination of phosphate concentration, it was prepared by dissolving 3.09 g  $(NH_4)_6Mo_7O_{24}.4H_2O$  and 0.84 g NaF in 50 mL of water. This solution was added with 25 mL of 2.0 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> before adjusting the volume to 100 mL with water. The reagent for the determination of total concentration of phosphate plus silicate was prepared by dissolving 3.09 g  $(NH_4)_6Mo_7O_{24}.4H_2O$  in 50 mL of water. This solution was added with 15 mL of 2.0 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> before adjusting the volume to 100 mL with water. The carrier solution of 0.020 mol L-1 ascorbic acid was obtained by dissolving 0.35 g L-ascorbic acid in 100 mL of water.

# 2.2 HSI Manifold and Operational Procedure

The manifold of HSI system for determination of phosphate and silicate is illustrated in Figure 2. All tubings used were of PTFE tubing connection network of three way connectors and tubings formed segments, A, B, C and D of defined volume of about 20 µL each. Solenoid pumps (Biochem valve, Inc., USA) and solenoid valves (Tagasago Electric, Inc., Japan) were used for propelling the solutions and control the flow direction, respectively. Absorbance was measured by a spectrophotometer (Spectronic 21, Bausch & Lomb, USA) equipped with a flow cell of 10 mm path length. A data collection and instrument control were done by using a data acquisition card (AT-MIO-16XE-50; National Instruments, Austin, TX) equipped with a personal computer. An in-house written program, which was created from LabVIEW<sup>®</sup> software (Version 7; National Instruments, Austin, TX), was used to operate the HSI system.



**Figure 2.** Schematic diagram of the HIS system: C, 0.020 mol L<sup>-1</sup> ascorbic acid; R1, 0.025 mol L<sup>-1</sup> molybdate solution in 0.50 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> with 0.20 mol L<sup>-1</sup> NaF; R2, 0.025 mol L<sup>-1</sup> molybdate solution in 0.30 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>; S, Sample; MC1, Mixing coil length 200 cm; MC2, Mixing coil length 100 cm; SP, Solenoid pump and SV, Solenoid valve (dense line = on, dash line = off).

Table 1. Operation sequence of HSI system.

Step	Pump		Valve*						Period	Description
	SP1	SP2	SV1	SV2	SV3	SV4	SV5	SV6	(sec)	
1	off	on	off	off	on	off	on	off	6	Draw up sample
2	off	on	off	off	off	on	on	off	6	Draw up reagent 1
3	on	off	on	off	off	off	off	on	50	Forward the mixture to
										detector (for phosphate
										determination)
4	off	on	on	off	on	on	off	on	8	Draw up reagent2
5	off	on	on	on	off	on	off	on	8	Draw up sample
6	on	off	off	on	on	on	on	off	15	Forward the mixture
										(for phosphate and silicate
										determination)
7	off	off	off	on	on	on	on	off	15	Stop the mixture to
										increase the reaction time
										of silicate to molybdate
										solution
8	on	off	on	on	on	on	on	off	70	Forward the mixture to
										detector (for phosphate
										and silicate determination)

"The word "on / off" mean that the valve was switched / stopped when observed from Figure 2.

Operation of the system was fully controlled by computer using the sequence as summarized in Table 1. The analysis included two major procedures; determination of phosphate concentration, and determination of total concentration of phosphate plus silicate. Two mixed reagent solutions; 0.025 mol L<sup>-1</sup> molybdate solution in 0.50 mol  $L^{-1}$   $H_2SO_4$  with 0.20 mol  $L^{-1}$  NaF (reagent 1) and 0.025 mol  $L^{-1}$  molybdate solution in 0.30 mol  $L^{-1}$   $H_2SO_4$  (reagent 2), were prepared for the determination of phosphate and total concentration, respectively. The reactions for molybdenum blue may involve [10]:

heptomolybdate + phosphate <u>fast</u> yellow heteropoly complex (a)

yellow heteropoly complex (Mo(VI)) + ascorbic acid <u>slow</u> molybdenum blue (Mo(V)↔Mo(III)) (b)

Ascorbic acid, a reductant solution, was also used as a carrier. Sodium fluoride (NaF) was mixed into molybdate solution as the masking agent to prevent the reaction between silicate and molybdate during the phosphate determination step [12]. The operation can be briefly described as follows: The system (Figure 2) was designed by separating two lines; a lower line for phosphate determination and an upper line for total concentration determination. First, standard/sample and reagent 1 were drawn up sequentially through solenoid valves (SV3 and SV4) with solenoid pump (SP2) to fill directly in segment A and B following with sequentially loading of reagent 2 and sample through solenoid valves (SV2 and SV3) in segment C and D, respectively. The stacked zone of sample and reagent 1 in the lower line was pushed by the carrier with SP1 into the detector to record the signal. Next, the stacked zone in the upper line was also pushed into the mixing coil (200 cm) and stopped to increase the reaction time before being pushed to the detector to record the signal. This was done due to the slower reaction rate between silicate and molydate. Samples were analysed using the two calibration graphs. Calibration graph for phosphate was constructed by plotting between peak height obtained and phosphate concentration. In the same way, calibration graph of total concentration of phosphate plus silicate was obtained by plotting between peak height and the total concentration. Silicate concentration was obtained by subtraction of phosphate concentration from the total concentration.

#### 2.3 Sample Preparation

Wastewater and fish pond water samples were collected for phosphate and silicate determination. After collection, all samples were filtered immediately through a  $0.45 \,\mu m$ membrane before analysis. A polyethylene bottle is a more desirable choice for storing a filtered sample than glassware to avoid interfering of silicate leached from the glassware. These samples were collected and analyzed within 24 h without any preservation.

#### 3. RESULTS AND DISCUSSION

By combination of tubings with three way connectors, well defined volume solution segments were created. The solutions could be introduced into these segments by the hydrodynamic injection [7] with the help of solenoid valves to control the direction of the solution flow. Extended from the hydrodynamic injection mode in FIA which only sample was injected, in the HSI both sample and reagent were injected to form stacked zones. Zone penetration similar to that in SIA was occurred while the stacked zones were pushed through a mixing coil to the detector, leading to producing of the reaction product to be measured at the detector. In SIA, volumes of sample and reagents can be variably and accurately aspirated using a high quality syringe pump and a selection valve. However, too large volume of the zones could reduce penetration of the sample and reagent zones lead to less reaction to occur [13]. In HSI, the volume of solution was fixed by the defined volumes of the tube connected between the T-connectors (about 20 µL each zone in this case), so a simple pump (solenoid pump) can be accepted for aspirating the solutions to fill the zones. Effect of the experimental variables such as acidity, concentration of reagents and stopping time was studied.

#### 3.1 Effect of Acidity

Although the interference of silicate could be reduced with an increase in the acid concentration, but the reaction sensitivity for phosphate is also concomitantly decreased. Moreover, under low acid concentration, even in the absence of orthophosphate, self-reduction of molybdate could be taken place [14]. So, the acidity effect is the important parameter and was studied first. Sulfuric acid  $(H_2SO_4)$  was chosen in this work and varied in the range of 0.050 - 0.60 mol L<sup>-1</sup>. A series of mixed standard solutions of phosphate (0.1-4.0 mg P L<sup>-1</sup>) and silicate (1.0-8.0 mg Si L<sup>-1</sup>) was injected to the system in order to construct calibration graphs for phosphate and phosphate plus silicate. A plot of the slopes of calibration graphs (sensitivity) versus the concentration of sulfuric acid is shown in Figure 3.



**Figure 3.** Effect of the acidity on slope of the calibration graphs of phosphate (0.1-4.0 mg  $P L^{-1}$ ), phosphate plus silicate (1.1-12 mg  $L^{-1}$ ) and silicate (1.0-8.0 mg Si  $L^{-1}$ ).

According to the results, low concentration of  $H_2SO_4$  resulted in low sensitivity for both phosphate and silicate determinations. At higher concentration of acid, higher sensitivity for phosphate was obtained, but sensitivity for silicate was decreased, due to the low reactivity of this reaction at low pH[15]. This fact should be useful to phosphate determination procedure as the increase in acid concentration would reduce possibility of interference due to silicate. For phosphate, signal began to be constant at  $H_2SO_4$  concentration higher than 0.40 mol

 $L^{-1}$ , while signal of silicate being practically constant for 0.20 to 0.40 mol  $L^{-1}$  H<sub>2</sub>SO<sub>4</sub>. Therefore, H<sub>2</sub>SO<sub>4</sub> concentrations of 0.50 mol  $L^{-1}$  and 0.30 mol  $L^{-1}$  were selected for phosphate and phosphate plus silicate determinations, respectively.

#### 3.2 Effect of Reagent Concentrations

Chromogenic reagent, molybdate solution was optimized for the suitable concentration by variation in the range of 0.001 - 0.040 mol L<sup>-1</sup>. The results were reported in Figure 4.



Figure 4. Effect of molybdate concentration on the determination of phosphate and phosphate plus silicate.

The signal of two curves increased rapidly with the increase of molybdate concentration but level off at 0.020 mol L<sup>-1</sup> molybdate. So, 0.025 mol L<sup>-1</sup>molybdate was selected for both cases. Ascorbic acid was used as a reducing agent. It was varied in the range of 0.001 -0.040 mol L<sup>-1</sup>. Peak height signal versus concentration of ascorbic acid was plotted as shown in Figure 5.



Figure 5. Effect of ascorbic acid concentration on phosphate and phosphate plus silicate determinations.

Higher peak signals for both of phosphate and phosphate plus silicate were obtained when higher concentration of ascorbic acid was used. However, as observed that 0.040 mol L<sup>-1</sup> ascorbic acid showed the highest signal in this study but highest blank signal was also obtained. Ascorbic acid of 0.020 mol L<sup>-1</sup> was selected although the signal was smaller than that 0.040 mol L<sup>-1</sup> ascorbic acid but smaller and smoother blank signal of 0.02 mol L<sup>-1</sup> was obtained (peak signals of blank of 0.02 mol  $L^{-1}$  and 0.04 mol  $L^{-1}$  ascorbic acid were 0.02 V and 0.08 V, respectively).

#### 3.3 Effect of Stopping Time

Due to the slow reaction rate of molybdate with phosphate and silicate, stopping the flow after mixing of the reagent and sample may help to increase the sensitivity. The effect of stopping time was investigated in the range of 0 - 45seconds. The results were reported in Figure 6.



Figure 6. Effect of stopping time on phosphate and phosphate plus silicate determinations.

From the results, stopping the flow to increase reaction time did not affect to phosphate signal. This may be because of higher reaction rate of phosphate than that of silicate [10]. Thus, stop flow is not necessary for phosphate determination step. However, for total concentration determination step, the higher peak signal could be obtained by the stopping the flow because of the lower reaction rate of silicate in mixture. Although the higher signal of total concentration obtained when the longer stopped-time, but blank also showed higher signal (peak signals of blank of stopping time at 15 and 30 seconds were 0.15 V and 0.17 V, respectively). From the graph, stopping time of 15 seconds should be suitable for the total concentration determination step.

#### 3.4 Analytical Characteristics

Under the selected condition, series of mixed standard solutions of phosphate and silicate was injected into the system. The signal profiles as shown in Figure 7 were obtained. Linear calibration graphs in the range of 0.1 - 4.0 mg L<sup>-1</sup> of phosphate (y = 0.0365x + 0.0609; R<sup>2</sup> = 0.9984) and 1.1 - 12.0 mg L<sup>-1</sup> of total concentration of phosphate plus silicate (y = 0.0542x + 0.0365; R<sup>2</sup> = 0.9971) were obtained with a sample throughput of 20 h<sup>-1</sup>. Detection limits calculated from 3 times of standard deviation of blank/slope of the calibration graphs were found to be 80 µg P L<sup>-1</sup>and 90 µg Si L<sup>-1</sup>, respectively.

Precision of the method was investigated by 11 replicate injections of 0.80 mg P L<sup>-1</sup> and 5.0 mg Si L<sup>-1</sup> under the selected condition. The relative standard deviations were 1.8% and 0.9% for phosphate and silicate, respectively. The procedure consumed 100  $\mu$ L of reagent and sample and 7.2 mL of carrier solution per operation cycle.



Figure 7. Signal profiles obtained from consecutive determinations of phosphate and phosphate plus silicate. Concentrations of phosphate and silicate: (I) 0,0; (II) 0.1,1.0; (III) 0.4,3.0; (IV) 0.8,4.0; (V) 2.0,6.0; (VI) 4.0,8.0 mg  $L^{-1}$ . (Signal was recorded only during steps 3 and 8 (see Table 1)).

# 3.5 Interference Study

Silicate was the main interference in spectrophotometric determination of phosphate. To study the tolerance limit of silicate on the phosphate determination, silicate was mixed together with a fixed phosphate concentration of 0.60 mg L<sup>-1</sup> ( $1.9 \times 10^{-5}$  mol L<sup>-1</sup>). The tolerance limit was investigated by using definition as the maximum concentration of a foreign ion causing a signal deviation of peak height of less than ±5% of the mean value of the peak due to standard solution without foreign ion. It was found that when NaF was added to reagent to mask silicate in the phosphate determination step, the tolerance limit of silicate was up to 1.1 mmol L<sup>-1</sup> for the ratio Si:P being 50:1.

Arsenate (As(V)) was also the important interference because of its ability to form complex with molybdate as molybdoarsenate [16]. Although it was found much lower concentration than phosphate and silicate in environmental samples but its effect should also be studied in the case of application to the area polluted with arsenic [14]. Katsaounos et. al suggested the prevention method of this interference by adding with an acidified sodium sulphite to reduce As(V) to nonreactive form As(III) [17]. The mentioned reducing agent was prepared form Na<sub>2</sub>SO<sub>3</sub> (anhydrous) and H<sub>2</sub>SO<sub>4</sub>. This could lead to the tolerance limit to arsenate up to 2.8  $\mu$ mol L<sup>-1</sup> for the ratio As(V):P being 0.15:1. So, the developed system would not be disturbed by the major interferences; silicate and arsenate, when applying to real samples.

Apart from the major interferences, the other foreign ions;  $NO_{2}^{-}$ ,  $NO_{3}^{-}$ ,  $ClO_{2}^{-}$ ,

ClO<sub>3</sub>,ClO<sub>4</sub>, SO<sub>4</sub><sup>2</sup>, F, Cl, Br, I, BrO<sub>3</sub>, IO<sub>3</sub>, CH<sub>3</sub>COO<sup>-</sup>, CO<sub>3</sub>, Na<sup>+</sup>, K<sup>+</sup>, Li<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Al<sup>3+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup> and Zn<sup>2+</sup>, of which known concentrations were added to a solution containing fixed concentrations of 19  $\mu$ mol P L<sup>-1</sup> (0.6 mg P L<sup>-1</sup>) and 0.11 mmol Si L<sup>-1</sup> (3.0 mg Si L<sup>-1</sup>). The effect of foreign ions was summarized in Table 2. From the results, it showed that none of these ions would affect the determination of phosphate and silicate.

Table 2. Maximum tolerance concentration ratios of diverse ions on the determinationof phosphate and silicate.

Species	Tolerance limit
F <sup>-</sup> , Cl <sup>-</sup> , Br <sup>-</sup> , I <sup>-</sup> ,SO <sub>4</sub> <sup>-2-</sup> , Na <sup>+</sup> , K <sup>+</sup>	$ m C_{ion}/ m C_{phosphate}$ 400
$BrO_{3}^{-}, Li^{+}, Mg^{2+}$	200
$CH_{3}COO^{2}$ , $CO_{3}^{2}$	100
$Si, NO_2, NO_3, ClO_2, Al^{3+}, Ni^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}$	50
$\text{ClO}_3^-, \text{ClO}_4^-, \text{Fe}^{3+}$	10
$Ca^{2+}, IO_3^{-}$	5
As(V)	0.15
F <sup>-</sup> , Cl <sup>-</sup> , Br <sup>-</sup> , I <sup>-</sup> , SO <sup>2-</sup> , NO <sup>-</sup> , Na <sup>+</sup> , K <sup>+</sup>	$\frac{C_{ion}}{70}$
$BrO_{3}^{-}, NO_{2}^{-}, Li^{+}, Mg^{2+}$	35
CH,COO	15
$CO_{3}^{2-}$ , $ClO_{2}^{-}$ , $Ca^{2+}$ , $Ni^{2+}$ , $Cu^{2+}$ , $Mn^{2+}$ , $Zn^{2+}$	8
$Al^{3+}, Fe^{3+}$	2
As(V), $IO_3^-$ , $ClO_3^-$ , $ClO_4^-$	0.2

#### 3.6 Analysis of Water Samples

Samples of wastewater and fish pond water were analysed for phosphate and silicate contents by the proposed method. All samples were prepared as described in the section 2.3. Concentration of phosphate in the sample solution was obtained from peak signal obtained using a calibration graph (y = 0.0365x + 0.0609;  $R^2 = 0.9984$ ) and total concentration of phosphate plus silicate was obtained from a calibration graph (y = 0.0542x + 0.0365;  $R^2 = 0.9971$ ). Then, silicate concentration was obtained by subtraction of phosphate concentration from total concentration. The phosphate and silicate contents in water samples are summarized in Table 3. Analyses of the samples by the standard batch spectrophotometric method based on molybdenum blue reduced by ascorbic acid for determination of phosphate and heteropoly blue method for determination of silicate [3] were also carried out for comparison.

The results obtained by the two

methods were compared by t-test at 95% confidence level. The tabulated t-value for seven degrees of freedom is 2.365 while the

Table 3. Analysis of phosphate and silicate in different samples.

calculated t-values of phosphate and silicate determination were 0.37 and 1.48, respectively. So, there are no significant differences.

Sample	Phosphate	(mg P L-1)	Silicate (mg Si L <sup>-1</sup> )		
-	Proposed	Standard	Proposed	Standard	
	method*	method	method*	method	
Wastewater 1	$5.0 \pm 0.2$	5.4	$3.1\pm0.2$	2.8	
Wastewater 2	$1.3\pm0.1$	1.0	$4.8\pm0.1$	5.0	
Wastewater 3	$0.9 \pm 0.1$	0.7	$6.0\pm0.3$	6.2	
Wastewater 4	N.D.	N.D.	$5.6\pm0.1$	5.9	
Wastewater 5	$1.9\pm0.1$	1.7	$6.1\pm0.1$	6.4	
Fish pond 1	N.D.	N.D.	$0.8\pm0.0$	0.7	
Fish pond 2	$5.3 \pm 0.3$	5.5	$7.4 \pm 0.2$	7.8	
Fish pond 3	N.D.	N.D.	$1.5\pm0.2$	1.4	

\*Mean of triplicate results; N.D.: not detected.

# 3.7 Recovery Study

Standard solutions of phosphate and silicate were added to all samples. From the results, recoveries were found to be 95 - 114 % and 94 - 115 % for phosphate and silicate, respectively.

#### 4. CONCLUSIONS

A fully automatic HSI system was developed for determination of phosphate and silicate based on molybdenum blue reaction. This simple flow system enables to determine two analytes consecutively, with low chemical consumption, fast, convenient and automatic operation. Stopped-flow mode was applied with the proposed system to increase reaction time in the step of determination phosphate plus silicate concentration because of slow reaction rate of silicate to molybdate reagent. Linear calibration graphs for phosphate and the total phosphate plus silicate concentration were established with the detection limits of 80  $\mu g$  P  $L^{\text{-1}}$  and 90  $\mu g$  Si  $L^{\text{-1}}.$  Frequency of the operation was 20 sample h<sup>-1</sup>. The system was successfully applied to the analysis of wastewater and fish pond water.

# ACKNOWLEDGEMENTS

The Thailand Research Fund (TRF), the Commission on Higher Education (CHE) and the Chiang Mai University are acknowledged for financial support. We thank the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC) for partial support.

#### REFERENCES

- Karthikeyan S., Hashigaya S., Kajiya T. and Hirata S., Determination of trace amounts of phosphate by flow-injection photometry, *Anal. Bioanal. Chem.*, 2004; 378: 1842-1846.
- [2] Daykin R.N.C. and Haswell S.J., Development of a micro flow injection manifold for the determination of orthophosphate, *Anal. Chim. Acta*, 1995; 313: 155-159.
- [3] Clesceri L.S., Greenberg A.E. and Trussell R.R., Standard Methods for the Examination of Water and Wastewater, 17<sup>th</sup> ed., American Public Health Association, USA, 1989.

- [4] Yaqoob M., Nabi N. and Worsfold P.J., Determination of silicate in freshwaters using flow injection with luminol chemiluminescence detection, *Anal. Chim. Acta*, 2004; 519: 137-142.
- [5] Zaporozhets O.A., Bas J.P., Kachan I.A., Zinko L.S. and Davydov V.I., Solid-phase spectrophotometric and test determination of silicate in natural water, *Talanta*, 2012; 90: 85-90.
- [6] Li Y., Muo Y. and Xie H., Simultaneous determination of silicate and phosphatein boiler water at power plants based on series flow cells by using flow injection spectrophotometry, *Anal. Chim. Acta*, 2002; 455: 315-325.
- [7] Ruzicka J. and Hansen E.H., *Flow Injection Analysis*, 2<sup>nd</sup> ed., John Wiley and Sons. Inc, New York, 1983.
- [8] Somnam S., Grudpan K. and Jakmunee J., Hydrodynamic sequential injection spectrophotometric system for determination of manganese in soil, *Spectro. Lett.*, 2008; 41: 221-227.
- [9] Buanuam J., Miro M., Hansen E.H., Shiowatana J., Estela J.M. and Cerda V., A multisyringe flow-through sequential extraction system for on-line monitoring of orthophosphate in soils and sediments, *Talanta*, 2006; 71: 1-10.
- [10] Grudpan K., Ampan P., Udnan Y., Jayasvati S., Lapanantnoppakhun S., Jakmunee J., Christian G.D. and Ruzicka J., Stopped-flow injection simultaneous determination of phosphate and silicate using molybdenum blue, *Talanta*, 2002; 58: 1319-1326.
- [11] Somnam S., Grudpan K. and Jakmunee J., Stopped-flow injection method for determination of phosphate in soils and fertilizers, *Maejo Int. J. Sci. Technol.*, 2008; 2: 172-181.

- [12] Morita K. and Kaneko E., Spectrophotometric determination of arsenic in water samples based on micro particle formation of ethyl violetmolybdoarsenate, *Anal. Sci.*, 2006; **22**: 1085-1089.
- [13] Tue-Ngeun O., Ellis P., McKelvie I.D., Worsfold P., Jakmunee J. and Grudpan K., Determination of dissolved reactive phosphorus (DRP) and dissolved organic phosphorus (DOP) in natural waters by the use of rapid sequenced reagent injection flow analysis, *Anal. Sci.*, 2005; 66: 453-460.
- [14] Morais I.P.A., Miro M., Manera M., Estela J.M., Cerda V., Souto M.R.S. and Rangel A.O.S.S., Flow-through solid-phase based optical sensor for the multisyringe flow injection trace determination of orthophosphate in waters with chemiluminescence detection, *Anal. Chim. Acta*, 2004; 506: 17-24.
- [15] Mihajlovic R.P., Kaljevic V.M., Vukasinovic M.P., Mihajlovic L.V. and Pantic I.D., Spectrophotometric method for the determination of phosphorus in natural waters using the bismuthphosphomolybdate complex, *Water SA*, 2007; 30: 513-517.
- [16] Borgnino L., Pfaffen V., Pfaffen P.J. and Palomeque M., Continuous flow method for the simultaneous determination of phosphate/arsenate based on their different kinetic characteristics, *Talanta*, 2011; 85: 1310-1316.
- [17] Katsaounos C.Z., Giokas D.L., Vlessidis A.G., Paleologos E.K. and Karayannis M.I., The use of surfactantbased separation techniques for monitoring of orthophosphate in natural waters and wastewater, *Sci. Total Environ.*, 2003; 305: 157-167.