*Full Paper*

# Stopped-flow injection method for determination of phosphate in soils and fertilisers

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**Abstract:** A stopped-flow injection system for the determination of phosphate has been developed. It involves the phosphate-molybdate-ascorbic acid reactions in the molybdenum blue method. The system is controlled by a semi-automatic stopped-FI analyser with a light emitting diode (LED)-colorimeter for monitoring the absorbance change relating to the concentration of a reaction product formed during the stopping period while the injected zone of a standard or sample is being in the flow cell. The slope of the FIAgram obtained is linearly proportional to the reaction rate, which depends on the phosphate concentration. Effects of concentration of reagents, viz. sodium molybdate, ascorbic acid and nitric acid, on the slope of the FIAgram were studied. The suitable concentration is 0.02 M, 0.25 %w/v and 0.15 M, respectively. A linear calibration graph in the range of 0.3-6.0 mg P L$^{-1}$ was employed for the determination of phosphate in soil and fertiliser samples. The results obtained agree well with those from a standard spectrophotometric method.

**Keywords:** phosphate, stopped-flow injection, molybdenum blue, fertiliser, soil

## Introduction

Phosphorus is an element which is widely distributed in nature. It is never found in a free or uncombined state because of its great affinity for oxygen [1]. The common species of phosphorus are phosphate ($PO_4^{3-}$), phosphorus trioxide ($P_2O_3$) or phosphorus oxide ($P_4O_6$), phosphorus tetraoxide

($P_2O_4$ or $PO_2$), and phosphorus pentoxide ($P_2O_5$ or $P_4O_{10}$). A condensed phosphate is formed by the combination of oxides of phosphorus and water [2].

In agriculture, phosphorus is one of the essential elements for plants. It is absorbed by plants preferably in the form of a phosphate ion ($HPO_4^{2-}$ or $H_2PO_4^-$). Under acidic conditions the latter is the dominant ion of the soil system. Plants need this nutrient for cell division, transformation of starch to sugar, seed germination, fruiting and flowering [3]. Thus, knowing the amounts of phosphate in soils or fertilisers is useful for monitoring the amounts of phosphorus for plants. Various methods for determination of phosphate have been reported such as ion chromatographic [4], batch [5], and flow injection (FI) [6-10] methods. Ion chromatography can quantitate different anions simultaneously at low concentration levels, but it requires an expensive and complicated instrument. Batch and FI spectrophotometric methods are used popularly for determination of phosphate by employing different chemical reactions such as molybdenum blue formation [10], complexation of orthophosphate with alizarin red sulphonate [11], malachite green ion association [12], and molybdate-crystal violet-phosphate reaction [13]. Both of the methods can provide simplicity and rapidness of analysis, but they suffer from interferences and low sensitivity. In order to improve sensitivity and selectivity of analysis, a stopped FI method is proposed in this research for determination of phosphate employing molydenum blue reaction.

The stopped FI method can increase sensitivity of the measurement by increasing the residence (reaction) time, the elapsed time after sample and reagent are mixed together prior to detection of the reaction product. By stopping the flow the residence time can be prolonged without increasing the length of the reaction coil, thus avoiding an increase of dispersion [14]. The absorbance signal due to the phosphomolybdenum blue product is continuously recorded during the stopping period. Improvement of selectivity is another advantage, since the response of an analyte increases with time whereas the background signal remains unchanged during the stopped-flow period. Therefore, by using the slope of the signal profile during the stopping period for analysis, interferences from coloured and colloidal substances present in the sample, which is a serious problem in the batch or normal FI method, can be eliminated.

## Materials and Methods

### Chemicals

Deionised water (Milli RX, Millipore) was used throughout. All reagents were of analytical grade, unless otherwise stated. Potassium dihydrogen phosphate (Merck) was used to prepare a stock standard solution of 1000 mg P $L^{-1}$, by dissolving 0.2197 g of the chemical in water, making up to a volume of 500 mL in a volumetric flask. Acidic molybdate reagent was prepared by dissolving sodium molybdate dihydrate (Fisher Scientific) (2.4195 g) in water. Then 5.4 mL of nitric acid was added before making up to a volume of 500 mL with water. Ascorbic acid (0.25 %w/v) was prepared freshly by dissolving 1.25 g of ascorbic acid in 500 mL water.
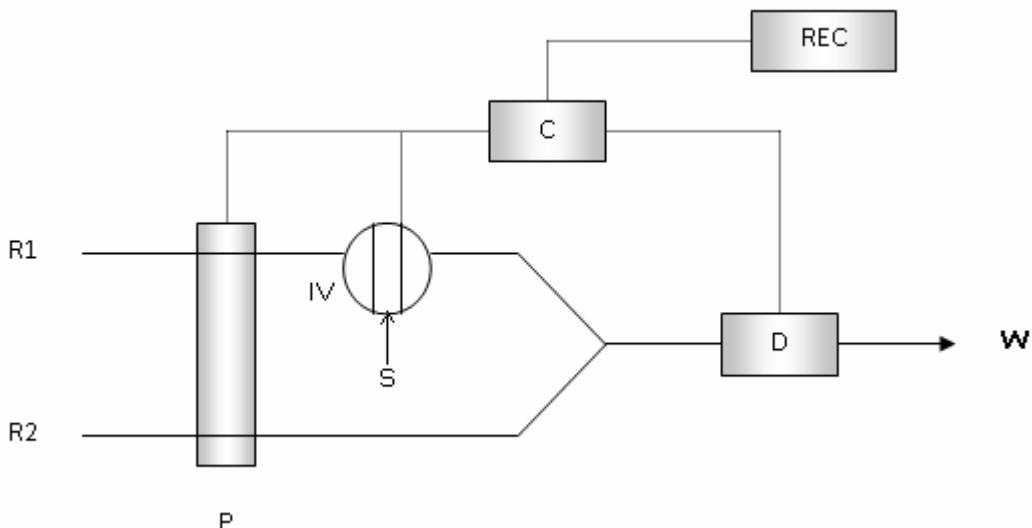
### Sample preparation

Soil samples were collected from different areas in Chiang Mai. Each sample was collected from at least 15 points by digging at the depth of 6 inches (15 cm) then combining together. The sample was

air- dried and ground to less than 300 μm particle diameter. A portion of 10 g of each sample was extracted with 25 mL of 0.8 M acetate buffer (pH 4.8) by shaking for 30 min and the mixture filtered through a filter paper and the final volume of the filtrate adjusted to 50 mL with water prior to analysis.

Fertiliser samples were obtained from local suppliers. An appropriate amount of the sample was dissolved with water prior to determination of available phosphate. The solution was filtered before analysis.

*Stopped FI setup*

The stopped FI system used is illustrated in Figure 1. A lab-built semi-automatic stopped-FI analyser as reported previously [10] was employed. It consists of a peristaltic pump (MP-3, Eyela, Japan), a 6-port injection valve (Upchurch, USA), a home-made colorimetric detector, a recorder (Philip, The Netherland) and a microcontroller for timing control of the pump and valve. The colorimeter had a light emitting diode (LED) as the light source and a photodiode as the light sensor. All tubings used were PTFE tubing with inner diameter of 0.5 mm, except for Tygon pump tubing (Saint-Gobain Performance Plastics, USA).



**Figure 1.** Stopped FI manifold for determination of phosphate: R1 = 0.02 M sodium molybdate in 0.15 M nitric acid, R2 = 0.25%w/v ascorbic acid, S = sample, P = Peristaltic pump, C = controller, IV = six-port injection valve, D = colorimetric detector, REC = recorder, W = waste

*Procedure*

A standard or sample (55 μl) was injected into a stream of 0.02 M sodium molybdate, which was then merged with a stream of 0.25%w/v ascorbic acid and flowed further to a colorimetric flow cell (see Figure 1). The flow rate of each stream was 2.0 mL min$^{-1}$. After injection, the injected zone was travelled for 3 s (travelling time) before being stopped for a period of 10 s (stopping time) in the flow cell at the detection point of the detector by stopping the pump. During this period, the colour intensity of the  phosphomolybdenum blue product increased continuously, and the absorbance at about 630 nm was recorded as a stopped FI profile (FIgram). Then the flow was started again to

propel a bolus of the solution mixture out of the flow cell for a period of 8 s (washing time). The slope of stopped-FIgram was used for phosphate determination by plotting slope versus phosphate concentration. A linear calibration graph was obtained, which could be utilised for the determination of phosphate in a sample. The whole cycle took about 40 s, with a consumption of 0.4 mL of each reagent.
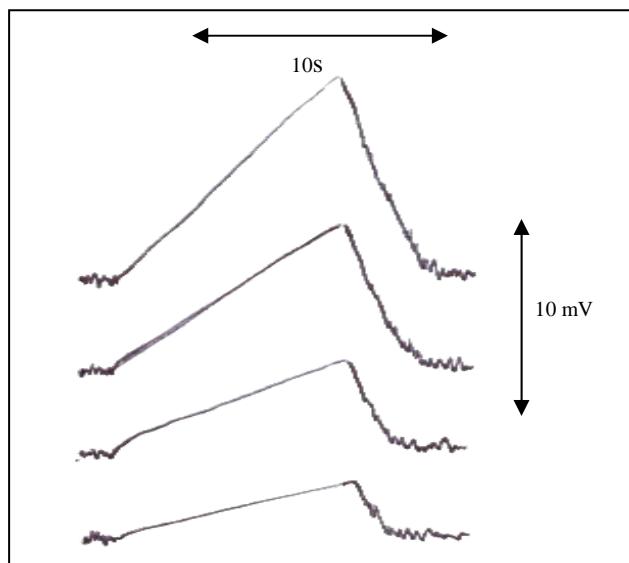
## Results and Discussion

The stopped-flow injection system for the determination of phosphate involves the phosphate-molybdate-ascorbic acid reaction to form a molybdenum blue product as shown [15].

$$PO_4^{3-} \quad + \quad \text{Acidic molybdate} \quad \rightarrow \quad \text{Heteropoly acid}$$

$$\text{Heteropoly acid} \quad + \quad \text{Ascorbic acid} \quad \rightarrow \quad \text{Molybdenum blue}$$

Ascorbic acid is selected as a reducing agent because it is efficient and more friendly to the environment. Tin (II) chloride can also be used, but it produces a heavy metal waste. With sodium sulfite as a reducing agent, the bubble of sulfur dioxide is evolved in the flow system, which may cause many problems.

In this work, a semi-automatic stopped FI-analyser was employed (see Figure 1). Via the controller, presetting values can be defined for travelling time (T), the period for sample to flow from the injection point to the detection point prior to the stopping of the flow and the monitoring of the reaction product. Also defined is stopping time (S), the period during which the flow is stopped, and washing time (W), the period during which the system is washed by restarting the flow (after stopping). Each operation cycle comprises these three periods. The analyser is on standby (the flow is halted) after each operation cycle is finished, ready for the user to start the next cycle. Due to the non-continuous flow, the stopped FI method consumes a smaller amount of reagent than the normal FI one.

During the stopping period, the change of absorbance due to the reaction product was recorded versus time, so that the kinetics of the reaction could be continuously monitored. Using the reagents mentioned above, the reaction of the phosphate was relatively fast compared to the silicate, and the kinetic data could be used for a discriminative determination of both of the species [10]. In this work, a stopping period of 10 s was selected in order to avoid interference from the silicate, which might be present in the soil extract at a high concentration. The stopped-FI signal profiles of standard solutions containing different concentrations of phosphate are illustrated in Figure 2. It can be noticed that the slope of the signal profile is linearly proportional to the phosphate concentration.
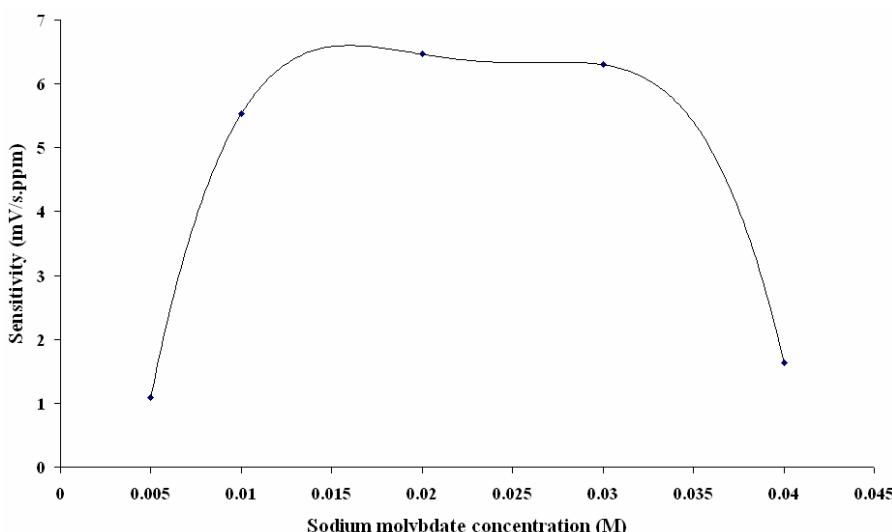
**Figure 2.** Stopped-FI profiles obtained with different concentrations of phosphate (0.3, 0.5, 1.0 and 2.0 mg P L$^{-1}$, respectively)

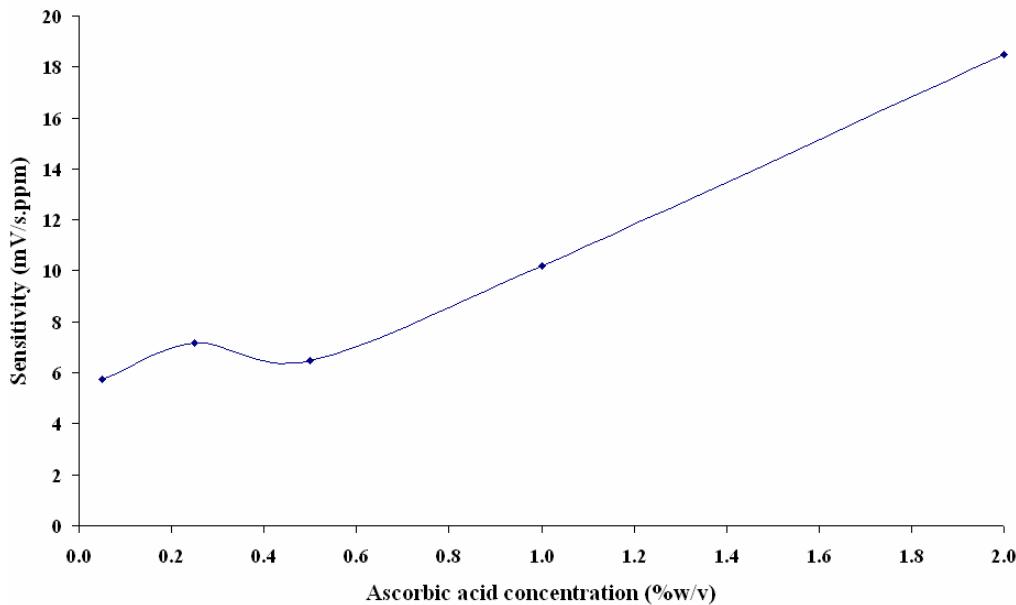*Effect of reagent concentration*

*1. Sodium molybdate*

The sodium molybdate concentration was varied from 0.005 to 0.04 M while ascorbic acid and nitric acid concentration was fixed at 0.5% (w/v) and 0.08 M, respectively. A series of standard phosphate solutions was injected and calibration graphs (plot of slope of signal versus phosphate concentration) were constructed.   A plot of slope of the calibration graph (sensitivity) versus concentration of sodium molybdate is illustrated in Figure 3, indicating that 0.02 M sodium molybdate should be selected.



**Figure 3.** Effect of sodium molybdate concentration

*2. Ascorbic acid*

Ascorbic acid concentrations in the range of 0.05 to 2%w/v were tried while the sodium molybdate and nitric acid concentration was fixed at 0.02 and 0.08 M, respectively. The effect of ascorbic acid concentration on the slope of the calibration graph is illustrated in Figure 4. Although 1 and 2% w/v ascorbic acid provided higher sensitivity than 0.05, 0.25 and 0.5% w/v, both of these concentrations gave a high noise signal that caused difficulty in measuring the slope of stopped-FIAgrams. Thus, ascorbic acid of 0.25% w/v was chosen because it provided higher sensitivity than 0.05 and 0.5% w/v.
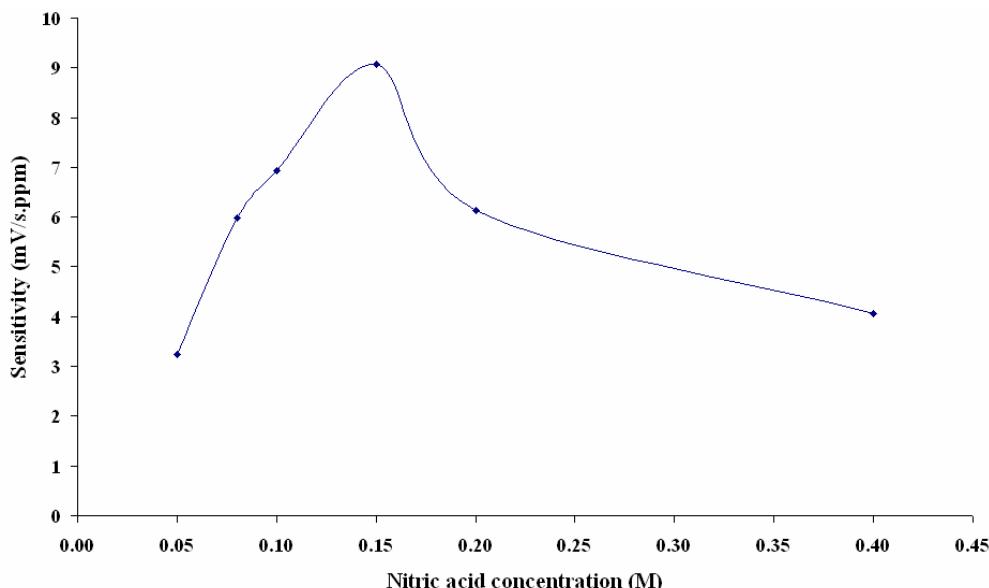


**Figure 4.** Effect of ascorbic acid concentration

*3. Nitric acid*

The effect of nitric acid concentration (0.05 to 0.4 M) was investigated while the sodium molybdate and ascorbic acid concentration was fixed at 0.02 M and 0.25% w/v, respectively. A sharp increase in sensitivity was observed when nitric acid concentration was increased up to 0.15 M, but at concentration higher than 0.15 M the sensitivity declined, as shown in Figure 5. In addition, when sulfuric acid of different concentrations was used, a similar trend was also observed, indicating that the reaction seemed to progress well in a narrow range of acid concentration.

*Calibration graph and precision*

Using the above selected conditions, a linear calibration graph in the range of 0.3 - 6 mg P L$^{-1}$ (y=10.091x + 5.5403, R$^2$=0.9987) is obtained. The detection limit calculated from the calibration data is found to be 0.02 mg P L$^{-1}$. A relative standard deviation obtained for 10 replicated injections of 2 mg P L$^{-1}$ is 2.6%. The method has a sample throughput of 90 h$^{-1}$, with a consumption of 0.4 mL of each reagent.

**Figure 5.** Effect of nitric acid concentration

*Application to real samples*

The developed method was applied to soil samples. The procedure for collecting and extracting soil has been described in the materials and methods section.  The accuracy of the proposed method was determined by comparing results obtained with those from a batch spectrophotometric method [16]. Comparative analyses of the same samples were carried out on the same day and the results are summarised in Table 1.  The phosphate contents obtained from the stopped-FI method (x) agree well with those obtained by the batch method (y), which is indicated by the value of the slope, the intercept and $R^2$ of the correlation graph between the two methods being closed to 1, 0 and 1, respectively ($y=1.0064x+0.0357$, $R^2=0.9997$).  According to the t-test at 95 % confidence [17], these methods correlate to each other well.

The proposed method was also applied to the determination of soluble phosphate in fertilisers. The results are shown in Table 2. Again, according to the t-test at 95 % confidence, the two methods correlate well to each other.

The determination of phosphate in soil and fertiliser samples by the stopped-FI method seems to have more advantages than the batch method. The former method can reduce the effect of interferences from such species as silicate and arsenate, because their reaction rates in the molybdenum blue reaction are slower than that of the phosphate.  By using the slope of FIAgram instead of peak height, the effect due to coloured and colloidal species usually found in these samples is also decreased.  In addition, the stopped-FI technique is simple, inexpensive, rapid, and has a good sensitivity. Also, very small amounts of reagents and sample are consumed.

**Table 1.** Content of available phosphate in soil samples found by stopped-FI and batch method

| Sample number | Physical appearance | [P] in soil sample (µg/g) [a] | | % Difference [b] |
|---|---|---|---|---|
| | | sFI method | Batch method | |
| 1 | Friable, wet | 11.2 ± 0.3 | 11.2 ± 0.0 | 0.0 |
| 2 | Friable, wet | 11.1 ± 0.2 | 11.0 ± 0.2 | 0.9 |
| 3 | Friable, wet | 13.3 ± 0.3 | 13.5 ± 0.2 | -1.5 |
| 4 | Friable, wet | 24.0 ± 1.2 | 24.1 ± 0.0 | -0.4 |
| 5 | Friable, wet | 15.2 ± 0.7 | 15.0 ± 0.2 | 1.3 |
| 6 | Laterite | 7.4 ± 0.2 | 7.4 ± 0.1 | -0.8 |
| 7 | Friable, wet | 12.8 ± 0.6 | 12.6 ± 0.1 | 1.6 |
| 8 | Friable, wet | 11.9 ± 0.5 | 11.8 ± 0.0 | 0.8 |
| 9 | Black soil | 120 ± 2 | 119 ± 1 | 0.8 |
| 10 | Sand | 42.0 ± 0.5 | 42.5 ± 0.0 | -1.2 |
| 11 | Friable, sand | 12.4 ± 0.5 | 12.6 ± 0.0 | -1.6 |
| 12 | Friable, wet | 5.7 ± 0.2 | 5.7 ± 0.1 | 0.0 |
| 13 | Hard, dry | 23.4 ± 1.2 | 23.6 ± 0.2 | -0.8 |
| 14 | Friable, wet | 11.0 ± 0.5 | 10.9 ± 0.1 | 0.9 |
| 15 | Friable, wet | 5.1 ± 0.3 | 5.0 ± 0.1 | 2.0 |
| 16 | Hard, dry | 24.0 ± 2.4 | 24.2 ± 0.3 | -0.8 |
| 17 | Friable, wet | 43.2 ± 0.6 | 43.1 ± 0.0 | 0.2 |
| 18 | Friable, wet | 14.5 ± 0.0 | 14.3 ± 0.0 | 1.4 |
| 19 | Mud | 3.0 ± 0.1 | 3.0 ± 0.0 | 0.0 |
| 20 | Mud | 5.0 ± 0.0 | 5.0 ± 0.1 | 0.5 |

[a] mean of triplicate results

[b] % difference = [(FIA value – batch value)/batch value] x 100

**Table 2.** Content of soluble phosphate in fertiliser samples found by stopped-FI and batch method

| Sample number | [P$_2$O$_5$] in fertiliser sample (mg/g) | | % Difference[a] |
|:---:|:---:|:---:|:---:|
| | sFI method | Batch method | |
| 1 | 85 ± 6 | 83 ± 2 | 3 |
| 2 | 69 ± 4 | 62 ± 1 | 10 |
| 3 | 50 ± 1 | 36 ± 1 | 37 |
| 4 | 396 ± 5 | 321 ± 0 | 23 |
| 5 | 63 ± 2 | 53 ± 2 | 19 |
| 6 | 76 ± 4 | 67 ± 2 | 13 |
| 7 | 98 ± 5 | 90 ± 2 | 8 |
| 8 | 136 ± 5 | 125 ± 2 | 9 |
| 9 | 0.7 ± 0.0 | 1.0 ± 0.0 | -26 |
| 10 | 0.8 ± 0.0 | 0.7 ± 0.0 | 13 |

[a] % difference = [(FIA value – batch value)/batch value] x 100

**Conclusions**

The stopped FI method based on the phosphate-molybdate-ascorbic acid reaction (the molybdenum blue method) has been developed for the determination of phosphate in soil and fertiliser samples. The effect of concentration of reagents was investigated. The suitable concentration for sodium molybdate, ascorbic acid and nitric acid, is 0.02 M, 0.25 %w/v and 0.15 M, respectively. A linear calibration graph (plot of slope of the stopped-FIgram versus phosphate concentration) in the range of 0.3-6.0 mg P L$^{-1}$ was employed for the determination of phosphate in samples. The developed method provides various advantages, including high sensitivity and selectivity, and a simple, fast and cheap analysis.

**Acknowledgements**

**References**

1. W. H. Waggaman, "Phosphoric Acid, Phosphate and Phosphatic Fertilizers", 2nd Edn., Hafner Publishing Company, New York, **1969**.

2.  O. Tue-Ngeun, "Development of flow injection analysis system for determination of phosphate, nitrite, nitrate, chromium, and calcium, *MS. Thesis*, **1997**, Chiang Mai University, Thailand, p 4-5.
3.  V. Sanchelli, "Chemistry and Technology of Fertilizers", Reinhold Publishing, New York, **1993.**
4.  M. T. Galceran and M. Diez, "Column-switching techniques in the analysis of phosphate by ion chromatography", *J. Chromatogr. A*, **1994,** *675*, 141-147.
5.  S. Motomizu, M. Oshima, and A. Hirashima, "Spectrophotometric determination of phosphorus in river water based on the reaction of vanadomolybdophosphate with malachite green", *Anal. Chim. Acta*, **1988**, *211*, 119-127.
6.  J. M. Estela and V. Cerda, "Flow analysis techniques for phosphorus: an overview", *Talanta*, **2005***, 66,* 307-331.
7.  H. Abderrazak, M. Dachraoni, and B. Lendl, "A novel flow injection procedure for determination of phosphate in industrial raw phosphoric acid", *Analyst*, **2000**, *125*, 1211-1213.
8.  Z. Chen, P. Grierson, and M. A. Adams, "Direct determination of phosphate in soil extracts by potentiometric flow injection using a cobalt wire electrode", *Anal. Chim. Acta*, **1998**, *363*, 191-197.
9.  R. J. Prieto and M. Silva, "Improved simultaneous reaction-rate determination of phosphate and silicate by use of the continuous addition of reagent technique", *Analyst*, **1998**, *123*, 2389-2394.
10. K. Grudpan, P. Ampan, Y. Udnan, S. Jayasvati, S. Lapanantnoppakhun, J. Jakmunee, G. D. Christian and J. Ruzicka, "Stopped-flow injection simultaneous determination of phosphate and silicate using molybdenum blue", *Talanta*, **2002**, *58*, 1319-1326.
11. A. M. Abdallah, M. E. Khalifa, and M. A. Akl, "Spectrophotometric determination of phosphate with alizarin red sulphonate"*, Anal. Chim. Acta*, **1991***, 251,* 207-213.
12. K. L. Linge and C. E. Oldham, "Interference from arsenate when determining phosphate by the malachite green spectrophotometric method", *Anal. Chim. Acta*, **2001***, 450,* 247-252.
13. D. Thorburn Burns, D. Chimpalee, N. Chimpalee, and S. Ittipornkul, "Flow-injection spectrophotometric determination of phosphate using crystal violet", *Anal. Chim. Acta*, **1991**, *254*, 197-200.
14. J. Ruzicka and E. H. Hansen, "Flow Injection Analysis", 2nd Edn., John Wiley & Sons, New York, **1988**, p156-166.
15. A. I. Vogel, "Quantitative Inorganic Analysis", 3rd Edn., Longmans, London, **1968**.
16. M. A. H. Franson, "Standard Methods for the Examination of Water and Wastewater", 17th Edn., American Public Health Association, Washington, DC, **1989**.
17. F. W. Fifield and D. Kealey, "Principles and Practice of Analytical Chemistry", 4th Edn., Blackie Academic & Professional, Glasgow, **1995**.