CHAPTER 1

INTRODUCTION

1.1 Stopped-flow injection analysis

Optimization of a flow injection system is a balancing act, where dispersion of the sample zone and its mixing with a reagent must be weighed against the time required to achieve a desired chemical conversion of an analyte into a detectable species. This is so because the parabolic profile established during the injection process expands further during sample zone transport through an open-tubular channel, ultimately leading to an undesired zone brodening and consequent loss of sensitivity and sampling frequency [1]. Using stopped-flow method can increase reaction time and decrease dispersion of product zone hence increase sensitivity. Moreover, it can provide kinetic discrimination, and eliminate background signal [2]. Stopped-flow mode is often used in flow injection analysis (FIA) to measure a reaction rate. In this mode, the slope of the signal can be used for measurement [3-5]. General stopped-FIgram is illustrated in Figure 1.1.

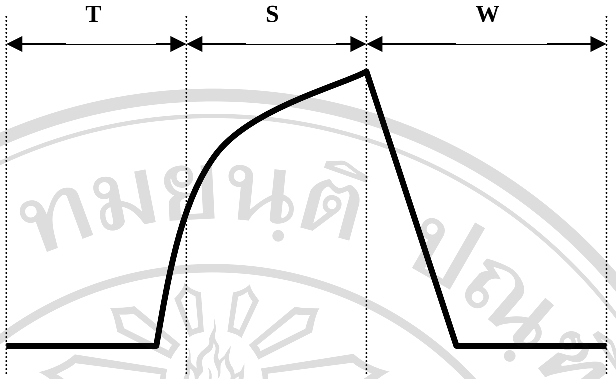

Figure 1.1 General stopped-FIgram : T = Travelling time, S = Stopping time, W = Washing time.

From Figure 1.1, travelling time (T) is the period of flow between the point of injection to the point at which the flow is stopped during an injected zone is being in a flow cell, so that the reaction development can be monitored at the flow cell. Stopping time (S) is the period during the flow stopped. Washing time (W) is the period when the stream is restarted to flow (after stopping) until this operation cycle ends and ready to start for the next cycle.

In this technique, to increase the sensitivity of measurement is done by increasing the residence time. Postulating that to increase the residence time, one should keep the reaction coil short, decrease flow rate and without increasing dispersion [4]. Response of an analyte increases whereas background signal remain unchanged during the stopped-flow period. In other words, sensitivity and selectivity can be enhanced by the stopped flow technique.

Kinetic discrimination particularly is used for simultaneous determination of different analytes for which behaviors are different under the same condition [5]. Stopped-flow method is employed to obtain kinetic information, based on differences in the reaction rates such as reaction of magnesium and calcium to cryptand (2,2,2) complexes [6], redox reaction between Hg(II) and Ag(I) catalyzed by hexacyanoferrate(II) and α,α'-bipyridyl with thiourea [7], the molybdenum blue method for phosphate and silicate [5].

1.2 Phosphate

1.2.1 General background

Phosphorus is the element that widely distributed in nature. It is never found in a free or uncombined state because of its great affinity for oxygen [8]. The common species of phosphorus are mentioned as follows:

1) Phosphate, PO_4^{3-}
2) Phosphorus trioxide, P_2O_3, or phophorus oxide, P_4O_6
3) Phosphorus tetraoxide, P_2O_4 or PO_2
4) Phosphorus pentoxide, P_2O_5 or P_4O_{10}

Relation between oxides and acids of phosphorus (or condensed phosphate [9]) are formed by the combination of oxides of phosphorus and water can be shown in Table 1.1 [10].

Table 1.1 Relation between oxides and acids of phosphorus.

Oxides	**Acids**
Phosphorus oxide, P_4O_6	Phosphorus acid, H_3PO_3 Pyrophosphorus acid, $H_4P_2O_5$ Metaphosphorus acid, $(HPO_2)_n$
Phosphorus tetraoxide, P_2O_4	Mixed acids, H_3PO_3 and H_3PO_4 Hypophosphoric acid, $H_4P_2O_6$
Phosphorus pentoxide, P_2O_5	Orthophosphoric acid, H_3PO_4 Pyrophosphorus acid, $H_4P_2O_7$ Metaphosphorus acid, $(HPO_3)_n$ Monoperphosphorus acid, H_3PO_5 Diperphosphorus acid, $H_4P_2O_8$ Hypophosphorus acid, H_3PO_2

In agriculture, phosphorus is one of the essential elements for plants. It is absorbed by plant preferably in the form of phosphate ion, HPO_4^{2-} or $H_2PO_4^-$. Under acid conditions the latter is the dominant ion of soil systems. Plants needed this nutrient for cell division, the transformation of starch to sugar, seed germination, fruiting and flowering [10]. Phosphate is needed for each plant in different levels. To know the amount of phosphate in soil is useful for caltivation, to estimate the enough amount for plants.

1.2.2 Methods for phosphate determination

Many methods for determination of phosphate based on molybdenum blue reaction has been reported. Phosphorus species are rapidly reacted with ammonium molybdate to produce a heteropolyacid complex that be slowly reduced by mild reducing agent such as ascorbic acid [5], tin (II) chloride [11], etc. The reaction may be presented as follows [12].

$$7PO_4^{3-} + 12(NH_4)_6Mo_7O_{24} + 36H_2O \rightarrow 7(NH_4)_3PO_4.12MoO_3 + 51NH_4^+ + 72OH^-$$

$$(NH_4)_3PO_4.12MoO_3 + \text{mild reducing agent} \rightarrow \text{Mo(IV) species (blue)}$$

Flow-based analysis systems with spectrophotometric detection have been applied for phosphate determination in various types of samples as summarized in Table 1.2.

Table 1.2 Flow-based analytical methods for determination of phosphate.

Analyte	Sample	Condition	Analytical characteristic	Ref.
Phosphate	Estaurine waters	-solving the problem of schlieren or reflextive index (RI) effect . -used sodium chloride solution of similar RI as a carrier instead of water.	-DL : 6 μg P l^{-1}	13

Table 1.2 (Continue)

Analyte	**Sample**	**Condition**	**Analytical characteristic**	**Ref.**
Phosphate	Industrial Raw phosphoric acid	-by measuring the decrease in absorption at 314 nm of $FeCl_3$ due to the formation of an iron (III) phosphate complex.	-LR : 0.1 to 20 g l^{-1} H_3PO_4 -TP : 30 sample h^{-1} -RSD : 1.4%	14
Phosphate	Natural water	-using inexpensive FI instrument. -a super bright light-emitting diode as the source and photodiode as the detector. -the tin (II) chloride-molybdate method coupled with in-line preconcentration anion-exchange column were used.	-LR : 0-100 μg P l^{-1} -DL : 0.6 μg P l^{-1} -RSD : 2.9% at 20 μg P l^{-1} and 0.5% at μg P l^{-1}	15
Phosphate and silicate	Running and bottle water	-based on kinetic reaction of SiO_3^{2-}/MoO_4^{2-}/thiamine and PO_4^{3-}/MoO_4^{2-}/thiamine -λ_{ex} = 375, λ_{em} = 440 nm	-LR of : PO_4^{3-} and SiO_3^{2-} : 30-600 $ngml^{-1}$ in ratio of 1:10 to 10:1	16
Phosphate	Industrial raw phosphoric acid	-based on the injection of sample into a stream of $FeCl_3$ and subsequent measurement of the decrease in absorption at 334 nm due to the formation of an Fe(II) phosphate complex	-LR : 0.1-20 gl^{-1} H_3PO_4 -TP : 30 sample h^{-1} -RSD : 1.4%	17

Table 1.2 (Condition)

Analyte	**Sample**	**Condition**	**Analytical characteristic**	**Ref.**
Fluoride and phosphate	Waters	-based on the analytes' inhibiting effect on the photooxidation of acridine catalyzed by Fe (III)	-LR : 0.76 to 9.5 μg F ml^{-1}, 0.95-9.5 μg P ml^{-1} -DL : 1.3x10^{-5} M (F), 3.2x10^{-6} M (P)	18
Phosphate and silicate	Sunflower	-The condition was used for improving the simultaneuos reactor-rate determination of phosphate and silicate -based on the formation of the corresponding heteropolymolybdate	-RSD : 2.5-4.5%	19
Total nitrogen and phosphate	Vegetables	-with gas diffusion unit to separate NH_4^+ from sample matrix -by partition of sample plug after the separation to ISE for NH_4^+ and photometer (700 nm) for P	-TP : 40-60 sample h^{-1} -RSD : 3.5%	20
Phosphate	Natural and waste waters	-based on formation of vanadomolybdate, Malachite Green and phosphomolybdenum blue methods	-LR : 18, 0.4 and 4.0 mg P l^{-1} -DL : 0.15, 0.01 and 0.01 mg P l^{-1} -RSD : 2.1%, 18% and 1.7%	21

A spectrophotometric method based on molybdenum blue for determination of phosphate is popularly used. Although the molybdenum blue reaction has been applied using conventional batch methods [22-23], the methods are tedious, time consuming and have high reagent consumptions. The stopped-flow mode should be employed for kinetic measurements, with rapid and reliable.

1.3 Chlorate

1.3.1 General background

Chlorate (ClO_3^-), an oxidized form of the chlorine atom, has an oxidation state of +5, making it a strong oxidant. It is usually combined with sodium or potassium to form salt. Chlorate is useful in the pulp and paper industry for bleaching process [24]. In addition, chlorate has been employed in agriculture as a herbicide and as a defoliant especially between the years 1930 and 1950 [25]. For Thailand, potassium chlorate is popularly used for promoting flowering and fruiting of longan. It is spectulated that chlorate ion has the effect to longan rather than potassium ion. Plants can absorbed chlorate in both leave and root [26].

There are some reports about the effect of chlorate causing damage to plants. The intake of chlorate in high amounts caused felling off leaves and dead in longan [26-27]. Aberg studied young wheat plants after they had been treated with chlorate and found a severe inhibition of growth in root and bleaching of leaves [28]. Chlorate acts better competitive than nitrate to be a substrate for the enzyme nitrate reductase, that reduces nitrate to nitrite in plants [25-26]. There was another experiment that plants growing in chlorate solutions are exposed to sunlight. Formation of the produced substances is accelerated and the injury resulting from the chlorate is increased. In addition, it was also suggested the possibility of differences in soil solidity affecting the action of chlorate on plants [29].

From the previously mentioned, knowing the amount of chlorate in soil is useful for controlling the effect of chlorate to plants and environment.

1.3.2 Methods for chlorate determination

Various methods for determination of chlorate has been reported such as ion chromatography [30], flow injection spectroscopy [31-32], spectrophotometry [33] and infrared spectrophotometry [34]. Ion chromatography and infrared spectrophotometry can quantitate anions at low concentration levels, but they require an expensive and complicated instruments. FIA and spectrophotometric method are used popularly for determination of chlorate based on various reaction. Both of the methods can provide simplicity and rapidness of analysis, but they are suffering from interferences and low in sensitivity. In this work, stopped-flow injection method was employed for chlorate determination to reduced the mentioned problems.

Spectrophotometric methods employ various chemistries, such as iodometric method [35], complexation of chlorate with rhenium-α-furildioxime [33] and method of decolorization of Indigo Carmine [36].

For the study, iodometric method was employed for determination of chlorate ion. The chlorate-iodide reaction is well known. In acidic solution, the following reaction occurs:

$$ClO_3^- + 6I^- + 6H^+ \rightarrow Cl^- + 3I_2 + 3H_2O$$

This reaction is very slow at low acidity. Thus, 6-12 M hydrochloric acid can be used to enhance the production of the iodine. Unfortunately, under these conditions iodide ion is readily oxidized by air :

$$4I^- + 4H^+ + O_2 \rightarrow 2I_2 + 2H_2O$$

To overcome the problem, the determination should be performed in close system. Under the stopped-flow conditions, severely acidic solution can be used because the flow system excludes air, and thus minimizes air oxidation of iodide ion [31,35,37]. Moreover, the stopped-flow system can increase sensitivity for the slow reaction.

1.4 Aims of the project

The aims of this research project are as follow:

To investigate simple, low cost, rapid and high sensitive methods using a stopped-flow injection technique for soil samples for (1) available phosphorus using molybdenum blue reaction and (2) chlorate using iodometric method.