CHAPTER 1
INTRODUCTION

1.1 The importance of phosphate determination

In aquatic systems phosphorus presents in various inorganic and organic forms. Some exhibits in dissolved colloidal or particulate form. A commonly found species is orthophosphate in either the mono or diprotonated form (HPO_4^{2-} or $H_2PO_4^-$). Phosphate is of great interest in environmental monitoring as it concerns nutrient for plant and algal growth. Increases in phosphate promote eutrophication effect [1] and especially phosphate in rainwater can indicate to the sources of pollutant (Table 1.1) [2]. In recent years, in semiconductors industry, large bulks of ultrapurified water are required for high-quality semiconductors manufacturing. Phosphorus existing event at trace/ultratrace amounts in the water would damage the quality of the semiconductors, and therefore the amounts must be as low level as possible. For next-generation semiconductor manufacturing, phosphorus is one of the impurities that must be determined in ultrapurified water [3].

Table 1.1 Primary associations in rain [2]

Origin	Associations
Marine inputs	Cl-Na-Mg-SO_4
Soil inputs	Al-Fe-Si-Ca-(K, Mg, Na)
Biological inputs	NO_3-NH_4-SO_4-K
Biomass burning	NO_3-NH_4-P-K-SO_4-(Ca, Na, Mg)
Industrial pollution	SO_4-NO_3-Cl
Fertilizers	K-PO_4-NH_4-NO_3

1.2 Analytical method

Various methods have been proposed for the determination of phosphate. Some examples are following:

1.2.1 Titrimetry

Titrimetric method has been proposed on the pharmaceutical standard method for phosphate assay. It is based on acid – base titration. Phosphoric acid and monobasic phosphate forms are determined with sodium hydroxide and phenolphthalein as indicator. Dibasic phosphate forms are assayed with hydrochloric acid and mixture solutions of bromocresol green and methyl red as indicator. [4-5]

1.2.2 UV-Visible spectrophotometry

This is the most popular standard method for phosphate determination. It is based either on the molybdenum blue method or molybdovanadophosphate method. [8-13]

1.2.3 Fluorescence spectrophotometry

The method is based on the redox reaction of vanadomolybdophosphate. Non fluorescence thiamine was used as a reducing agent. Thiochrom as fluorloginic product may be employed. For the indirect fluorometry, the reactions are based on the quenching of the fluorescence of Rhodamine B derivatives or Rhodamine 6G through to the formation of ion association compounds with phosphate. [14-16]

1.2.4 Chemiluminiscence photometry

This method involves the detection of luminol chemiluminiscence. The molybdophosphoric heteropoly acid formed from the reaction between reactive phosphate and ammonium molybdate in an acid condition can generate chemiluminiscence emission via the oxidation of luminol. [17]

1.2.5 Potentiometry

Using the potentiometric titration for the phosphate assay (dibasic and tribasic phosphate forms) in pharmaceutical preparations, hydrochloric acid is employed as a standard solution. [4-5]

1.2.6 Voltammetry

It is based on the change in electron transfer rate constant of the redox reaction on electrode surface. This technique has been reported for the determination of phosphate. Both of direct and indirect measurements have been proposed. Application of enzyme immunoassay by modified electrode surface with 3-indoxyl phosphate was proposed for the determination of alkaline phosphatase and horseradish peroxidase activity. [18-19]

1.2.7 Amperometry

The amperometric detection of phosphate can be performed with a conventional three-electrode potentionstatic system. Ag/AgCl and platinum wires were used as reference and counter electrodes, respectively. The working electrode is glassy carbon or modified electrodes with the specific activity of the

enzymes such as immobilizing pyruvate oxidase, bienzyme of alkaline phosphatase and horseradish peroxidase, trienzyme of maltose phosphorylase, mutarotase, and glucose oxidase mixture, each kind of enzyme electrodes can be used for measurement of the current in the presence of phosphate. Phosphate analysis is usually monitored by potentiostating the reduction of phosphate with acidic molybdate at glassy carbon working electrode versus the Ag/AgCl reference electrode. [20-22]

1.2.8 Ion chromatography

This technique is commonly be applied for anions analysis. Several literatures have proposed the determination of phosphate in real samples using an anion column and a conductivity detector. [23-30]

1.2.9 Miscellaneous methods

Reveres phase high performance liquid chromatography, capillary electrophoresis, atomic absorption spectroscopy, fourier transform infrared spectrometry, radio luminescence, mass spectrometry and ^{31}P nuclear magnetic resonance spectrometry have been reported for phosphorus analysis. [31-37]

Flow based techniques are now frequently be used for determination of phosphate. A brief summary of the phosphate determination is presented in Table 1.2.

Table 1.2. Brief summary of phosphate determination

Technique	Detector	Linear range	Detection limit	Correlation	Sample	Reference
Flow injection	UV	0 – 50 mg l^{-1}	-	0.9998	-	[38]
	Spectrophotometer	1.55 – 350 ng ml^{-1}	0.3 ng ml^{-1}	-	Natural water	[39]
	Spectrophotometer	0.5 – 1.5 µg l^{-1}	0.2 µg l^{-1}	-	Real water	[40]
	Spectrophotometer	0.01-6.00 ppm	0.01 ppm	0.9995	Citified reference material	[41]
	Spectrophotometer	30 nM-30 µM	30 nM	-	Ultra pure water	[42]
	Spectrophotometer	0.007 – 1.5 mg l^{-1}	7 µg l^{-1}	-	Soil and water	[43]
	Spectrophotometer	8 – 250 ng l^{-1}	8 ng l^{-1}	-	Ultra pure water	[44]
	Chemiluminescence	0.5-9 µM	0.14 µM	0.999	Sediment pore water	[45]
	Radio luminescence	-	-	-	-	[46]
	Fluorescence	0.2-1.6 µg	10 µg	-	natural water	[47]

Table 1.2. (Continued)

Technique	Detector	Linear range	Detection limit	Correlation	Sample	Reference
Flow injection	Voltammetric detector	0.3-3.0 ppb	0.3 ppb	-	-	[48]
	Amperometric detector	$4 \times 10^{-8} - 10^{-6}$ M	3×10^{-8} M	0.9996	Kinetic of acid phosphatase activity	[49]
	Amperometric detector	50-1000 ppb	0.18 ppb	0.9998	Fresh and marine water	[50]
	Semiconductor laser	1.0 – 50 µg l^{-1}	0.6 µg l^{-1}	0.999	Wastewater and natural water	[51]
	Biosensor	$5x10^{-7} - 8x10^{-4}$ M	2.5 x 10-7 M	0.999	Environmental and food sample	[52]
Sequential injection	Spectrophotometer	0.2-7.0 ppm	0.1 ppm	0.9998	water and sediments	[53]
	Spectrophotometer	0.3-20 ppm	100 ppb	0.9965	Beverages, waste water and urine	[54]
	Spectrophotometer	20 – 400 mg l^{-1}	-	0.9998	Vegetable, milk, juice and wines	[55]
	Spectrophotometer	0.01 – 4 mg l^{-1}	0.01 mg l^{-1}	0.9996	Drinking waters and waste waters	[56]

Table 1.2. (Continued)

Technique	Detector	Linear range	Detection limit	Correlation	Sample	Reference
Sequential injection	Spectrophotometer	0.5-30 M	-	0.996	Urine	[57]
	Voltammetric detector	0.06-300 mg	0.15 mg	-	-	[58]
All injection	Spectrophotometer	-	-	-	Soil and sediment samples	[59]
Micro-Flow injection	Spectrophotometer	1 – 10 µg ml^{-1}	0.1 µg ml^{-1}	0.9952	-	[60]
Monosegmented flow system	Spectrophotometer	5.0-75 ppb	0.70 ppb	0.9992	natural water	[61]

The Table indicates that the flow based system can be hyphenated with other detection techniques. The reaction of phosphomolybdenum blue has commonly been employed with good sensitivity and precision. Nevertheless, the seriously interferences by arsenate and silicate ions may be associated with. This problem may be avoided by using the other detection techniques. Electroanalytical technique has been aimed to be developed with flow system for phosphorus (phosphate) determination. Reports on this so far have not been applied for trace amounts. Phosphate in the environmental sample such as the rainwater was found to be in trace level. Thereby, the development of flow injection electrochemistry for monitoring environmental is still essential and especially in a place with conditions like in Thailand.

1.3 Research aims

This research work was aimed to develop flow injection involving amperometric detector for determination of phosphate in ppb and sup-ppb levels. The aims of this work as follows :-

1. To develop flow injection amperometric system with sample pretreatment column for the determination of orthophosphate.
2. To apply the developed system for determination of orthophosphate in water samples.