CHAPTER 3

RESULTS AND DISCUSSION

3.1 Determination of iron by FIA using salicylic acid

3.1.1 Preliminary studies

3.1.1.1 Absorption spectra of Fe(III)-salicylate complexes

The absorption spectra of complexes of Fe(III)-salicylate were studied in the pH range of 1.5-7.0. Mixtures with 3 pH values (1.5, 6.5 and 7.0) of Fe(III) (10 mg Fe l^{-1} or 1.79×10^{-4} M) and salicylic acid (0.1 M) were prepared. The absorption spectrum of each solution was run. The spectra are shown in Fig. 3.1.

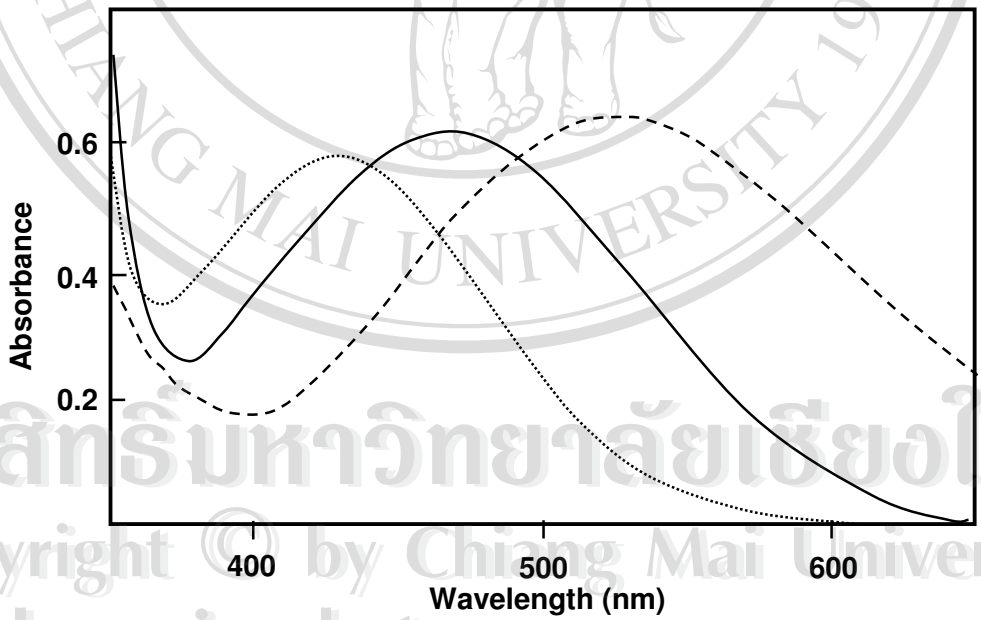
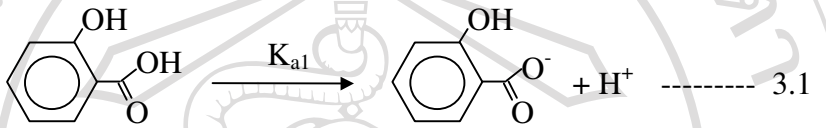


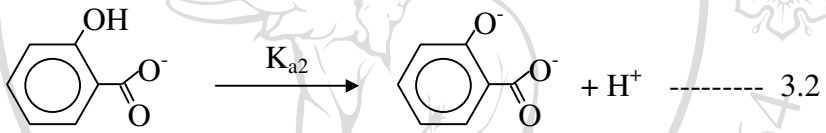
Figure 3.1 The absorption spectra of mixtures containing of Fe(III) (10 mg Fe l^{-1} or 1.79×10^{-4} M) and salicylate (0.1 M) of (a) pH 7.0 (b) pH 6.5 and (c) pH 1.5 (using 0.1 M salicylate as a reference solution)

From the preliminary study, it was found that the λ_{max} of Fe(III)-salicylate complexes was a pH dependent parameter. It can be seen that absorption maxima of Fe(III)-salicylate complexes varied with pH, i.e, 423, 467 and 528 nm for pH values of 7.0, 6.5 and 1.5, respectively.

The structural formula and dissociation of salicylic acid are presented in the equations 3.1 and 3.2.

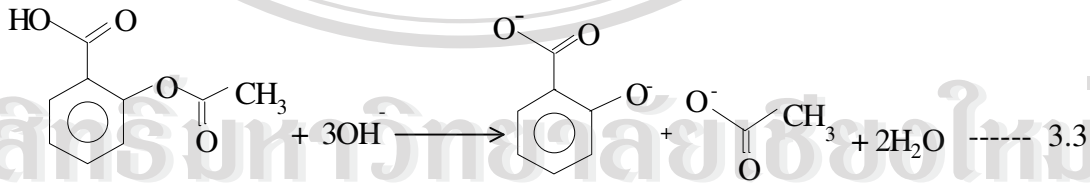


--------- 3.1



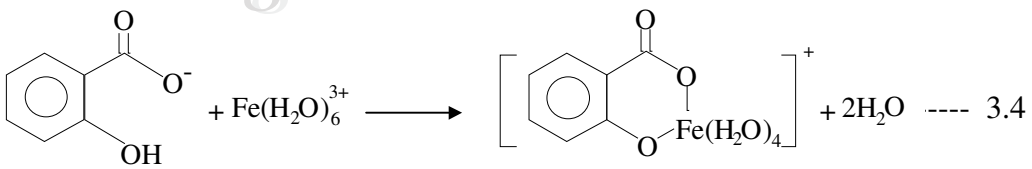
--------- 3.2

Salicylic acid contains two of different protons with two pKa values of 2.97 and 13.70. Hydrolysis of acetyl salicylic acid in basic solution yields salicylate (equation 3.3) [52].



------ 3.3

Salicylate reacts with Fe(III) to yield soluble tetraaquasalicylatoiron(III) [52].



---- 3.4

This intensely purple solution due to such a complex was reported to exhibit a very strong absorbance at 520 nm [52] and was observed in this study to be 528 nm as previously mentioned. The composition of this complex is sensitive to pH. If pH is more than 2 the formation of di and trisalicylato complexes would occur [52].

3.1.1.2 Study of the molar absorptivity of Fe(III)-salicylate complex

The molar absorptivity of Fe(III)-salicylate complex was evaluated. Three series of Fe(III) standards (3.58×10^{-6}, 7.17×10^{-6}, 1.43×10^{-5} and 3.58×10^{-5} M for each series) were prepared in 0.1 M salicylate at pH 4, 6 and 9, respectively (without buffer). The results indicate that at 467 nm, the condition with pH 6 results in the highest sensitivity (due to molar absorptivity: the slope of the calibration (Fig. 3.2)). This supports the study in the previous section (3.1.1.1).

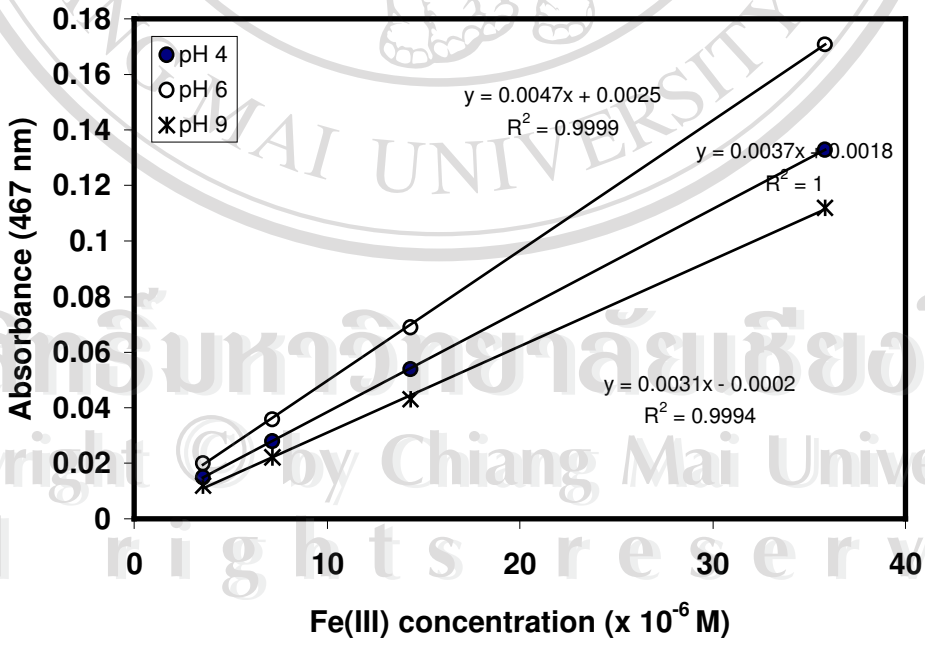


Figure 3.2 Plots of absorbances vs Fe(III) concentrations in 0.1 M salicylate

From the graphs the molar absorptivity (at 467 nm) of the complex at pH 4.0, 6.0 and 9.0 were 3,663, 4,697 and 3,120 l mol^{-1} cm^{-1}, respectively. The results confirmed that at the wavelength of 467 nm the absorption of the Fe(III)-salicylate complexes at pH 6.0 should be more than at pH 4.0 and 9.0. It should be noted that a molar absorbtivity of Fe(II)-1,10-phenanthroline was observed to be 8,500 at 510 nm (0.151 abs. at 1.79×10^{-5} M). It indicated that the sensitivity of the signal from Fe(III)-salicylate complex is about 2 times lower than the Fe(II)-1,10-phenanthroline complex, which is mostly used for iron determination. Salicylic acid/salicylate should be proposed as a color reagent for high-level iron determination as it is cheaper than 1,10-phenanthroline (see Table 3.1). Salicylate solution can be prepared from acetyl salicylic acid, which is usually available from a drug store for its antipyretic powder. Also another advantage is that there is no toxic waste from this chemical.

Table 3.1 Price comparisons of chemicals for iron determination (From ALDRICH Handbook of Fine Chemicals and Laboratory Equipment [61])

Weight (g)	Price (US$)			
	Salicylic acid	Sodium salicylate	Acetyl salicylic acid	1,10-phenanthroline
25	-		8.10	83.30
100	21.00	13.00	-	240.70
500	46.20	-	28.20	-
1000		69.40	46.60	-

In this work, acetyl salicylic acid was obtained from antipyretic, aspirin, available in any drug stores. It costs 1 Baht per 0.65 g (or about 0.02 US$ for 0.65 g).

3.1.1.3 FIA system set up and study for conditions

The FIA system in Fig. 2.1 for iron determination using salicylate was set-up. A simple one-line manifold was first tried. The effects, namely concentration, flow rate and pH of acetyl salicylate reagent solutions, mixing coil length and mixing coil types were investigated. The size of sample loop (35 μl) and Fe(III) standard (0.5 mg Fe l^{-1}) were fixed for all studies. Salicylate were obtained by hydrolysis of acetyl salicylic acid in 2 M sodium hydroxide and boiled in water bath for 15 minutes.

Various concentrations (0.01, 0.05, 0.1 and 0.5 M) of salicylate were tried by using a flow rate of 4.0 ml min^{-1} and mixing coil lengths of 92 and 200 cm. It was found that for a concentration more than 0.01 M salicylate, Schlieren effect was observed in spite of a higher peak height obtained. A 0.01 M salicylate concentration was selected for future studies. Effect of pH was then investigated next by varying pH values of salicylate from 4.2 to 10.2. It was found that there were no significant changes in peak heights. This could be due to that a blue LED light source used is not monochromatic so the effect of pH could be compensated. Then the pH of the reagent used could be in the range of 4.2 to 10.2 without any buffer to control pH.

Effects of flow rate of the salicylate stream were next studied. It was found that the peak height increased from 0.68 to 0.75 cm as flow rate decreased from 8.8 to 4.0 ml min^{-1}. A flow rate of 4.0 ml min^{-1} was then selected. A flow rate lower than 4.0 ml min^{-1} was not considered, as a lower sample throughput would be obtained.

Under such the conditions, a peak obtained was observed to be a split shape. So a glass bead-mixing device (0.3 mm glass beads packed in 0.3 mm i.d. × 5 cm Tygon tube, in which both ends were plugged with 0.3 mm o.d. × 2.0 cm Tygon tubing for the connection to the system tubings) was introduced instead of a mixing coil. A length of 5 cm was found to give a reasonably good peak shape. The conditions employed for Fe(III) determination using salicylate reagent are summarized in Table 3.2.

Table 3.2 The conditions for Fe(III) determination using salicylate reagent

Condition	Value
Salicylate (Reagent) concentration, M	0.01
Injection volume, µl	35
Glass bead mixing length, cm	5
Reagent Flow rate, ml/min	4.0
Recorder range, fsd, V	1.0

Using the employed conditions, the FIAgram and the calibration graph as shown in Fig. 3.3 and 3.4, respectively, resulted in a linear calibration, which was constructed by using five standards in the range of 1.0-20.0 mg l^{-1}. A higher concentration resulted in curvature. The linear regression equation obtained was:

peak height (cm) = 0.4618[Fe(III)(mg l^{-1})] + 0.1627, r^2 = 0.9997 -------- 3.5

Relative standard deviations (n = 3) over this range (1– 20 mg l^{-1}) varied from 1.4-5.4%. A detection limit (3σ of the blank signal [53]) of 0.46 mg l^{-1} was obtained.

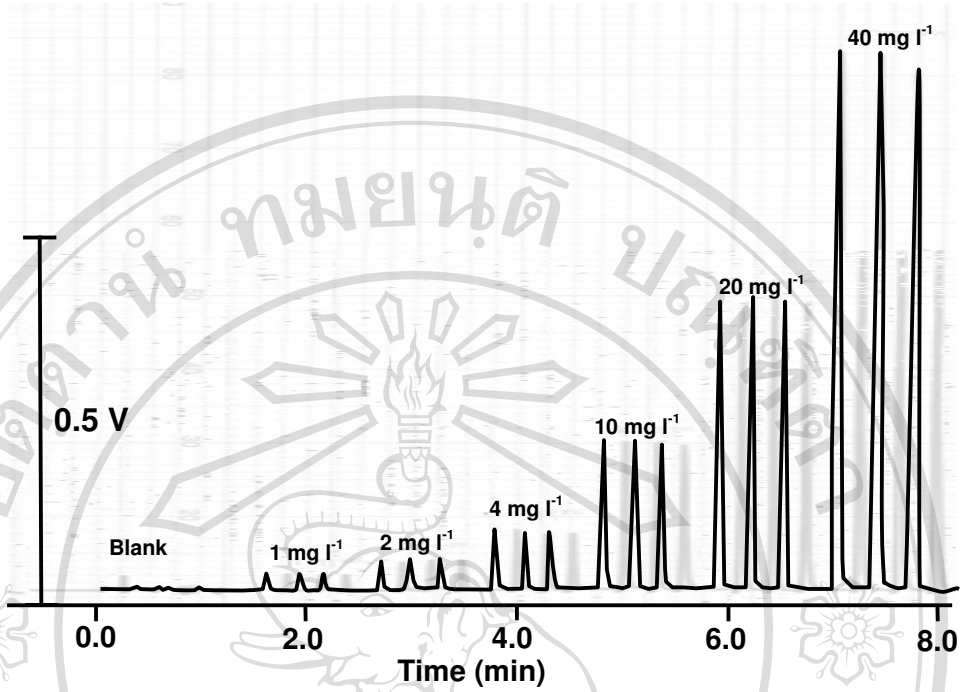


Figure 3.3 FIAgram for the determination of Fe(III) using salicylate

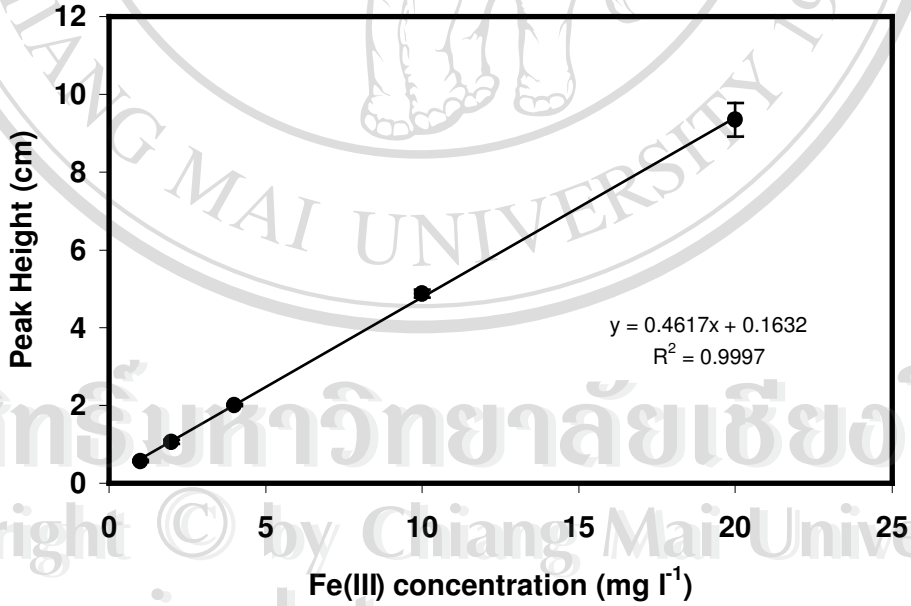


Figure 3.4 Calibration graph of Fe(III) determination using salicylate

3.1.2 Application of the developed method to pharmaceutical preparation samples and method validation

Pharmaceutical preparation samples were taken from local drug stores. Two procedures for sample preparation were employed. The first procedure was modified from AOAC 977.30 [54] as followed. Twenty tablets of a drug sample were weighed and powdered. A portion of the powder, equal in weight to one tablet of each drug, was accurately weighed and transferred to a 250 ml flask. A 50 ml of water and 2 ml conc. hydrochloric acid were added into it. The mixture was boiled in a stream bath for 30 min before cooling to a room temperature and was diluted with water to a volume of 100 ml. The mixture was filtered through Whatman No. 1 paper. A 1 ml aliquot of the filtrate was transferred to a 50 ml volumetric flask, and 0.5 ml of hydrogen peroxide was added before making to a volume of 50 ml with water.

The second procedure is as followed. Twenty tablets of a drug sample were weighed and powdered. A portion equal to the weight of one tablet was transferred to a 250 ml flask. A 25 ml volume of water, 15 ml conc. nitric acid and 5 ml conc. perchloric acid were added into it. The mixture was boiled on a hot plate until nearly dry, then cooled to room temperature before adding 4 ml conc. hydrochloric acid and 10 ml water and making to a volume of 100 ml with water. The mixture was filtered through Whatman No. 1 paper. A 1 ml aliquot of the filtrate was transferred to a 50 ml volumetric flask and diluted to the mark with water.

The samples after being treated by the above two preparation procedures were analysed by the proposed method. The USP method was used as a reference method. The procedures followed the USP 24 [55] for the drug samples in the form of fumarate, gluconate and sulfate.

Fumarate form [55]

Not less than 20 tablets of a sample were weighed and finely powdered. A portion of the powders with the accurate weight equal to about 0.5 g of Fe(II) fumarate, was transferred to a 250 ml beaker. A 25 ml volume of water, 25 ml conc. nitric acid and 7.5 ml conc. perchloric acid were added. The mixture was heated to the production of strong fumes. Then the solution was cooled. The watch glass and the sides of the beaker were rinsed with water and evaporated in a hood to near-dryness. A 2 ml portion of conc. hydrochloric acid and a small volume of water were used to wash down the watch glass and the sides of the beaker. The residue was slightly warmed if necessary, to dissolve and transferred to a glass-stoppered, 250-ml conical flask. A 2 ml volume of conc. hydrochloric acid and not more than 20 to 25 ml of water were used to repeat the washing and complete the transfer to the flask was made. A 4 g portion of potassium iodide was added to the flask and a stopper was inserted before allowing to stand in the dark for 5 min. Then 75 ml of water was added to the solution before titration with 0.1 M sodium thiosulphate was performed using starch as indicator. Each 1 ml of 0.1 M sodium thiosulphate is equivalent to 16.99 mg of $C_4H_2FeO_4$.

Gluconate form [55]

Not less than 20 tablets of a sample were weighed and finely powdered. A portion of the powders with accurate weight equal to about 1.5 g of Fe(II) gluconate, was transferred to a 300-ml conical flask containing a mixture of 75 ml of water and 15 ml of 4 M sulfuric acid. A 250 mg portion of zinc dust was added before closing the flask with a stopper containing a Bunsen valve. The mixture was allowed to stand

at room temperature for 20 min or until the solution became colorless. The solution was filtered through a filtering crucible containing a thin layer of zinc dust. The crucible and contents were washed with 10 ml of 4 M sulfuric acid, followed by 10 ml of water. Orthophenanthroline was added before titration with 0.1 M ceric sulfate. A blank determination was performed for a necessary correction. Each 1 ml of 0.1 M ceric sulfate is equivalent to 44.6 mg of $C_{12}H_{22}FeO_{14}$.

Sulphate form [55]

Not less than 20 tablets of a sample were weighed and finely powdered. A portion of the powders with accurate weight equal to about 0.5 g of Fe(II) sulfate was dissolved and transferred to a beaker, which contained a mixture 20 ml of 4 M sulfuric acid and 80 ml of freshly boiled and cooled water. The solution was rapidly filtered as soon as all soluble ingredients in the tablets were dissolved. The container and the filter were washed with a small portion of a mixture of 20 ml of 4 M sulfuric acid and 80 ml of freshly boiled and cooled water. Orthophenanthroline was immediately added before titrating the combined filtrate with 0.1 M ceric sulfate. Each ml of 0.1 M ceric sulfate is equivalent to 15.19 mg of $FeSO_4$.

Citrate form

As there is no USP method for a sample in a form of citrate, in this work a similar procedure to that for sulfate form was used for determination of iron.

The results are summarized in Table 3.3.

Table 3.3 Determination of iron in pharmaceutical preparation samples by the proposed FIA method and USP method

Sample	Form of iron	Label mg tab^{-1}	USP method [55]		The proposed FIA method			
					Sample preparation procedure			
					I		II	
			mg g^{-1}	mg tab^{-1}	mg g^{-1}	mg tab^{-1}	mg g^{-1}	mg tab^{-1}
1	Fumarate	200	458.9	221	454.6	219	443.4	213
2	Fumarate	90	67.7	90	69.8	93	72.0	96
3	Fumarate	200	446.1	214	436.4	209	481.4	231
4	Fumarate	200	389.6	197	375.6	190	395.3	200
5	Fumarate	400	424.4	415	404.8	396	400.1	391
6	Fumarate	200	351.5	194	354.7	196	375.2	208
7	Gluconate	200	509.1	330	296.2	192	329.6	213
8	Gluconate	150	230.8	161	204.6	157	179.4	138
9	Sulphate	200	322.1	149	438.0	202	444.5	205
10	Citrate*	470.9	1093.5	604	687.8	380	908.6	502

* For iron citrate sample, a similar procedure to that for sulfate form was used for determination of iron.

It was found that the results from the proposed FIA methods using both sample preparation procedures were in good correlation with the USP method by considering from t test (t = 1.37 for that of the sample preparation procedure I and t =

1.44 for the procedure II at 95% confidence level). And there was no significant difference between the two procedures for sample preparation, by considering t test (t = 0.08 at 95% confidence level). However, although the results from t test indicated that there were no significant differences between the methods it can be seen from Table 3.3 that the mg per tablet obtained by the USP method of the sample numbers 7-10, of which iron contents are in the form of gluconate, sulfate and citrate, differed from that of the labels, whereas that of the proposed method were nearly the same except for sample number 10 (citrate form). The errors from the USP method for gluconate and sulfate forms may be from the filtration step, which took a period of time. The fine powders of zinc dust and drug powder may clog the filter paper and there is difficulty in end point observation. For the citrate form, both the procedures for sample preparation are not suitable in the determination of iron. However, the proposed method can well applied to the samples, in which iron contents are in the forms of fumarate, gluconate and sulfate.

It should be noted that the precipitation of Fe(OOH) was not observed. This could probably be due to that it was in a medium of pH being lower than 7.0 and that complex formation constant of Fe(III)-salicylate is high enough.

3.2 Determination of Fe(II) and Fe(III) by Capillary Electrophoresis and Voltammetry

3.2.1 Capillary Electrophoresis

The capillary electrophoresis system as shown diagrammatically in Fig. 2.5 was used. All separations were performed in untreated fused silica capillaries, 75 μm i.d. (Polymicro Technologies, Phoenix, AZ, USA). The total length of the capillaries

was 45 cm. The distance between the injection and the detection site was 30 cm. A 0.1 M borate buffer pH 9.0 was used as a carrier. First the capillaries were filled with borate buffer using the pressure injection mode, for which the pressure used was 30 mbar and waited until the drop at the opposite end was observed (using a syringe pump to push the buffer passing through the capillary). The electrokinetic injection was used for sample injection, which were performed at the anode end. The other conditions used are summarized in the Table 3.4.

Table 3.4 The conditions for capillary electrophoresis

Condition	Value
Injection voltage (kV)	10
Injection time (s)	10
Analysis voltage (kV)	10
Analysis time (min)	20
Detection wavelength (nm)	540

Each of a mixture of Fe(II) and Fe(III) standards with PAR was prepared from 0.01 M ferrous sulfate, 0.01 M ferric chloride and 0.005 M PAR. The final concentrations of Fe(II), Fe(III) and PAR were 6.67×10^{-5}, 6.67×10^{-5} and 1.67×10^{-4} M, respectively. The injections were performed using the conditions as shown in Table 3.4. The data were recorded and processed using computer programs. The results are represented in the Fig.3.5.

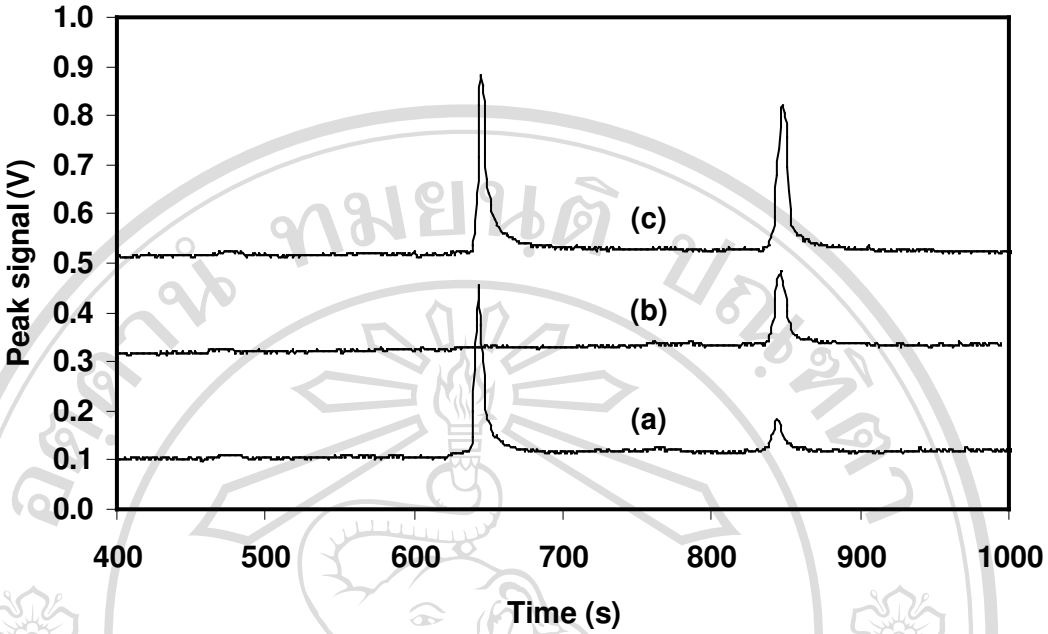


Figure 3.5 Electropherograms obtained by an injection of a mixture solution of (a) 1.67×10^{-4} M PAR with 6.67×10^{-5} M Fe(III), (b) 1.67×10^{-4} M PAR with 6.67×10^{-5} M Fe(II) and (c) 3.34×10^{-4} M PAR with 6.67×10^{-5} M Fe(II) and 6.67×10^{-5} M Fe(III)

The structural formula of PAR shown in the Fig. 3.6 can be represented with H_2L. It dissociates in an aqueous solution to obtain major species as shown in the distribution profile (Fig. 3.7).

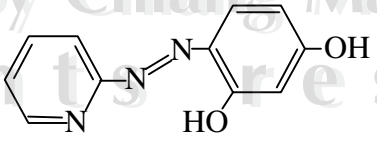


Figure 3.6 The structural formula of PAR [56]

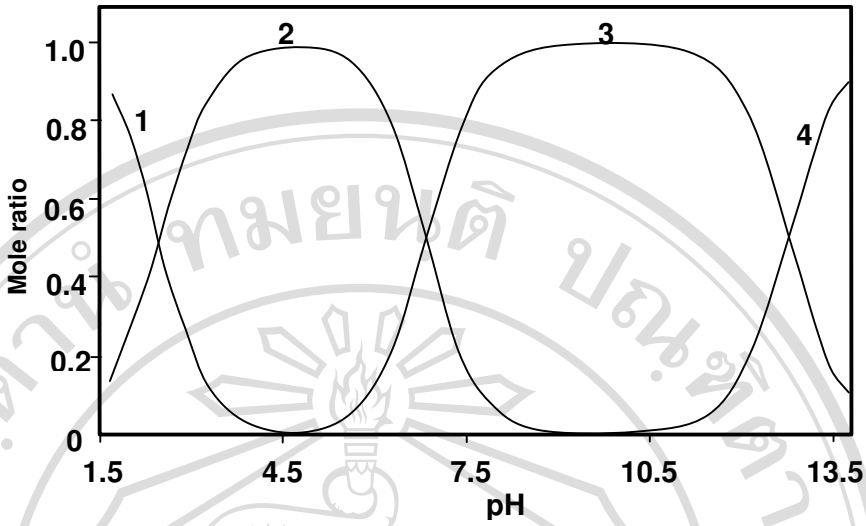


Figure 3.7 Distribution profile of major species of PAR, (1) H_3L^+, (2) H_2L, (3) HL^-, (4) L^{2-}, as a function of pH [56]

The forms of PAR are varied from H_3L^+ to L^{2-} as the pH increases from 1 to 14 [56].

$$H^+ + H_2L \rightleftharpoons HL^- + H^+$$

$$H_3L^+ \qquad\qquad L^{2-} + H^+$$

In the buffered solution of pH 9, PAR should exist in the form of HL^- [56] which should form a complex with Fe(II) and Fe(III). Under the run conditions, i.e., sample injection made at the anode, a peak due to Fe(II) in an electropherogram was observed at about 850 s (Fig.3.5 (b)) while peaks obtained from a mixture of Fe(III) and Fe(II) were at 650 and 850 s, respectively (Fig.3.5 (c)). The peak at 650 s should be due to Fe(III). This could be explained by the formation of the complex of Fe(II)-PAR (in the HL^- form in pH 9 solution) should have less net positive charge than that of Fe

(III)-PAR complex so that the Fe(III)-PAR complex move faster from anode (positive electrode) to cathode (negative electrode). It can be observed that for the electropherogram obtained from the solution of Fe(III) (Fig. 3.5 (a)), apart from the main peak at 650 s due to Fe(III); there was also a small peak at 850 s. As Fe(III) forms complex with PAR by the ratio of 1:2 (Fe(III) : PAR) so the formation at pH 9 could be expressed as follows:

$$Fe(III) + HL^{-} \rightleftharpoons [Fe(III)\text{-}HL]^{2+} \qquad K_1 \quad \text{---------- 3.6}$$

$$[Fe(III)\text{-}HL]^{2+} + HL^{-} \rightleftharpoons [Fe(III)\text{-}(HL)_2]^{+} \qquad K_2 \quad \text{------ 3.7}$$

The peak at 850 nm would not be other form of Fe(III) complexes as they have charge to mass ratios more than $[Fe(III)\text{-}HL]^{2+}$, of which the retention time should be less than 650 s. So this peak should be due to Fe(II) which could possibly arise from a reduction of Fe(III) by PAR which has an oxidizable resorcinol moiety as part of its structure [18]. The above studies should be confirmed further by the conditions (described in Fig. 3.5) with more excess of PAR.

From the results it was found that the complexes of Fe(II)-PAR and Fe(III)-PAR can be separated under the conditions used. Fe(II)-PAR retained in the capillaries longer than the Fe(III)-PAR complex.

Despite the successful separation of Fe(II)-and Fe(III)-PAR chelates, the resulting electropherograms clearly indicate that this method has an intrinsic analytical drawback as the Fe(III) chelate is partly reduced to the Fe(II) chelate.

3.2.2 Voltammetric study for possibilities of speciation of Fe(II) and Fe(III)

The voltammetric analyzer consisting of a 693 VA processor, 694 VA Stand and the accessories including the cell (Fig. 2.6) (Metrohm, Switzerland) was used for all studies. The conditions used are summarized as follows.

Conditions:

Working electrode: SMDE (Static Mercury Drop Electrode)

Reference electrode: Ag/AgCl # Auxiliary electrode: Platinum

Running mode: Differential pulse polarography

Potential range: 0 to –1300 mV # Scan rate: 100 mV/s

0.1 M sodium pyrophosphate in ammonia ammonium chloride buffer was first used as supporting electrolyte and complexing ligand. The solution was prepared by transfering 10 ml of 0.1 M sodium pyrophosphate in ammonia ammonium chloride buffer to the voltammetric cell. The solution was purged with nitrogen for 3-5 min before the first voltammogram was recorded. The same solution was further added at each time with 200 µl of Fe(II) or Fe(III) standard solution for higher concentrations.

The free ions of Fe(II) and Fe(III) can be reduced at different potentials as follows [33].

$$Fe^{2+} + 2e^- \rightarrow Fe \quad E^0 = -0.44\ V \text{ ---------- } 3.8$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad E^0 = 0.77\ V \text{ ---------- } 3.9$$

In a solution with some complexing ligands the standard reduction potential of these ions will shift to more negative values. Fig. 3.8. depicts recorded voltammograms.

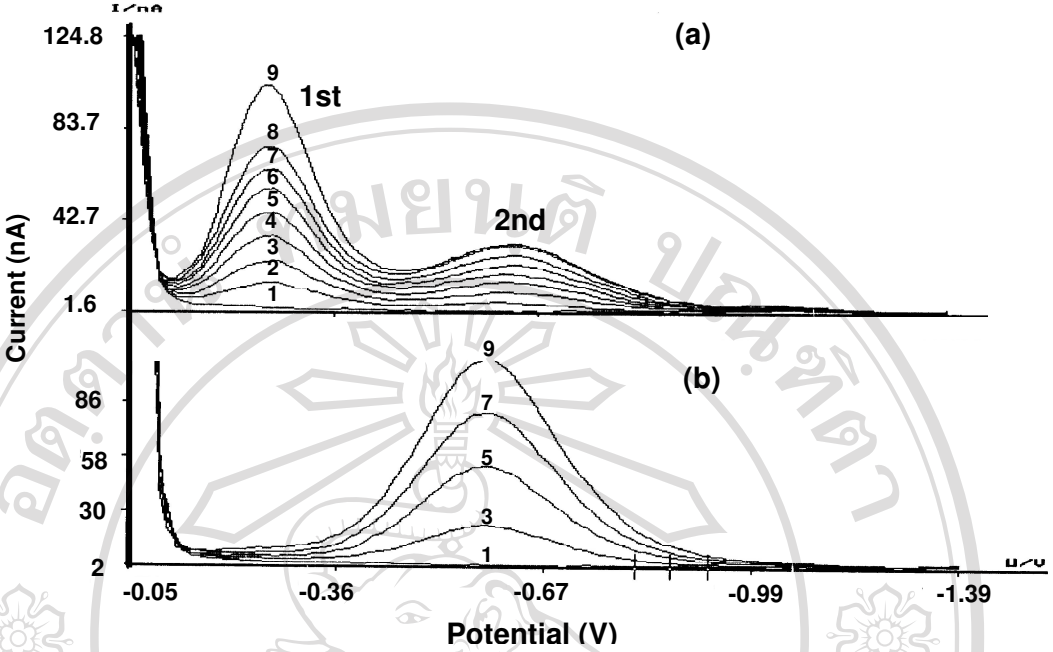


Figure 3.8 Voltammograms obtained by serial mixture solutions of (a) Fe(II) and (b) Fe(III) in 0.1 M sodium pyrophosphate and ammonia ammonium chloride buffer: 1) blank 2) 2 mg l^{-1} 3) 4 mg l^{-1} 4) 6 mg l^{-1} 5) 8 mg l^{-1} 6)10 mg l^{-1} 7) 12 mg l^{-1} 8) 14 mg l^{-1} 9)16 mg l^{-1}

The voltammograms in Fig. 3.8 (a) show that under the conditions used the reduction peaks of Fe(II) at –220 mV (1st) and –590 mV (2nd) were obtained, while for Fe(III) solution (Fig. 3.8(b)) there was only one observed reduction peak at –590 mV. In the voltammograms in Fig. 3.8(a) the 1st peak should be expected to be the reduction of Fe(II)-pyrophosphate complex, -220 mV and the 2nd peak should be due to the reduction of Fe(III)-pyrophosphate complex, as the peak occurred at the same potential as Fe(III)-pyrophosphate complex, -590 mV. The 2nd peak was presumed to be Fe(III)-pyrophosphate complex reduction. As Fe(III) can form a stronger complex with pyrophosphate than that of Fe(II), the peak potential will shift to higher negative

potential than that of Fe(II)-pyrophosphate. For the 2nd peak appearing in Fig. 3.8(a) it could be due to step reactions of some other free Fe(II) species, which did not from complex with pyrophosphate in the solution. As soon as the slight potential was applied, these species can be oxidized to Fe(III) at the electrode surface and from a stable complex with surrounding pyrophosphate to give a peak signal next to that of Fe(II)-pyrophosphate. Or it could also due to that Fe(II) can be rapidly oxidized in the solution to produce Fe(III). This led to two observed peaks in the voltammogram obtained from the mixture solutions of Fe(II). A pyrophosphate medium has been used earlier for the determination of iron of the two oxidation states using differential pulse polarography [6]. Similar results were observed. Both reduction and oxidation processes might occur in the pyrophosphate medium. A previous study [27], of which differential anodic stripping voltammetric run was made in a flow-through cell with a glassy carbon electrode, indicated that the mechanism on glassy carbon and SMDE may be different.

It can be concluded that these conditions studied in this work are not useful for simultaneous determination of Fe(II) and Fe(III).

The supporting electrolyte was changed to 1.0 M dipotassium hydrogen phosphate. The procedures and the conditions used were similar to that of 0.1 M sodium pyrophosphate in ammonia-ammonium chloride buffer. The recorded voltammograms are shown in Fig. 3.9.

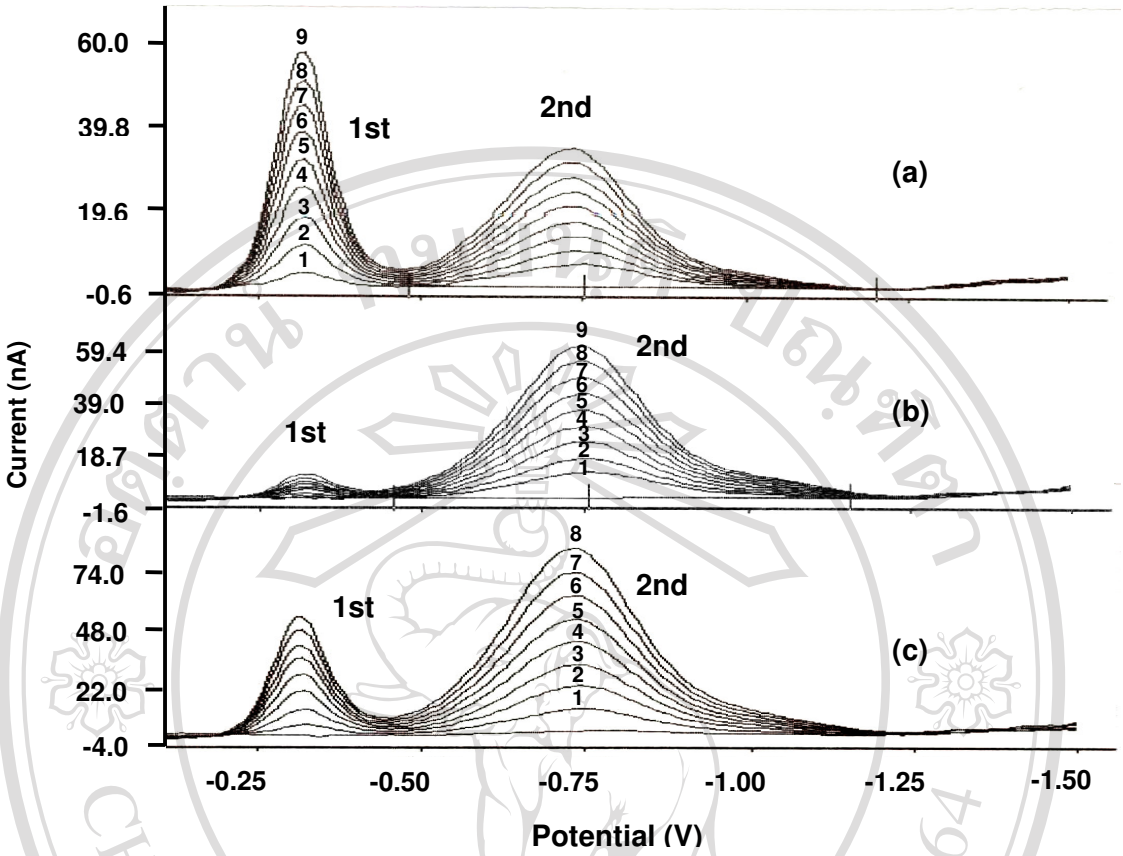


Figure 3.9 Voltammograms obtained by a serial mixture solutions of (a) Fe(II) and (b) Fe(III) and (c) Fe(II) + Fe(III) in 1.0 M dipotassium hydrogen phosphate: 1) 2 mg l^{-1} 2) 4 mg l^{-1} 3) 6 mg l^{-1} 4) 8 mg l^{-1} 5)10 mg l^{-1} 6) 12 mg l^{-1} 7) 14 mg l^{-1} 8)16 mg l^{-1}

The results showed that the voltammograms obtained from dipotassium hydrogen phosphate solution are in similar pattern of that of Fe(II) or Fe(III) in pyrophosphate solution. Only the change in peak potentials (-300 mV for Fe(II) complex and -700 mV for Fe(III) complex) leading to improvement of peak separation and peak shape. Although the results in this present study have not yet led to speciation of both iron species but it indicates possibility for simultaneous determination of Fe(II) and Fe(III). Further studies should be made.

3.3 Phosphate determination using flow injection analysis with electrochemical detection

Orthophosphate can be determined as 12-molybdophosphate in an aqueous acidic molybdate solution by linear-sweep voltammetry and differential pulse voltammetry at a glassy carbon electrode in a static system [37]. The mechanism, which produces the current can be explained as the reduction of 12-molybdophosphate at 0.14 V [35] or adsorption process [57].

The FIA manifolds illustrated diagramatically in the Fig.2.2 were used. A laboratory-made FIA workstation, consisting of peristaltic pumps (Ismatec, CA5E), an injection valve (Rheodyne 5041, USA), and an electrochemical detector (Princeton Applied Research model 400, PAR, USA) were employed. PTFE tubing of 0.5 mm i.d. was used as mixing coils and another parts which connected to the pump tubings. The FCS computer program (A-Chem Technologies, Australia) was used to control the system and collect the data from the detector. Evaluation was made by using Microsoft Excel software. The injection valve was actuated by the pressure from a nitrogen gas cylinder. The flow through cell consisted of a glassy carbon working electrode, a silver/silver chloride reference electrode and stainless steel cell body auxiliary electrode arrangement.

3.3.1 Direct FIA system for phosphate determination

For the manifold in Fig. 2.2(a) (FI system A), standard/sample was injected into a stream of acidic molybdate, which acts as a reagent and a supporting electrolyte for amperometric detection. For the manifold in Fig. 2.2(b) (FI system B), standard/sample was injected into potassium chloride before merging with an acidic

molybdate, leading to better mixing of sample and reagent. Flow rates of the solutions, potassium chloride and molybdate were 2.0 and 0.9 ml min^{-1}, respectively. The potential applied to the glassy carbon working electrode was fixed at 220 mV vs Ag/AgCl reference electrode. The sensitivity range of the detector was set at 0.2 μA for the FI systems A and B whereas at 0.1 μA for the FI system C. A series of orthophosphate standards in concentration range of 10 to 1000 μg P l^{-1} was triplicately introduced into the FI system.

By using the FI system A, employing acidic molybdate reagent only, a high negative peak of blank was obtained possibly due to the difference in ionic strength of the solutions. This led to a higher detection limit (22.4 μg P l^{-1}), which was calculated using the criteria of three times the standard deviation of the blank described by Miller and Miller [53]. Whereas the FI system B using 2 reagent streams, potassium chloride and acidic molybdate, provided a lower peak of blank and a lower detection limit (3.4 μg P l^{-1}). Potassium chloride served as an electrolyte to promote the electrical conduction and decrease the difference in electrical conduction between sample plug and the acidic molybdate. The effect of potassium chloride on the polarogram of 12-molybdophosphate was reported by another study [38]. It was described that potassium ion and ions of similar size greatly increase the surface area of 12-molybdophosphate. The effect of potassium ion on the voltammogram of 12-molybdophosphate may be associated with this.

The linearities of calibration graphs of both systems were obtained in concentration ranges of 50 to 1000 μg P l^{-1}, of which the equations were: $y = 1.0181x - 31.753$, $R^2 = 0.9995$ and $y = 0.7834x - 2.4996$, $R^2 = 0.9995$ for the FI systems A

and B, respectively. The FI system B giving better analytical performance was then selected for further investigation. The conditions used are summarized in Table 3.5.

Table 3.5 Conditions for determination of phosphate using the FIA electrochemical detection

Condition	Value
Potassium chloride (R1) concentration, M	0.1
Molybdate (R2) concentration, %(w/v) in 2.5%(v/v) H_2SO_4	0.5
Injection volume, µl	50
Mixing coil length, cm	60
R1 Flow rate, ml min^{-1}	2.1
R2 Flow rate, ml min^{-1}	0.9
Applied potential, mV	220
Detector range, µA	0.1

3.3.1.1 Study of the reproducibility of the signal

The FI system B was then studied further for stability by 50 replicate injections of 50 and 500 μg P l^{-1} of phosphate standards. Fig. 3.10 shows that at a lower concentration of phosphate (50 μg P l^{-1}), the peak-height responses were quite reproducible. The mean and relative standard deviation of peak heights were 21.3 mV and 1.3%, respectively. For a higher concentration of phosphate (500 μg P l^{-1}), more fluctuation of peak-heights was obtained. The mean and standard deviation of peak heights were 182.6 mV and 9.5%, respectively. From the results, it could be that, when a higher concentration of phosphate was injected to the system, phosphomolybdate was formed. The molybdenum blue complex from reduced phosphomolybdate could precipitate and clog at the flow cell as it can be observed some of blue precipitate in the flow cell. This may lead to fluctuation in the peak-height responses when the system was applied to a high concentration of phosphate.

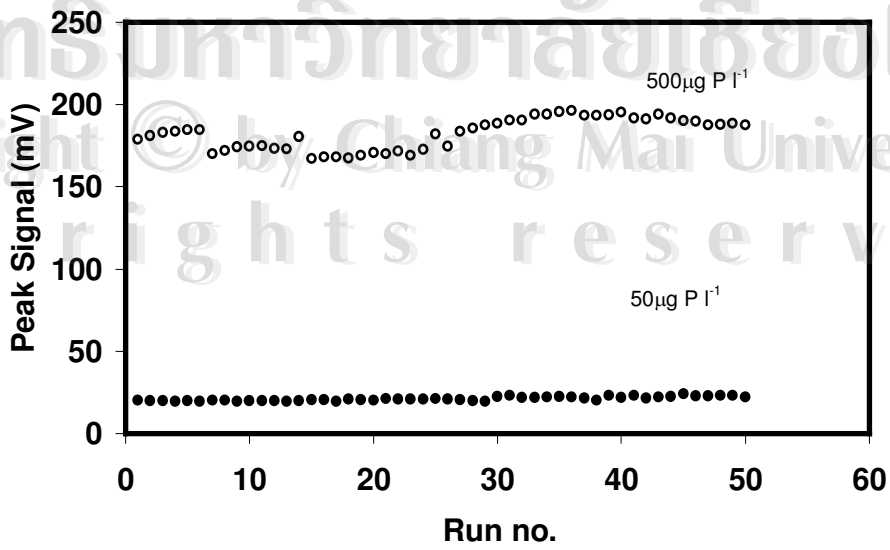


Figure. 3.10 Reproducibility of phosphate peak height concentration of 50 and 500 μg P l^{-1}. Detector range: 0.2 μA

3.3.1.2 Interference study

Some potential interferent species have been studied.

Silicate

Silicate solutions of different concentrations were injected to the system. It was found that at a concentration lower than 50 mg l^{-1} no peak signal was obtained. However, silicate usually found in natural water is not more than 20 mg l^{-1} so it could be concluded that there should be no effect or interference due to silicate for the determination of phosphate in natural water by this method. And from cyclic voltammograms of phosphate and silicate in a mixture solution of acidic molybdate and potassium chloride it was found that silicate does not give peak signal at 220 mV under this condition. This will support that there is no interference from silicate.

Sulfide

Sulfide is one of the interferents in phosphate determination by the common spectrophotometric molybdenum blue method. Sulfide can be reduced at 220 mV (vs Ag/AgCl) at the working electrode and give a negative peak (opposite direction to the phosphate peak). It was found that 5 mg l^{-1} of sulfide will have some effects on the peak-height responses of phosphate (50-1000 μg P l^{-1}). But no interference was

observed for 2 mg l^{-1} of sulfide at which the concentration does not exceed in natural water.

Organic phosphates

Five organic phosphate species: adenosin-5-monophosphate, 2-aminoethyl phosphonic acid, glycerophosphate, phenyl phosphate and phytic acid were employed to study whether they interfered. It was found that 100 μg l^{-1} of each of organic phosphate did not give any peak signal on the FIAgrams.

Effect of salinity

The effect of sodium chloride was also investigated. It was found that sodium chloride concentration up to 4% (typical concentration in sea water) does not affect the peak-height responses of phosphate in the range of 50-1000 μg l^{-1}. This method can then apply to seawater samples without any salinity compensation. This is superior to the FI determination of phosphate using photometric detection for the molybdenum blue method for which high salinity of sea water samples can cause the problem of so called Schlieren or refractive index (RI) effect in the flow injection with photometric detection system [42].

3.3.1.3 Samples determination and method validation

The method was applied to 4 reference materials: fresh waters, NLLNCT-Round 7: bottle 1 and bottle 3, and seawaters, NLLNCT-Round 7: bottle 5 and bottle 7 (Queensland Health Scientific Services Australia). The results as shown in Table 3.6 indicate that there is no adverse effects from sample salinity.

Table 3.6 Analysis of reference fresh water and sea water

Water type	CRM NLLNCT-round 7	Certified value ($\mu g\ P\ l^{-1}$)	Found ($\mu g\ P\ l^{-1}$)
Fresh water	bottle1	27.0 ± 0.8	29.4 ± 1.4
	bottle 3	97.9 ± 1.1	86.8 ± 1.1
Sea water	bottle5	27.7 ± 1.2	23.1 ± 0.3
	bottle 7	11.8 ± 0.9	13.6 ± 1.7

The proposed method was also applied to 8 sediment water samples, which were collected from the water over the sediment at various depths in the laboratory cylinders. The results (Table 3.7) agreed well with those obtained by FIA-spectrophotometric method (t- test at the 95% of confident: t-table = 2.365, t-calc = 0.11).

Table 3.7 Analysis of sea water sample

Sample	Found ($\mu g\ P\ l^{-1}$)	
	Proposed procedure	Conventional FIA [42]
BB12	146	138
BB13	319	337
BB14	408	389
BB15	393	380
BB16	417	419
BB17	417	418
BB18	435	436

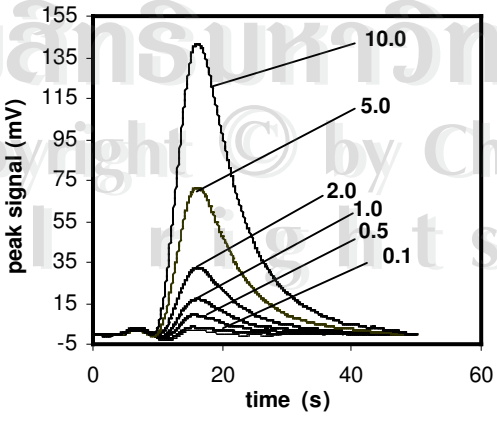
(Detection limit = 13 $\mu g\ P\ l^{-1}$ for the Conventional FIA)

3.3.2 Develop method for on-line preconcentration of phosphate

An ion-exchange mini-column was employed using Bio-Rad AG1-X8 (Bio-Rad, USA.) of 200-400 meshes, chloride form. The resin was placed in the column (5 mm long × 1.5 mm i.d.), of which both ends were plugged with nylon net and connectors.

3.3.2.1 The system used and calibration data

The FI system C (Fig. 2.2) was used for preconcentration and determination of orthophosphate in a concentration lower than 10 μg P l^{-1}. By using 2 min preconcentration time at a loading flow rate of 0.9 ml min^{-1}, the detection limit, defined as three times standard deviation of the blank [53] was found to be 0.18 μg P l^{-1}. The linearity of a calibration graph (Fig. 3.11) was between 0.1 to 10 μg P l^{-1}. The concentration efficiency (CE) [58] was 2.3.



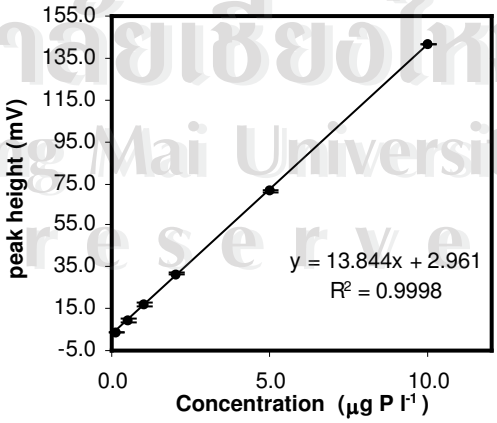


Fig. 3.11 FI gram and calibration graph of phosphate standard concentrations 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 μg P l^{-1} (The peak signals were obtained from computer (reading in mV) connecting to electrochemical detector (reading in nA))

From the graph the detection limit was found to be 0.18 μg P l^{-1}. The linearity was between at least 0.1 to 10 μg P l^{-1} (R^2 = 0.9998).

3.3.2.2 The effect of chloride on phosphate adsorption

Potential competition for ion-exchange site by chloride ion present in the sample was investigated by analyzing a series of 5 μg P l^{-1} standards containing chloride 0, 50, 100, 200, 500 and 1000 mg l^{-1}. The results were reported in Fig. 3.12.

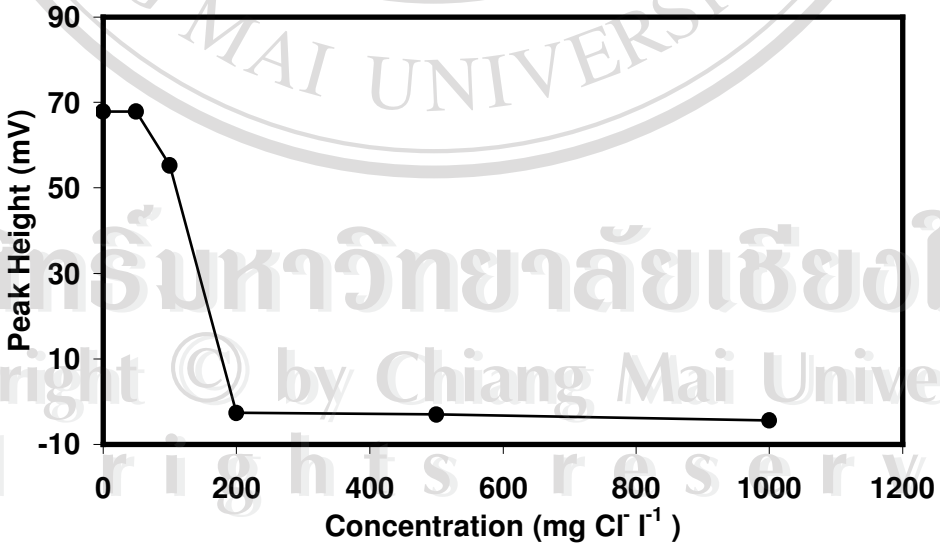


Figure 3.12 The effect of chloride on peak signal response of 5 μg P l^{-1}

The results show that chloride ion concentration more than 50 mg l^{-1} can reduce the peak height of phosphate. As chloride concentration increased up to 200 mg l^{-1} the peak height was equal to blank. The results indicate that the method is not suitable for a sample with some salinity.

3.3.2.3 Samples determination and method validation

As the salinity of tap water usually less than 10 mg l^{-1} so this method was tried for tap water samples. Four tap water samples were spiked with phosphate standard for the concentrations of 0.5, 1.0, 3.0 and 5.0 μg l^{-1}. By using the same conditions, the samples were injected into the FIA system. The results are summarized in Table 3.8.

Table 3.8 Recoveries of spiked dissolved reactive phosphorus in tap water samples, using preconcentration manifold. These data were computed from the calibration equation: $y = 12.989x-0.4932$, $r^2 = 0.999$

Sample	Concentration (μg P l^{-1})		% Recovery *
	Added	Found	
A	-	0.74[a]	-
	3.00[b]	3.50[c]	92
B	-	1.05[a]	-
	5.00[b]	6.83[c]	116
C	-	0.66[a]	-
	1.00[b]	1.92[c]	125
D	-	0.50[a]	-
	0.50[b]	1.09[c]	118

* %Recovery = [(c-a)/b] x 100

From Table 3.8, it was found that the recovery of spiked phosphate was in the range of 92-125%.

3.3.2.4 Single standard calibration

The possibility of applying a single standard calibration was investigated using 5 and 10 μg P l^{-1} phosphate standards by varying the preconcentration time (or loading time) for a sample. A calibration graph, which is a plot of μg P vs. peak height, is shown in Fig. 3.13. The μg P was calculated by flow-rate (ml min^{-1}) x loading time (min) x standard solution concentration (μg P l^{-1}) x 10^{-3}. It was found that the data points of 5 μg P l^{-1} are on the same line as that of 10 μg P l^{-1}. That means a single standard calibration can be applied in this method.

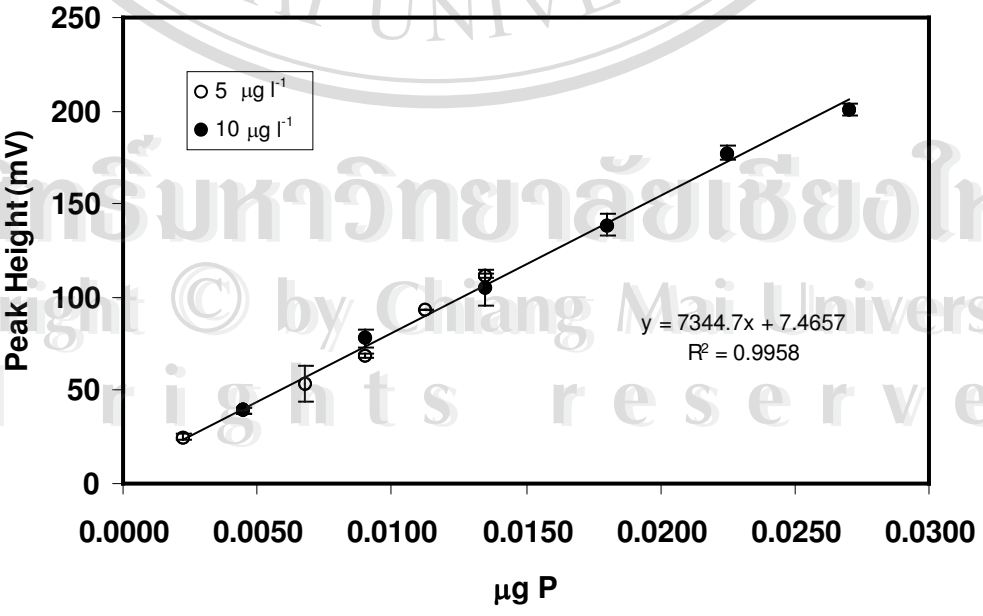


Figure 3.13 Single standard calibration using 5.0 and 10.0 μg P l^{-1}

3.4 On-line sulfide removal for phosphate determination

The FIA manifold in Fig. 2.3 was employed. A homemade FIA workstation consisted of peristaltic pumps, an injection valve and spectrophotometer CECIL model CE 1010. The FCS computer program was used to control pumps and injection valve, and to collect the data from the detector. The flow rates of the carrier, acidic molybdate solution and reducing solution were 2.0, 0.9 and 0.9 ml min^{-1}, respectively. The 0.5 mm i.d. TPFE tubings were used in the manifold and mixing coils.

A 0.01 M permanganate solution was obtained by dissolving 0.40 g of potassium permanganate in 250 ml water. Phosphate standard solutions (10 to 1000 μ g P l^{-1}) were prepared by diluting the intermediate 5 mg P l^{-1}, which was diluted from the 100 mg P l^{-1} stock solution (0.2197 g KH_2PO_4 in 500 ml deionized water). A 200 mg l^{-1} sulfide solution was prepared by dissolving 0.1529 g of sodium sulfide

nonahydrate in 100 ml water. The intermediate 20 mg l^{-1} sulfide solution was prepared by diluting a 200 mg l^{-1} sulfide solution.

The pore water samples were taken from intact sediment cores collected from Lake Victoria in the Gippsland Lakes, Victoria, Australia. 11 cm diameter sediment cores were collected in perspex tubes. The tubes were approximately half filled with the sediment and the other half with water. During transport, the cores were kept upright and as close as possible to the in situ temperature. Back in the laboratory, the cores were extruded vertically and sliced under a nitrogen atmosphere. The slices were placed in a centrifuge tube and spun at 10,000 rpm for 20 minutes. The supernatant was then collected and frozen at –20° C prior to analysis.

3.4.1 Analytical wavelengths for the determination of phosphate by the molybdenum blue method with various reducing agents

Different reducing agents used for the determination of phosphate by using the molybdenum blue method were reported to exhibit different absorption maxima [60], namely 690, 810 and 882 nm for tin(II) chloride, ascorbic acid, and ascorbic with antimony, respectively as depicted in Fig. 3.14.

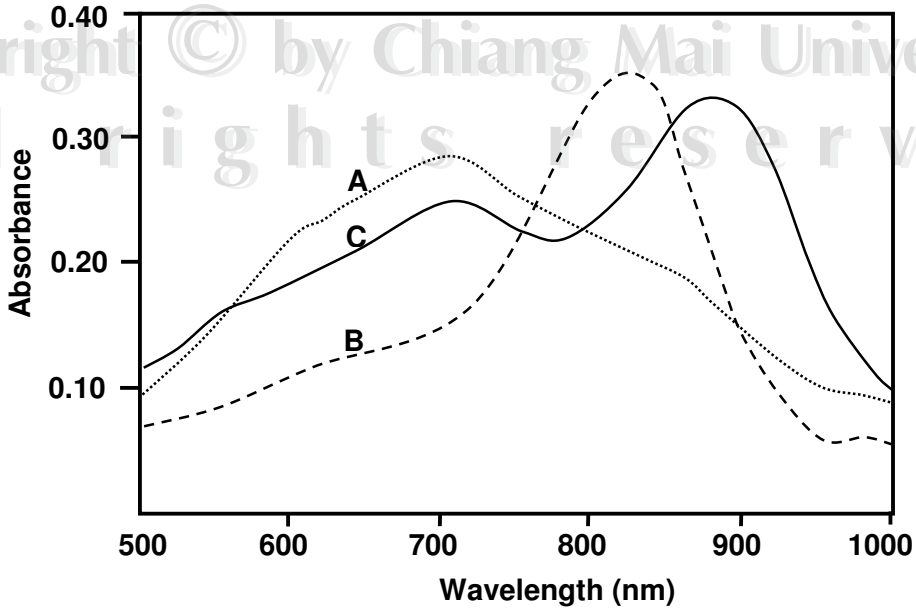
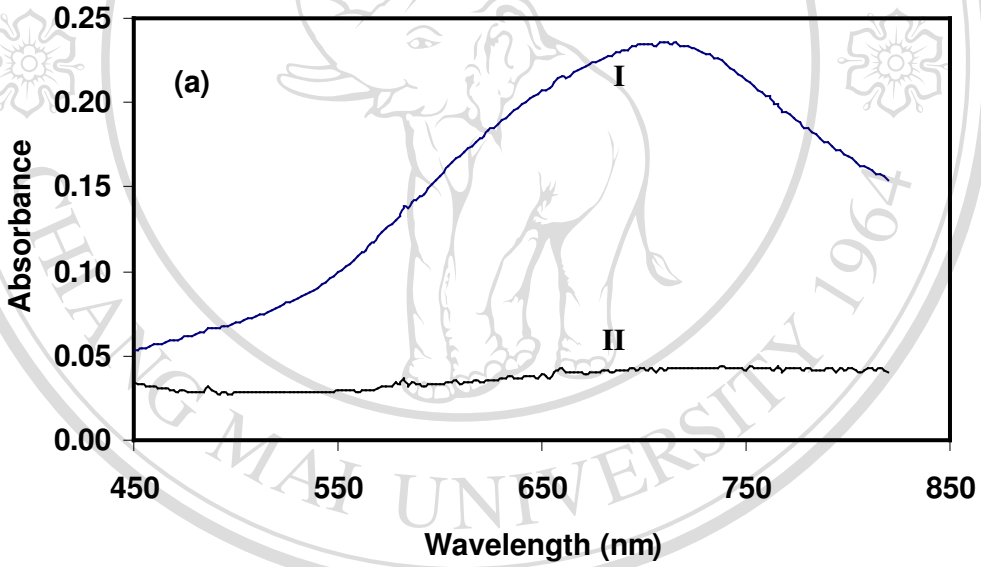


Figure 3.14 Absorption spectra for molybdenum blue formed with various reducing agents (3.0 μg P as PO_4^{3-} in 50-ml flasks; 7.62-cm cell): A) reduced with tin(II) chloride, B) reduced with ascorbic acid and C) reduced with ascorbic acid and antimony [60]

Absorption spectra recorded as represented in Fig. 3.15 indicate that tin(II)



chloride has faster kinetics in reduction than ascorbic acid does.

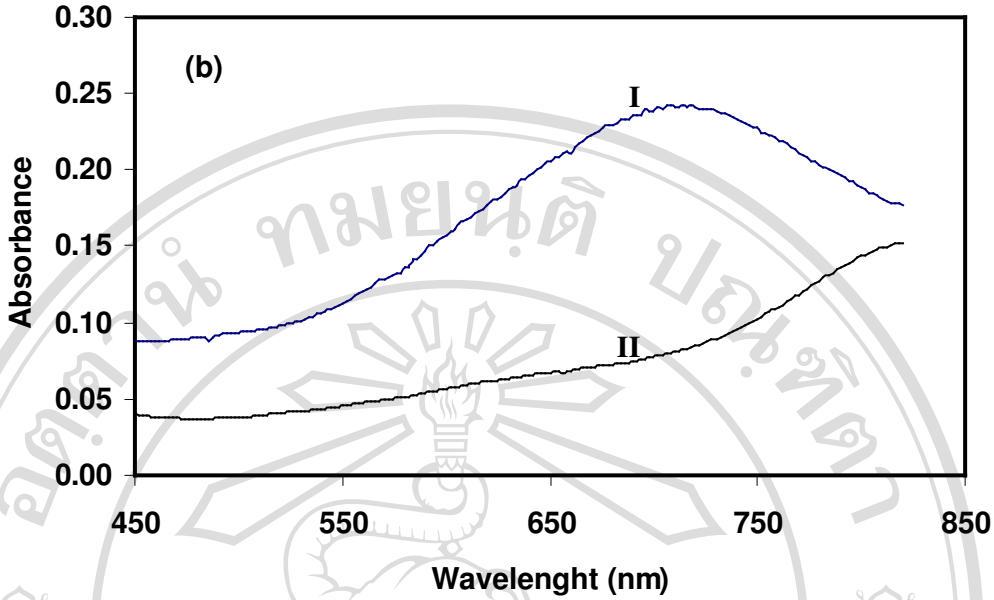


Figure 3.15 Absorption spectra obtained by using phosphate (1000 µg P l^{-1}) in the molybdenum blue method with reducing agents of (I) tin(II) chloride or (II) ascorbic acid after mixing the solutions for (a) 2 min and (b) 10 min

Fig. 3.16 shows that under the employed conditions with ascorbic acid reducing agent, maximum peak height would be obtained at a wavelength of 750 nm.

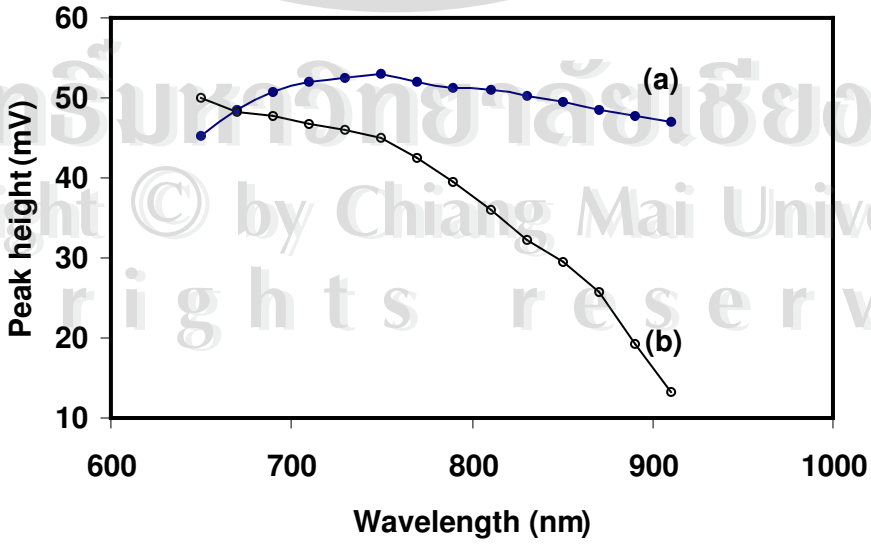


Figure 3.16 Peak heights of signals obtained in a molybdenum method for: (a) phosphate (1000 μg P l^{-1}) and (b) sulfide alone (20 mg S l^{-1}) by using ascorbic acid as reducing agent

3.4.2 Quantify sulfide interference using tin(II) chloride and ascorbic acid as reducing solutions

A series of 30 mixtures containing phosphate and sulfide was prepared by fixing phosphate concentration at a time (0, 50, 100, 200, 500 and 1000 μg P l^{-1}), and varying sulfide concentrations (0, 2, 5, 10 and 20 mg S l^{-1}). All of the mixture solutions were injected into the FIA system (Fig. 2.3) in which the flow rates of the carrier, acid molybdate and tin(II) chloride reducing solution were 2.0, 0.9 and 0.9 ml min^{-1}, respectively, and the absorbance at 690 nm was measured. This was following the WRC conditions (or modified [42]). Similarly for the system with ascorbic acid reducing solution, 5 standard sets of phosphate concentrations of 0, 50, 100, 200, 500 and 1000 μg P l^{-1} were prepared. The concentrations of sulfide added were varied 0, 0.1, 0.5, 1.0 and 2.0 mg S l^{-1}. All of the solutions were injected into the FIA system (Fig. 2.3) and absorbance was measured at 750 nm.

From the results (Fig 3.17, Fig.3.18, Table 3.9 and Table 3.10) it was found that sulfide affected the phosphate determination when using either tin(II) chloride or ascorbic acid, but more pronounced with the ascorbic acid system. Sulfide did not interfere the determination of phosphate when using tin(II) chloride as a reducing solution until the concentration of sulfide was more than 5 mg l^{-1}. It was considered to interfere when it gave change of more than 10% of the average signal. And the

interference of sulfide will affect the low phosphate at lower concentration more than that of a high concentration of phosphate. In ascorbic acid system, only 0.5 mg l^{-1} sulfide would affect the peak signal of phosphate as indicated in Fig. 3.18 and Table 3.10. Ascorbic acid is one of the most frequently used reducing solutions for phosphate determination by molybdenum blue method as it is convenient to prepare a solution, non-toxic and cheaper. So further development was made by using ascorbic acid system.

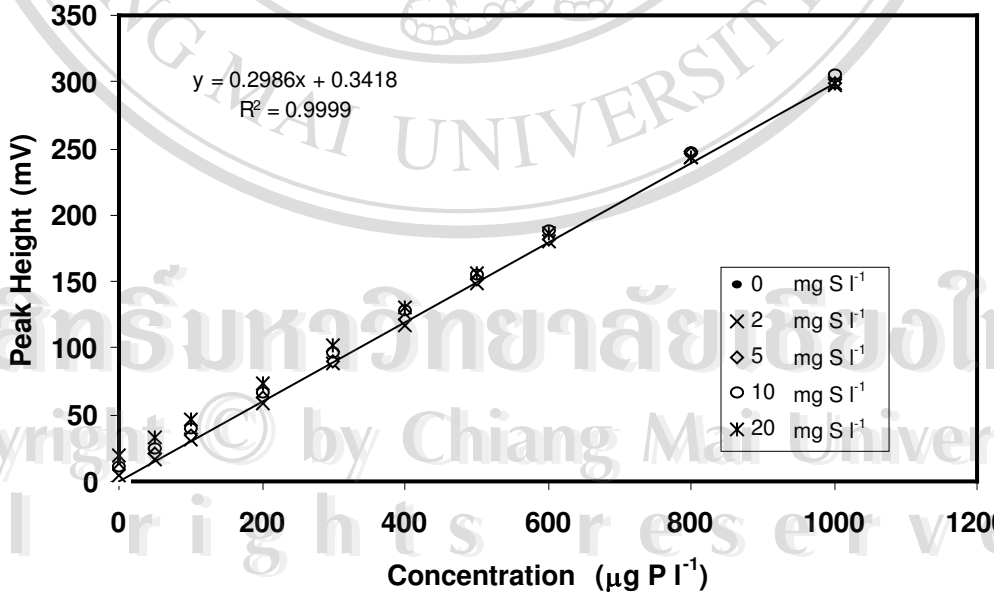


Figure 3.17 The effect of sulfide on phosphate determination using tin(II) chloride as a reducing solution

Table 3.9 Percent change of orthophosphate standards in concentration range of 50-1000 μg l^{-1} which contained sulfide of various concentrations. Using tin(II)chloride as a reducing agent

Expected P μg l^{-1}	% Change of P in the present of sulfide (mg l^{-1})			
	2.0	5.0	10.0	20.0
50	11.4	35.7	57.9	114.1
100	3.7	14.7	29.3	54.0
200	-2.5	5.5	8.9	22.0
300	-1.9	0.8	6.9	13.1
400	-1.8	1.0	6.2	8.9
500	-1.0	1.3	3.7	4.8
600	0.6	1.3	4.3	4.1
800	1.6	2.8	2.8	1.8
1000	-0.3	0.1	2.1	-0.1

* % Change = (Calculated P/Expected P) × 100

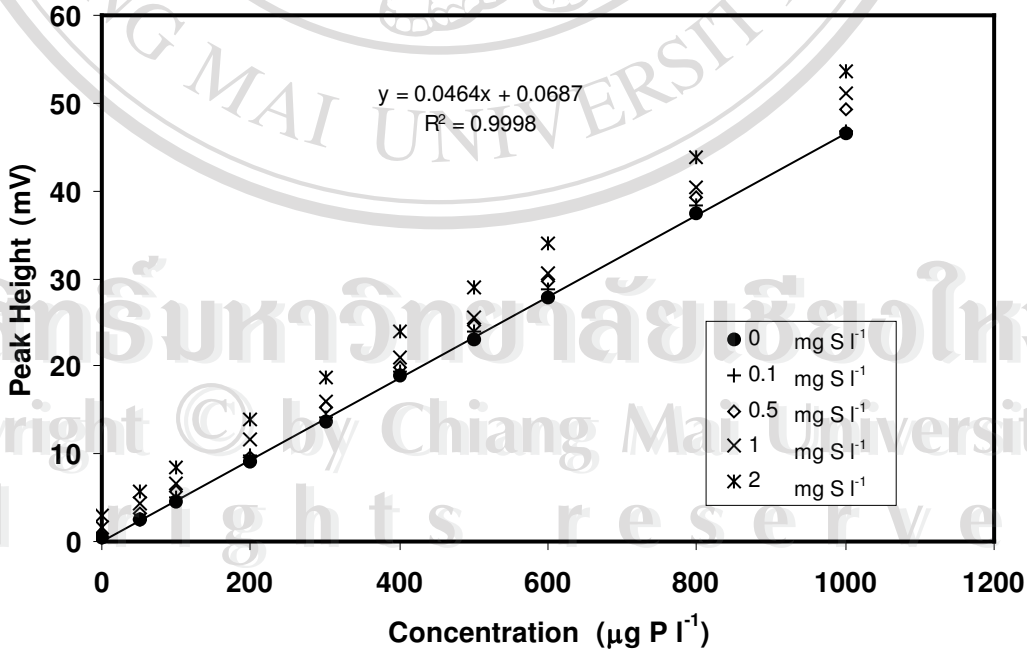


Figure 3.18 The effect of sulfide on phosphate determination using ascorbic acid as a reducing solution

Table 3.10 Percent change of orthophosphate standards in concentration range of 50-1000 μg l^{-1} which contained sulfide of various concentrations. Using ascorbic acid as a reducing agent

Expected P μg l^{-1}	% Change of P in the present of sulfide (mg l^{-1})			
	0.1	0.5	1.0	2.0
50	4.8	33.7	82.0	143.6
100	6.5	19.6	39.7	82.8
200	4.2	3.4	25.4	48.3
300	1.3	9.3	14.9	33.6
400	3.8	6.3	13.0	28.7
500	2.5	5.4	9.6	24.6
600	2.8	6.6	9.7	21.6
800	3.3	5.7	8.4	18.1
1000	0.8	5.8	9.9	15.3

3.4.3 Test effectiveness of batch acidifying to remove sulfide

To test the effectiveness of batch pretreatment to remove sulfide, 0.01 and 0.05 M sulfuric acid solution was used to acidify sulfide before determining of phosphate. For the experiments with 0.01 M sulfuric acid, 3 standard sets with phosphate concentrations of 0, 50, 100, 200, 500 and 1000 μg P l^{-1} were prepared. The first set contained only phosphate in 0.01 M sulfuric acid but without sulfide. The second and third sets contained of 2 and 5 mg S l^{-1}, respectively. The solutions were purged with nitrogen gas for 15 minutes to expel hydrogen sulfide before injecting into the FIA system with 0.01 M sulfuric acid as a carrier. Similarly, the experiments with 0.05 M sulfuric acid were performed.

The results in Fig. 3.19, Fig. 3.20, Table 3.11 and Table 3.12 show that to remove sulfide concentration up to 5 mg l^{-1} from phosphate standards effectively, the concentration of acid used must be more than 0.01 M. A 0.01 M sulfuric acid solution cannot remove sulfide effectively (% change of P is in the range of ±10). A 0.05 M sulfuric acid in final solution can remove sulfide effectively (% change of P is in the range of ±10) under the used conditions.

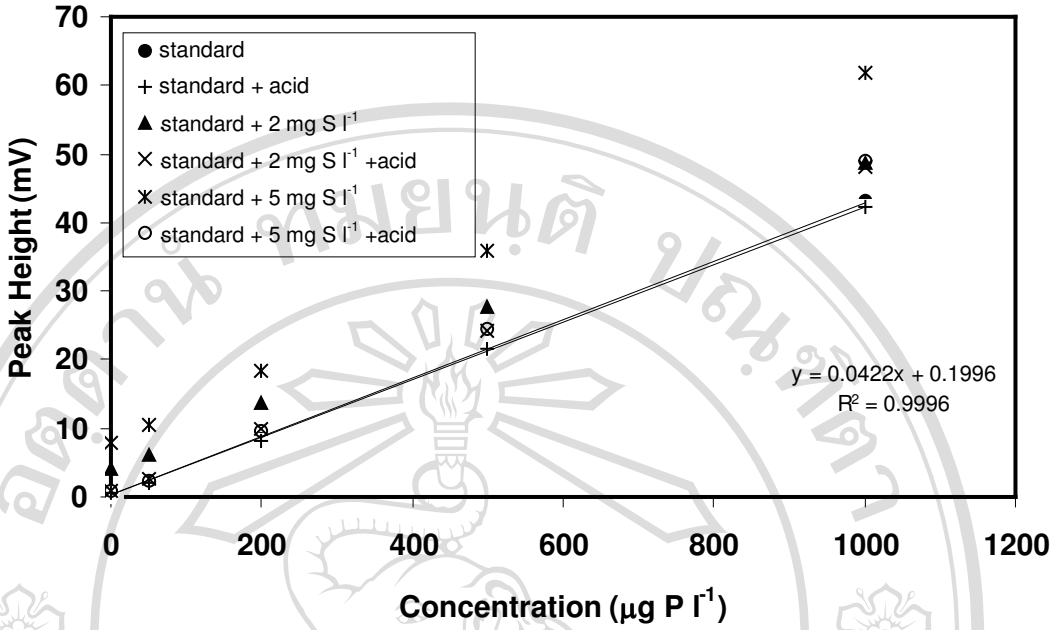


Figure 3.19 Acidification of sulfide in phosphate standard using 0.01 M sulfuric acid

Table 3.11 Test of the effectiveness of batch pretreatment to remove sulfide using 0.01 M sulfuric acid

Expected P	% Change of P			
($\mu g\ l^{-1}$)	2 mg S l^{-1}	2 mg S l^{-1} + acid	5 mg S l^{-1}	5 mg S l^{-1} + acid
50	178.5	15.7	387.3	7.1
200	57.9	14.0	112.4	11.3
500	28.2	14.1	67.0	15.2
1000	13.3	13.6	43.9	15.6

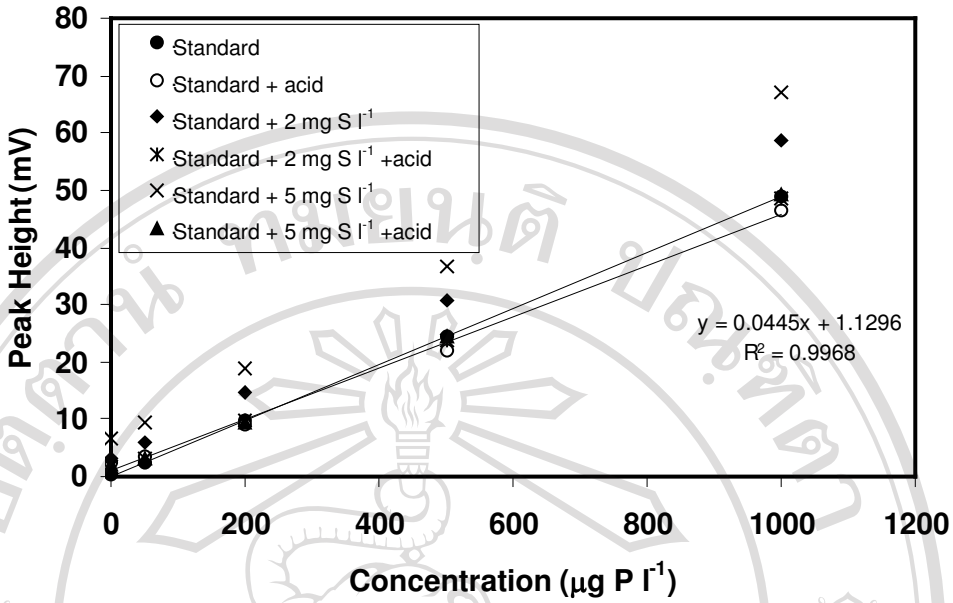


Figure 3.20 Acidification of sulfide in phosphate standard using 0.05 M sulfuric acid

Table 3.12 Test of the effectiveness of batch pretreatment to remove sulfide using 0.05 M sulfuric acid

Expected P	% Change of P			
($\mu g\ l^{-1}$)	2 mg S l^{-1}	2 mg S l^{-1} + acid	5 mg S l^{-1}	5 mg S l^{-1} + acid
50	120.7	-5.6	275.7	-9.2
200	50.9	-4.4	98.2	-6.5
500	33.7	2.2	60.5	5.0
1000	29.0	6.6	48.5	7.8

3.4.4 On-line oxidation of sulfide using potassium permanganate

As sulfide is a reducing agent, the easy way to remove sulfide from the sample is to use some oxidizing agent to oxidize sulfide on-line. Potassium permanganate was proposed as an on-line oxidizing solution in a new method as it is easily available, cheap and less toxic comparing to other oxidants.

The manifold was similar to the one previously described but potassium permanganate was used as carrier and the MC1 being 60 cm instead of 30 cm (to ensure that the oxidation of sulfide is nearly complete). The flow rate of potassium permanganate, acid molybdate and ascorbic acid were 2.0, 0.9 and 0.9 ml min^{-1}, respectively. The optimum concentration of potassium permanganate was 0.01 M. To test the method, two sets of phosphate standards were prepared by diluting the 5 mg P l^{-1} intermediate solution to the final concentration of 0, 50, 100, 200, 500 and 1000 μg P l^{-1}. The first set was used as control solutions. The second set, each solution was added with 200 mg S l^{-1} solution to become the final concentration of 5 mg S l^{-1}. All the solutions were injected into the FIA system.

The phosphate standards, which contained 5 mg l^{-1} sulfide, were used to test the method. Fig. 3.21 and Table 3.13 show that potassium permanganate can remove sulfide from phosphate standard effectively as the two calibration graphs were nearly the same and % change of P is in the range of ±10. The linearity of the calibration was determined using six standards for the range 0-1000 μg l^{-1}. The linear regression equation (using different data from Fig. 3.21) obtain was

peak height (mV) = 0.0409[P(μg l^{-1})] + 0.2169, r^2 = 0.9998 --------- 3.10

The relative standard deviation (n = 3) over this range varied from 0.3-12.4%. The detection limit of 17.3 μg l^{-1} was calculated [52].

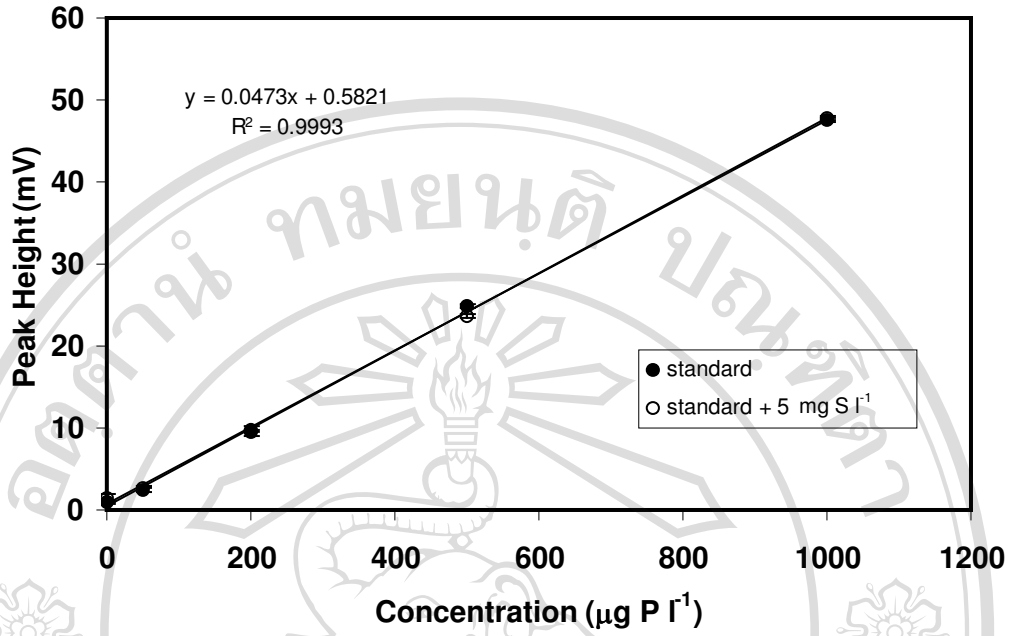


Figure 3.21 On-line oxidation of sulfide using potassium permanganate

Table 3.13 On-line oxidation of sulfide using potassium permanganate

Expected P µg l^{-1}	S^{2-} Added mg l^{-1}	Apparent P µg l^{-1}	% Change of P
50	5	45.6	-8.8
200	5	191.3	-4.4
500	5	489.6	-2.1
1000	5	996.8	-0.3

The on-line oxidation of sulfide with potassium permanganate is very simple. By only changing from water carrier to 0.01 M potassium permanganate and increasing the length of the first mixing coil to 60 cm, the method can work effectively. There was no effect from the color of potassium permanganate as it can react with excess ascorbic acid and give a clear solution before the absorbance was measured. A 2% (W/V) ascorbic acid was sufficient to reduce both phosphomolybdic acid and potassium permanganate.

3.4.5 Test for organic phosphate

Five species of organic phosphate; phytic acid, phenyl phosphate, adenosin-5-monophosphate, glycerophosphate and 2-aminoethyl phosphonic acid, were used to test for the oxidation power of potassium permanganate solution. Each was100 mg l^{-1}.

The Fig. 3.22 shows that the peak signal from organic phosphate solutions did not come from the oxidation of organic phosphate but from the impurity of the chemical used. The net peak height of all organic phosphates in the water carrier system and potassium permanganate system are not significantly different. So no significant interference was observed due to 100 mg l^{-1} of phytic acid, phenyl phosphate, adenosin-5-monophosphate, glycerophosphate and 2-aminoethyl phosphate.

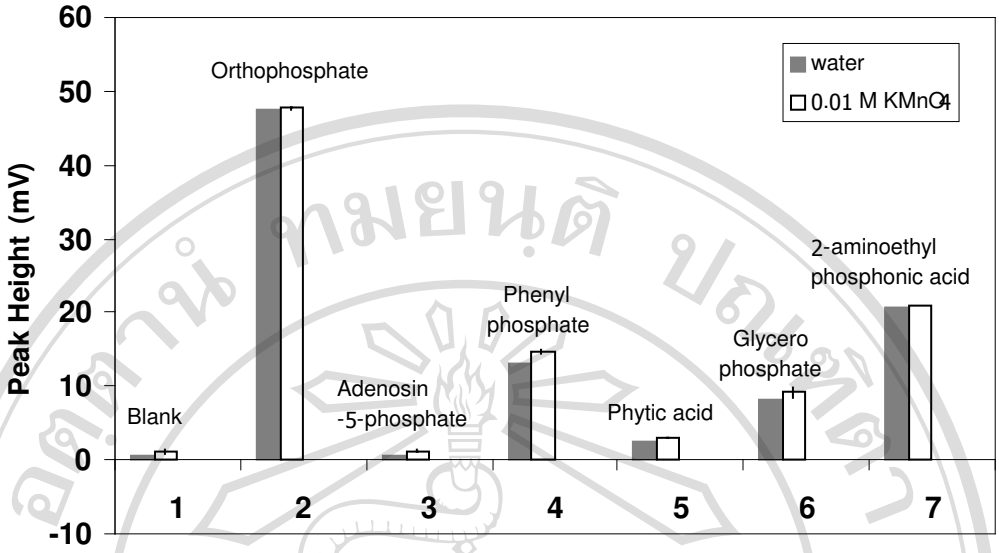


Figure 3.22 Test of the oxidation potential of potassium permanganate on organic phosphates (each of 100 mg l^{-1}) comparing to orthophosphate (1mg l^{-1} - **one hundredth of the concentration** of the organic phosphate species) (see the table below)

100 mg l^{-1} organic phosphate	Peak height (mV)		net peak height (mV)	Peak height comparing to the peak of 1 mg l^{-1} orthophosphate (%)
	A	B	C	
Blank	0.46	1.13	0.67	1.4
1 mg l^{-1} Orthophosphate	47.63	47.73	-	-
Adenosin-5-phosphate	0.53	1.22	0.69	1.4
Phenyl phosphate	13.12	14.7	1.58	3.3
Phytic acid	2.49	3.11	0.62	1.3
Glycerophosphate	8.17	9.14	0.97	2.0
2-aminoethyl phosphonic acid	20.63	20.97	0.34	0.7

A = using water as a carrier
B = using 0.1 M $KMnO_4$ as a carrier
C = B-A

3.4.6 Method validation and sample determination

The proposed method of on-line oxidation of sulfide for phosphate determination was applied to 8 samples of sediment water, which were spiked with sulfide 4 mg l^{-1}. The results (Table 3.14) were compared with the batch acidifying

method discussed above and a conventional FIA method [42]. Studies on recovery were made by adding 455 μg P l^{-1} into the sediment water samples.

It was found that the results from the proposed method were in good correlation with the acidifying FI method by t test (t = 1.3689 at 95% of confidence), but significantly different from the conventional FI method (Table 3.14). As there was no sulfide removal step in the conventional FI method, the phosphate concentrations obtained by this procedure were higher than the others. Table 3.15 shows the percent recovery of the spiked phosphate in sediment water samples. The recoveries were in the range of 93-104%.

The proposed method can be applied well to water samples, which contained high concentration of sulfide.

Table 3.14 Samples determination using potassium permanganate method and comparison of the results to the other methods. All samples were spiked with 4 mg l^{-1} sulfide

Samples	P found (μg l^{-1})		
	Conventional FI method*	Acidifying FI method	$KMnO_4$ FI method
BB21	52	45	44
BB22	239	189	172
BB23	367	324	316
BB24	497	449	436
BB25	498	441	461
BB26	555	458	501
BB27	556	471	501
BB28	518	433	473

* Without sulfide removal

Table 3.15 Recoveries of spiked dissolved reactive phosphorus to water samples. Using potassium permanganate method. These data were computed from the calibration equation: $y = 0.0409x-0.2169$, $r^2 = 0.9998$

Samples	Concentration (µg P l^{-1})		%Recovery* (d)
	Added	Found	
BB12	-	126[a]	94
	455[b]	552[c]	
BB13	-	307[a]	93
	455[b]	729[c]	
BB14	-	354[a]	99
	455[b]	801[c]	
BB15	-	345[a]	99
	455[b]	795[c]	
BB16	-	381[a]	101
	455[b]	839[c]	
BB17	-	380[a]	100
	455[b]	833[c]	
BB18	-	396[a]	104
	455[b]	870[c]	

* %Recovery (d) = [(c-a)/b] x 100

3.5 Simultaneous determination of phosphate and silicate using stopped FIA

The proposed method for simultaneous determination of phosphate and silicate is based on the formation of molybdenum blue complex as the following reactions [33].

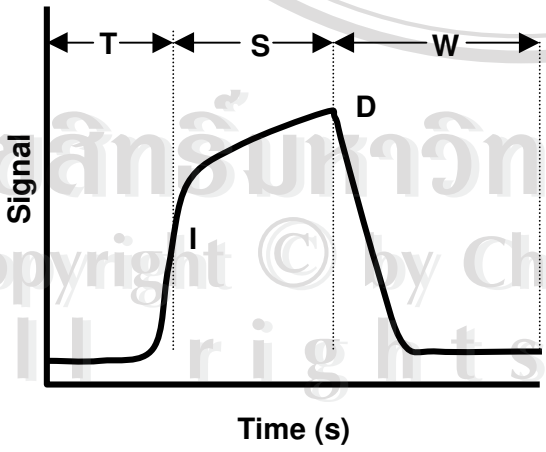
PO_4^{3-}, SiO_3^{2-} + Acidic molybdate $\rightarrow$ Heteropoly acid --------- 3.11

Heteropoly acid + Ascobic acid $\rightarrow$ Molybdenum blue ------- 3.12

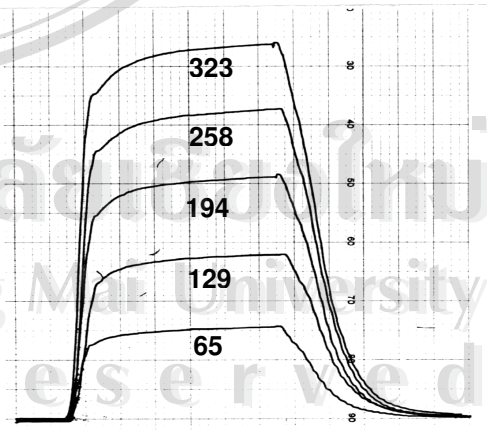
The rate of reaction due to phosphate is faster than that of silicate [48]. Simultaneous determination of phosphate and silicate using molybdate reagent for the kinetic characteristic differentiation by stopped FIA was then investigated. In this work the semi-automated stopped-FI-Analyzer (Fig. 2.4) was employed. Signal profiles are illustrated in Fig. 3.23. Via the controller, presetting can be made for travelling time (T), the period of flow between the point of injection to the point at which the flow is stopped so that the reaction development can be monitored at the flow-cell; stopping time (S), the period during the flow stopped; washing time (W), the period when the stream is re-started to flow (after stopping) until this operation cycle ends and ready to start for the next cycle. I and D, the signals at the first and last stopped points, respectively will be given as digital read-outs on the photometer. Figs. 3.23 (b)-(d) demonstrate stopped-FI-grams of phosphate, silicate and mixtures of the standard solutions. The conditions employed are summarized in Table 3.16.

Table 3.16 Conditions for simultaneous determination of phosphate and silicate using the semi-automated stopped-FI-Analyzer

Condition	Value
Molybdate concentration, M (in 1.3% (V/V) nitric acid)	0.025
Ascorbic acid concentration, % w/v	0.5
Injection volume, µl	35
Mixing coil length, cm	50
Flow rate (each line), ml/min	3.2 ± 0.2
Travel time, s	2.5
Stop time, s	20
Wash time, s	15
Light source (LED)	Red



(a)



(b)

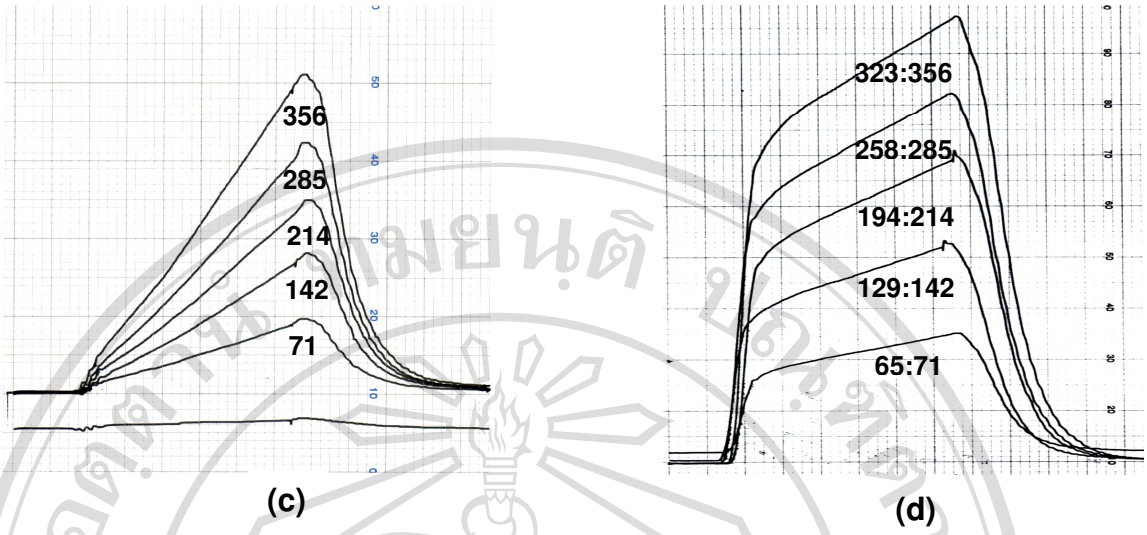


Figure 3.23 Stopped-FI profiles obtained (a) in general, (b) phosphate, (c) silicate, (d) mixture of phosphate and silicate: T = travelling time, S = stop time, W = washing time, I = signal at the first stopped point, D = signal at the last stopped point before going, the numbers refer to concentrations (mM) as the ratio of phosphate and silicate

3.5.1 Simultaneous determination of phosphate and silicate

Employing the conditions summarized in Table 3.16, and the criteria of I and D for simultaneous determination of phosphate and silicate, the calibration plots were obtained as shown in Fig. 3.24 and 3.26.

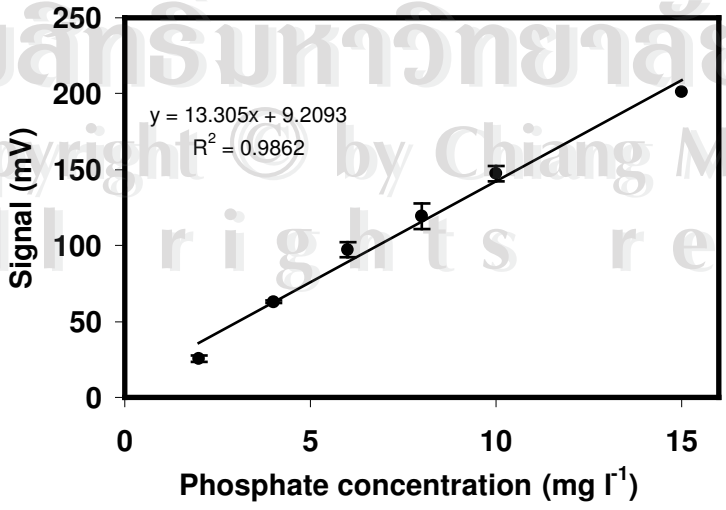


Figure 3.24 Calibration graph of phosphate

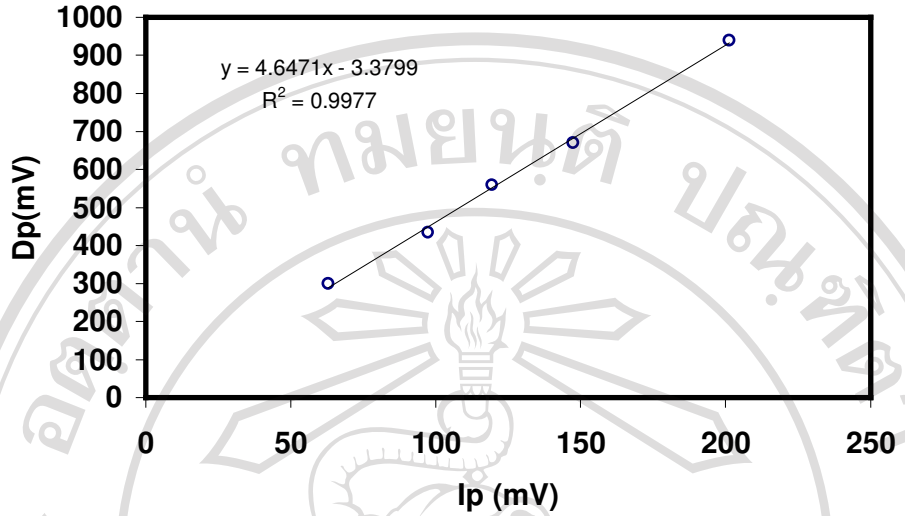


Figure 3.25 The correlation between I_p and D_p

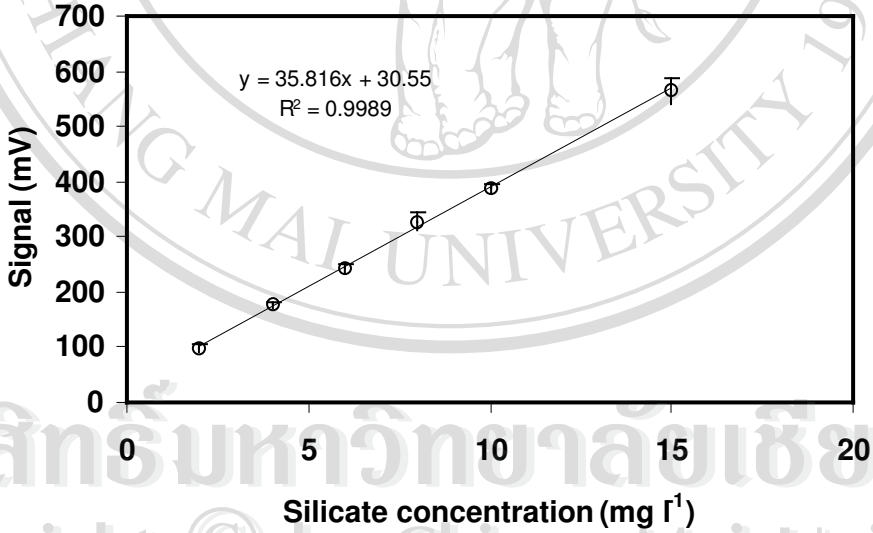


Figure 3.26 Calibration graph of silicate

From the calibration graphs it will be obtained that:

Phosphate standard (up to 15 mg Pl^{-1}), $I_p = 13.31[P] + 9.20$, $r^2 = 0.986$, ---- 3.13

Correlation of D_p (mV) and I_p (mV), $D_p = 4.65I_p - 3.38$, $r^2 = 0.998$, ----- 3.14

Silicate standard (up to 15 mg Si l^{-1}), $D_{Si} = 35.82[Si] + 30.55$, $r^2 = 0.999$, ---- 3.15

The concentration of phosphate in a mixture or a sample is obtained from the calibration plot of phosphate standards (equation 3.13) by using I_{mix} from the mixture or the sample for I_p (as at the first stopped point, there should be no contribution due to silicate). Then D_p is calculated from equation 3.14. In the single profile of the mixture/sample, calculation for signal due to silicate only at the last stopped point, $D_{Si,mix}$ is made from the correlation:

$$D_{Si,mix} = (D_{mix} - D_{P,\,mix}) + 0.996\, I_p \quad \text{----------} \quad 3.16$$

$D_{P,\,mix}$ is the contributed signal due to phosphate in the mixture and can be evaluated from the equation 3.14. The last term ($0.996I_P$) is for correction. Substituting the value of $D_{Si,mix}$ for D_{Si} in the equation 3.16 and obtains the concentration of silicate in the mixture /sample.

3.5.2 Sample determinations and method validation

The method was applied to 5 water samples and phosphate spiked water samples, with sampling from natural source of water. The results are compared to these obtained by the standard method [50], as summarized in Table 3.17.

Table 3.17 Determination of phosphate and silicate by the proposed stopped-FI method and the standard method

Water sample	Phosphate content (mg P l^{-1})		Silicate content (mg Si l^{-1})		Phosphate added (mg P l^{-1})	% recovery of phosphate**	
	stopped-FI method (A)	standard method (B)	stopped-FI method	standard method	(C)	stopped-FI method (D)	standard method (E)
Reservoir	n.d.(1)	n.d.(1)	8.2	8.6	-	-	-
	1.9(2)	1.8(2)	9.4	8.7	2.0	95	90
Pond	n.d.(1)	n.d.(1)	7.6	7.9	-	-	-
	4.1(2)	3.5(2)	7.5	8.0	4.0	102	88
Irrigation	n.d.(1)	n.d.(1)	8.3	8.2	-	-	-
Canal	6.9(2)	5.7(2)	7.8	8.3	6.0	115	95
Moat	n.d.(1)	n.d.(1)	8.1	9.0	-	-	-
	8.7(2)	7.9(2)	7.3	8.9	8.0	109	99
Drainage	1.1(1)	1.2(1)	11.9	13.9	-	-	-
(from a	3.5(2)	3.1(2)	11.2	13.9	2.0	120	95
dormitory)							

** % recoveries evaluated by: D = (A(2)-A(1)/C)x 100 and E = (B(2)-B(1)/C)x 100

From t-test it was found that the results of phosphate and silicate contents by both methods were in good correlation (t = 0.11417 at 95% of confidence for phosphate and 0.115 for silicate).