# CHAPTER 3

# RESULTS AND DISCUSION

This work was to develop an amperometric determination of trace phosphate by enhancive the sensitivity and selectivity employing FI with pretreatment (in-valve) column. The first part was to study cyclic voltammetry involving the molybdenum blue reactions, the second part was development of FI-amperometric set-up and the last part involved the FI set-up with pretreatment (in-valve) column.

The reactions are based on :

$$PO_4^{3-} + \text{Acidic molybdate} \rightarrow \text{12-phosphomolybdate complex} \quad \text{------(1)}$$

$$\text{12-phosphomolybdate complex} + \tilde{e} \rightarrow \text{Molybdenum blue} \quad \text{------(2)}$$

$$\text{or for step (2):} \quad Mo(VI) + \tilde{e} \rightarrow Mo(V) \quad \text{------(3)}$$

Usually, ascorbic acid or $SnCl_2$ is employed for the reduction in an acidic medium, in the step (2). The reaction product (molybdenum blue) is usually detected spectrophotometrically (at 660 nm). Silicate and arsenate may interfere due to their similar reaction products which have similar absorption spectral characteristics.

Electroanalytical techniques such as voltammetry or amperometry may be an alternative for the phosphate determination using the same chemistry but the reaction products (the molybdate complexes of phosphate, silicate and arsenate) may have different electrochemical properties.

### 3.1 Voltammetric study

Using the conditions observed in 2.3.1, solutions containing phosphomolybdate containing orthophosphate of 0, 5, 10, 15, 20 and 25 ppm $PO_4$-P in 0.1 M KCl electrolyte solution, 0.5% (w/v) ammonium molybdate in 2.5% (V/V) $H_2SO_4$ were investigated.

### 3.1.1 Cyclic voltammetry of $PO_4^{3-}$

Cyclic voltammograms (Figure 3.1) were obtained. Scanning with cathodic direction, reduction peaks at 220 mV (versus Ag/AgCl) were observed. The peak currents were proportional to the phosphate concentrations ($y = 8x10^{-9}+3x10^{-8}$, $R^2 = 0.9991$). With anodic direction scanning, a peak potential of 240 mV was found. Peak current was also proportioned to the orthophosphate concentration ($y = 7x10^{-9}+3x10^{-8}$, $R^2 = 0.9978$). The current changes could be to the reaction of Mo(VI) ➔ Mo(V) [46]

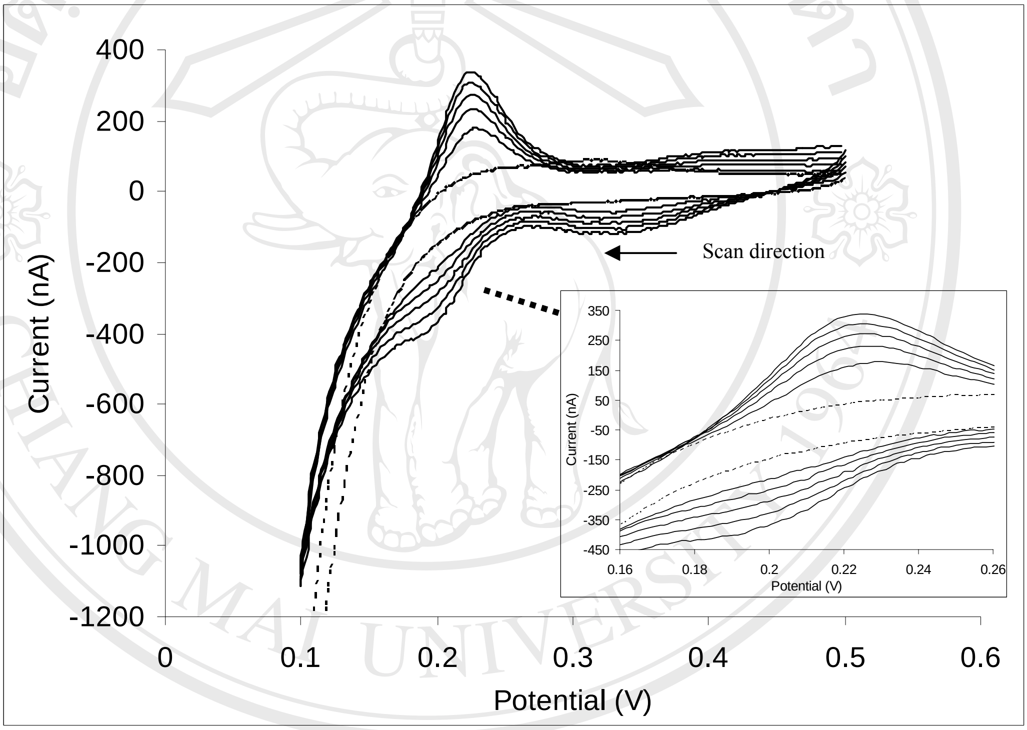

**Figure 3.1** Cyclic voltammogram orthophosphate of 0, 5, 10, 15, 20 and 25 ppm $PO_4$-P ( potential scan : start from 0.5 V to cathodic direction to 0.1 V and back to 0.5 V) .

### 3.1.2 Batchwise amperometric standard calibration graph and peak profiles of orthophosphate

From the cyclic voltammetry study in section 2.3.1 and 3.1.1, batch wise experiment for amperometric determination of phosphate was studied: applied potential (220 mV versus Ag/AgCl), electrolytic (0.1 M KCl) and reagent 0.5 % (w/v) acidic molybdate in 2.5 % (v/v) $H_2SO_4$. A phosphate standard solution (1000 ppm) was added for an increment of 50 µl to result in 5, 10, 15, 20 and 25 ppm $PO_4$-P Figure 3.2 represents amperogram while Figure 3.3 shows the calibration graph. A detection limit [64] was found to be 2.6 ppm $PO_4$-P.

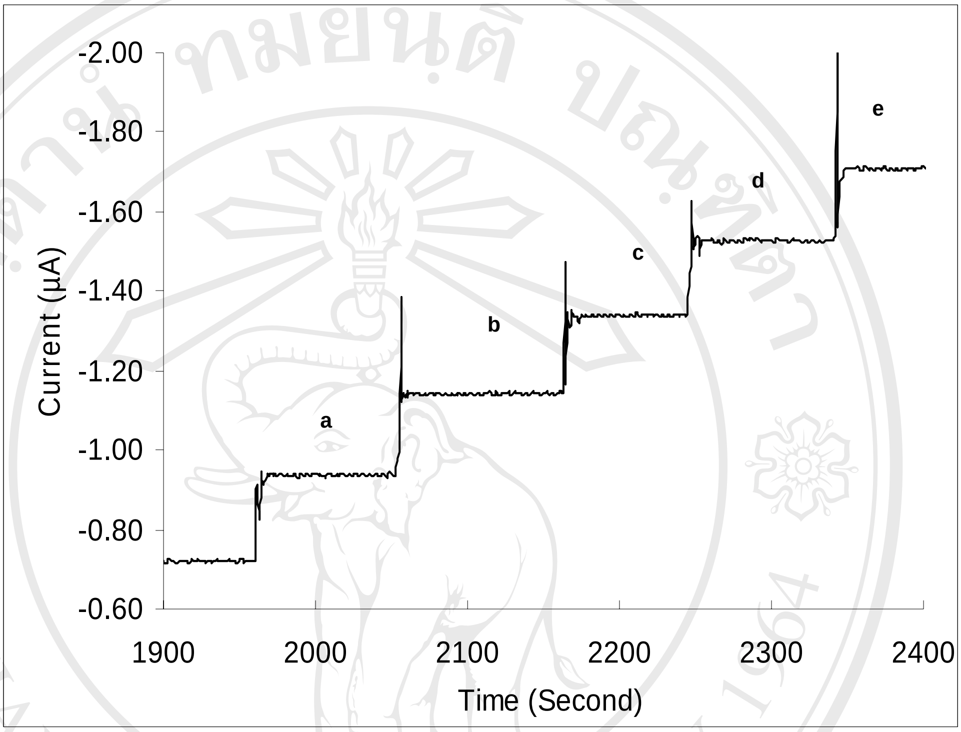

**Figure 3.2** Batchwise amperometric profiles for determination of orthophosphate; addition of phosphate solution into 0.1 M KCl to obtain (a) 5 ppm, (b) 10 ppm, (c) 15 ppm, (d) 20 ppm and (e) 25 ppm $PO_4$-P in final solution. Current measurement on glassy carbon electrode with applied potential at 220 mV versus Ag/AgCl.

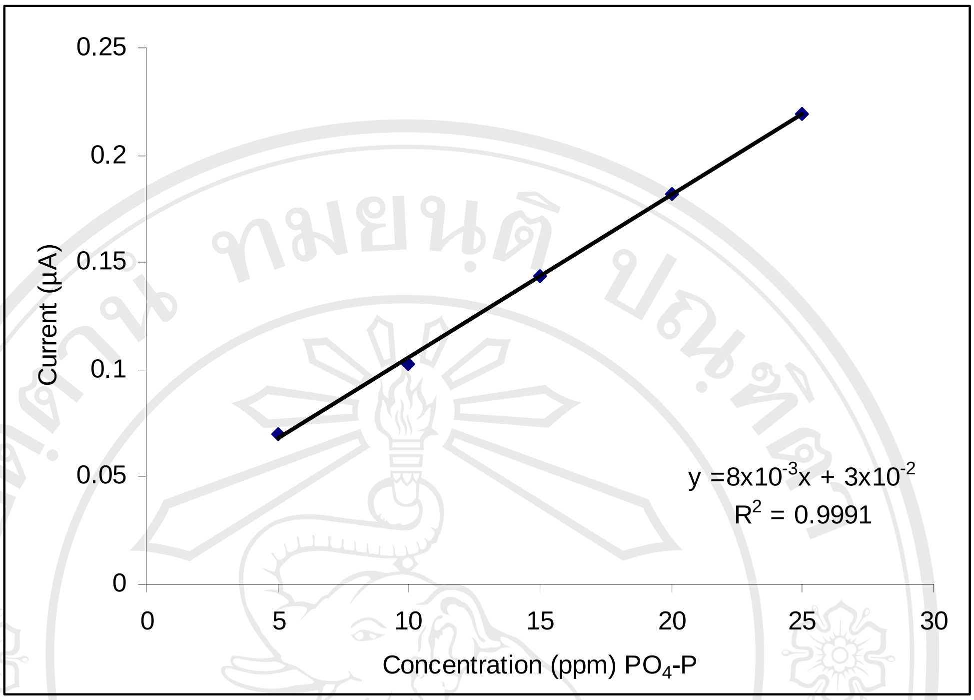

**Figure 3.3** Calibration graph of standards $PO_4$-P (glassy carbon electrode with applied potential at 220 mV versus Ag/AgCl).

### 3.1.3 Cyclic voltammetry $SiO_3^{-2}$

Under the same conditions but with silicate (0, 5, 10, 15, 20 and 25 ppm $SiO_3^{-2}$) instead of phosphate, cyclic voltammograms in Figure 3.4 were obtained. No peak due to any reactions was obtained. Currents in the range of 200-500 mV (versus Ag/AgCl) were observed to be very low. This indicates that silicate would not give any interference in the orthophosphate determination using the above conditions.

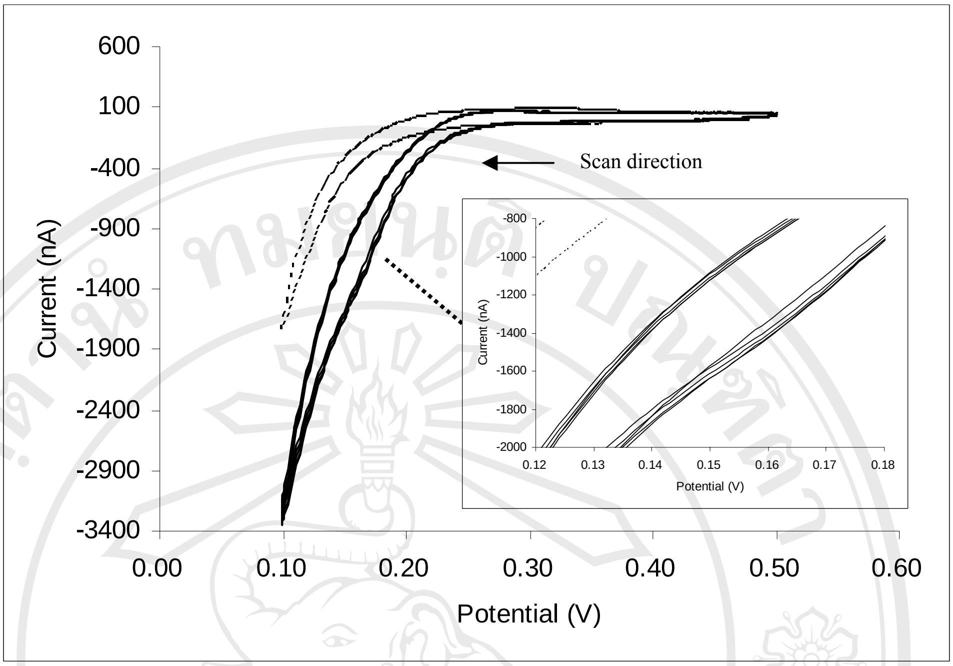

**Figure 3.4** Cyclic voltammogram of silicate of 0, 5, 10, 15, 20 and 25 ppm $SiO_3^-$

### 3.1.4 Cyclic voltammetry of $AsO_4^{3-}$

Similarly, investigation for arsenate was made. Cyclic voltammograms are represented in Figure 3.5. Peak potentials were observed at 250 and 450 mV (Ag/AgCl). The current peaks were proportional to the arsenate concentrations. This indicated that interference due to arsenate may be observed in the determination of phosphate under the employed condition. Figure 3.6 shows the overlaid cyclic voltammogram due to reagent blank, 25 ppm $PO_4$-P and 25 ppm arsenate. Although it indicated the interference due to the arsenate to the determination of $PO_4$-P but arsenate contents in natural water are usually very low comparing to the phosphate contents[62-63]. In real application, the interference from arsenate to the determination of phosphate under the conditions should be negligible.

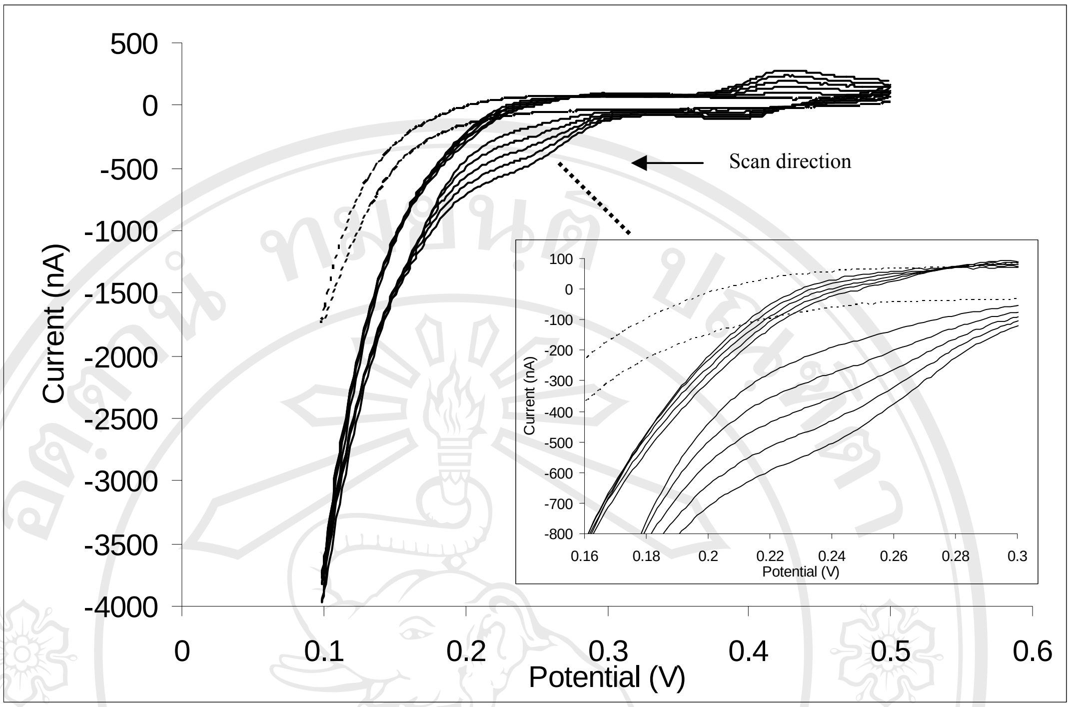

**Figure 3.5** Cyclic voltammogram of arsenate of 0, 5, 10, 15, 20 and 25 ppm $AsO_4^{3-}$

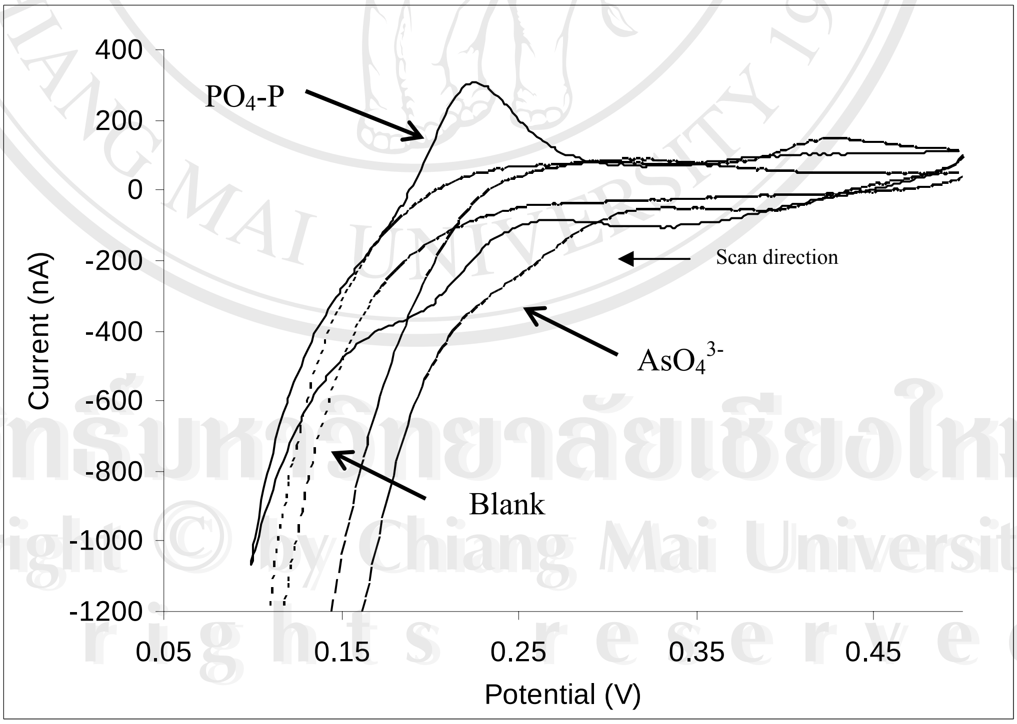

**Figure 3.6** Cyclic voltammograms of $PO_4^{3-}$ (25 ppm) and $AsO_4^{3-}$ (25 ppm) standards solution.

## 3.2 FI-amperometric system for determination of orthophosphate

### 3.2.1 FI-amperometric system for determination of orthophosphate

Employing the FI-amperometric set-up illustrated in Figure 2.2, a sample/standard was injected via an injection valve (V) into a stream of 0.1 M KCl and merge with a stream of 0.5 % (w/v) acidic molybdate in 2.5 % (v/v) $H_2SO_4$ before flowing into a mixing coil and a flow-through cell of an amperometric detector (D) hooking up with a computer (C) for signal/data acquisition.

A set of conditions (in Table 3.1[50]) was followed.

**Table 3.1** The selected conditions for the FI-amperometric system

| **Parameter** | **Condition** |
|---|---|
| Electrode | Glassy carbon electrode |
| Mixing coil | 50 cm |
| Flow rate | |
| -KCl (0.1M) | 2.0 ml/min |
| -Acidic molybdate 0.5 % (w/v) in 2.5% (v/v) $H_2SO_4$ | 0.9 ml/min |
| Potential applied | 220 mV |
| Sample volume | 100 µl |

### 3.2.2 Effect of applied potential

Employing the set of conditions in Table 3.1 but varying applied potentials (210, 220 and 230 mV) for a set of phosphate standards (0.1, 0.4, 0.8 and 1.2 ppm $PO_4$-P). The peak current results are summarized in Table 3.2 and figure 3.7.
The conditions with the applied potential of 220 mV (versus Ag/AgCl) resulted in the highest slope (i.e. the best sensitivity). It was then chosen to be an applied potential for further work.

**Table 3.2** Effect of applied potential

| Concentration of $PO_4$-P (ppm) | Peak current (nA) at applied potential of (n=2) | | |
|---|---|---|---|
| | 210 mV | 220 mV | 230 mV |
| 0.1 | 39.38 | 104.84 | 30.51 |
| 0.4 | 90.19 | 295.58 | 65.01 |
| 0.8 | 210.74 | 586.58 | 196.70 |
| 1.2 | 313.58 | 881.98 | 285.93 |
| Equation | $y = 3\times10^2x + 3$ | $y = 7\times10^2x + 20$ | $y = 2\times10^2x - 8$ |
| $R^2$ | 0.9919 | 0.9991 | 0.9787 |

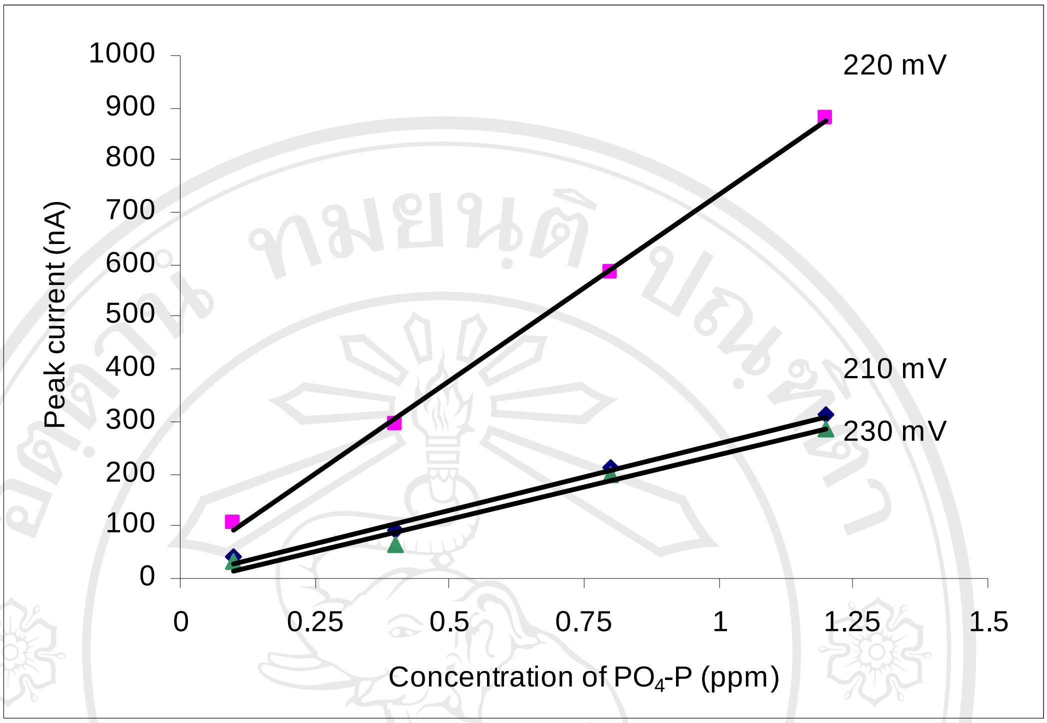

**Figure 3.7** Effect of applied potential

### 3.2.3 Effect of acidic molybdate concentrations

Using condition in Table 3.1 and Ep = 220 mV, effect due to concentration of molybdate solution was studied by employing phosphate standards (0.5 – 1.2 ppm $PO_4$-P). The results are summarized in Table 3.3. Figure 3.8 suggests that a slope of calibration obtained by employing molybdate of ≥ 0.4 % (w/v) becomes practically constant. A concentration of 0.5 % (w/v) was the chosen. A higher concentration may lead to precipitate.

**Table 3.3** Effect of acidic molybdate concentrations

| Concentration of $PO_4$-P (ppm) | Peak current (μA) for molybdate concentration of (n=2) | | |
|---|---|---|---|
| | 0.2 %(w/v) | 0.4 %(w/v) | 1 %(w/v) |
| 0.5 | 0.08 | 0.08 | 0.10 |
| 0.8 | 0.14 | 0.15 | 0.17 |
| 1.0 | 0.18 | 0.20 | 0.22 |
| 1.2 | 0.21 | 0.25 | 0.26 |
| Equation | $y = 1.85\times10^{-1}x - 7.91\times10^{-3}$ | $y = 2.32\times10^{-1}x - 3.14\times10^{-2}$ | $y = 2.33\times10^{-1}x - 1.57\times10^{-2}$ |
| $R^2$ | 0.9935 | 1.0000 | 0.9970 |

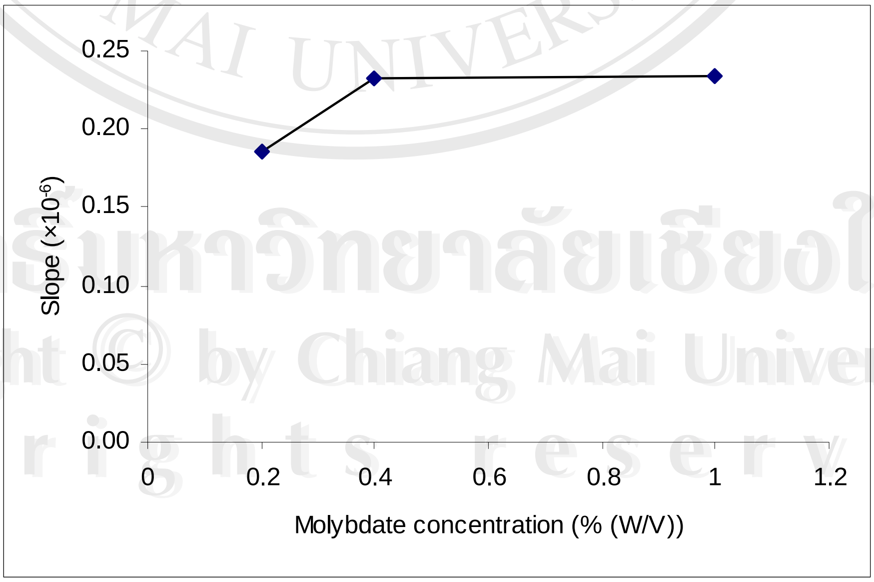

**Figure 3.8** Effect of molybdate concentration.

### 3.2.4 Precision

Precision of the FI amperometry under the condition described in 3.2.3 each phosphate standard (60 and 80 ppb). The result (estimation of the concentration via a calibration graph) are represented in Table 3.4. RSDs were found to be 4.8% and 7.0% for 60 and 80 ppb $PO_4$-P respectively.

**Table 3.4** Precision of 60 and 80 ppb of $PO_4$-P (n=7)

| Inject | 60 ppb $PO_4$-P | 80 ppb $PO_4$-P |
|---|---|---|
| | Concentration of $PO_4$-P (ppb) | Concentration of $PO_4$-P (ppb) |
| 1 | 59.0 | 82.4 |
| 2 | 63.9 | 86.5 |
| 3 | 66.8 | 85.6 |
| 4 | 61.7 | 74.1 |
| 5 | 61.0 | 72.0 |
| 6 | 60.8 | 84.4 |
| 7 | 58.1 | 81.3 |
| **Average** | 61.6 | 80.9 |
| **SD** | 2.9 | 5.7 |
| **RSD** | 4.8 | 7.0 |

### 3.2.5 Standard calibration graph of orthophosphate

Using the conditions described in 3.2.3, calibration graph was obtained in the range of 20 – 100 ppb $PO_4$-P. Figure 3.9 and 3.10 illustrate peak profiles and calibration graph respectively. A detection limit of 7 ppb was found.

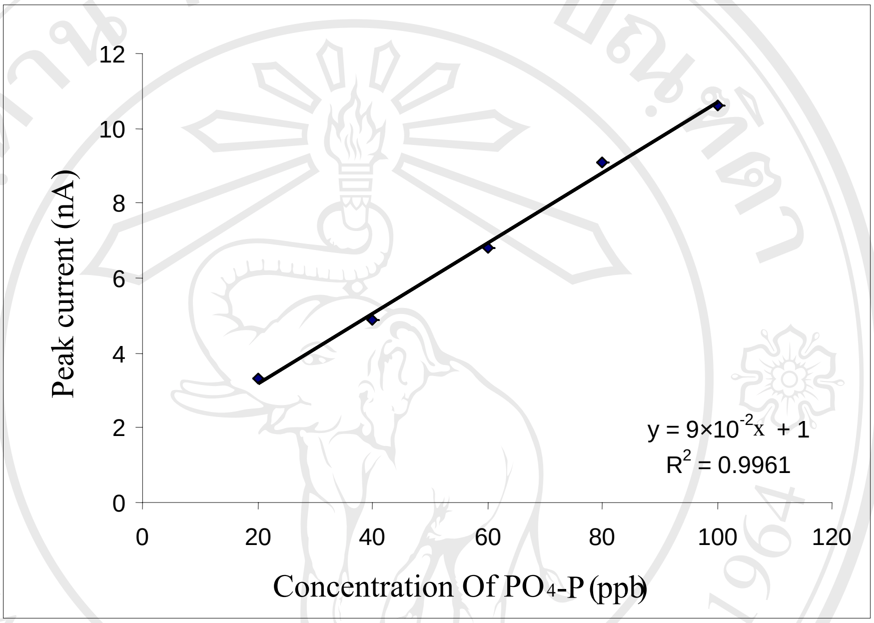

**Figure 3.9** Calibration graph of 20-100 ppb $PO_4$-P

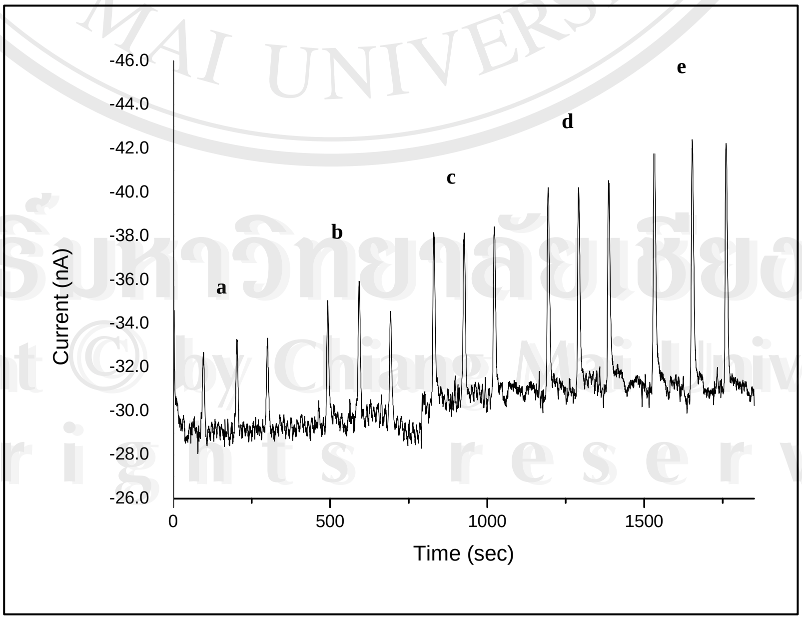

**Figure 3.10** Peak profile of $PO_4$-P (a) 20 ppb, (b) 40 ppb, (c) 60 ppb, (d) 80 ppb and (e) 100 ppb

### 3.3 FI-amperometric system with column preconcentration for determination of orthophosphate

Employing the FI-amperometric set-up (Figure 2.3) and the conditions described in Table 3.5 which were obtained from previous study with Sequence A of the operation. (Note: Sequence A: see Table 2.1)

**Table 3.5** The selected conditions for FI-amperometric system with preconcentration column for determination of orthophosphate

| **Parameter** | **Condition** |
|---|---|
| Electrode | Glassy carbon electrode |
| Mixing coil | 50 cm |
| Flow rate | |
| -Std/sample | 1.0 ml/min |
| -HCl (0.1M) | 1.0 ml/min |
| -Acidic molybdate 0.5 % (w/v) in 2.5% (v/v) $H_2SO_4$ | 0.6 ml/min |
| Applied potential | 220 mV |
| Sample volume | 100 µl |

### 3.3.1 Effect of eluent type

Two eleuent solutions, namely 0.1 M KCl and 0.1 M HCl were investigated for the FI-amperometric determination with pretreatment column, using the condition described in reaction 3.4 with the operation sequence A. Loading a phosphate standard (10, 30, 50 or 70 ppb $PO_4$-P) for 5 min at a flow rate of 1 ml/min. A set of peak profiles (in Figure 3.11) were obtained. A peak that of 0.1 M KCl is not in a good shape, although the height of the peaks is the same.

**Table 3.6** Effect of eluent type

| Concentration of $PO_4$-P (ppb) | Peak current (nA) (n=1) | |
|---|---|---|
| | HCl | KCl |
| 10 | 8.02 | 7.44 |
| 30 | 43.40 | 35.50 |
| 50 | 85.00 | 88.70 |
| 70 | 141.00 | 150.00 |
| Equation | y = 2x - 20 | y = 2x - 30 |
| $R^2$ | 0.9890 | 0.9754 |

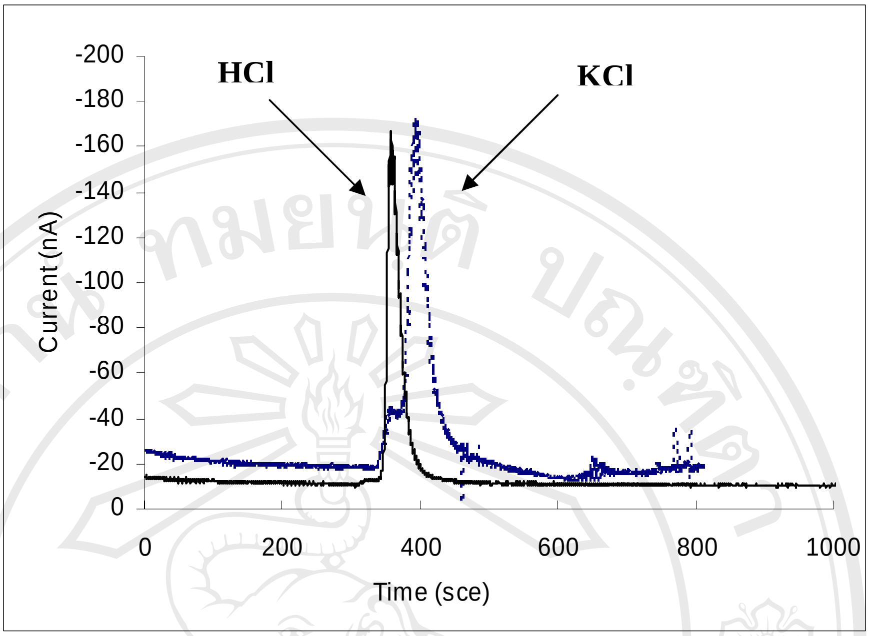

**Figure 3.11** Effect of eluent type (120 ppb $PO_4$-P)

### 3.3.2 Effect of sequence for regenerate column

Using the operation sequence A (as recommended by the resin supplier), it takes 30 min per run cycle. An operation sequence B (see Table 2.1) was introduced. A series of phosphate standard (10-30 ppb $PO_4$-P) was loaded for 5 min of a flow rate of 1 ml/min. A calibration graph, a plot of current (nA) versus concentration, employing the conditions with operation sequence B (see Table 3.7 and Figure 3.12) is compared to the one with the condition using the operation sequence A. Although sequence A provide a better slope, the condition with sequence B was chosen due to the less operation time and less reagent consumption.

**Table 3.7** Effect of sequence for regenerate column

| Concentration of $PO_4$-P (ppb) | Peak current (nA) (n=1) | |
|---|---|---|
| | (Sequence A) | (Sequence B) |
| 10 | 14.40 | 10.20 |
| 30 | 72.70 | 48.70 |
| 50 | 134.00 | 96.20 |
| 70 | 191.00 | 124.00 |
| Equation | y = 3x- 20 | y = 2x - 8 |
| $R^2$ | 0.9998 | 0.9904 |

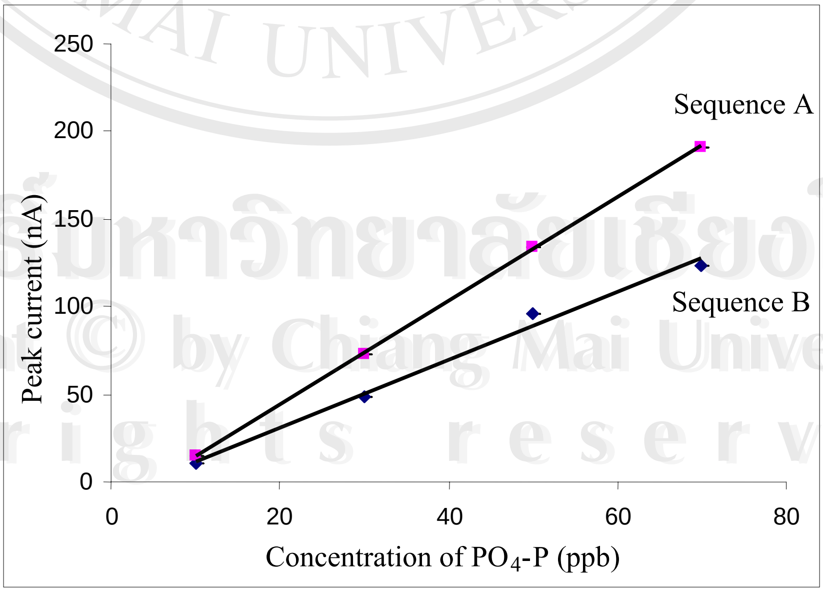

**Figure 3.12** Effect of sequence for regenerate column

### 3.3.3 Effect of resin type

Two types of resin, Dowex 1-X8 and Bio Rad AG1-X8 were investigated. Using the conditions described previously. The results using 5 ppb $PO_4$-P are represents in Figure 3.13. The peak due to Dowex 1-X8 is 3 times higher than that of Bio Rad AG1-X8. Dowex 1-X8, should be then suitable.

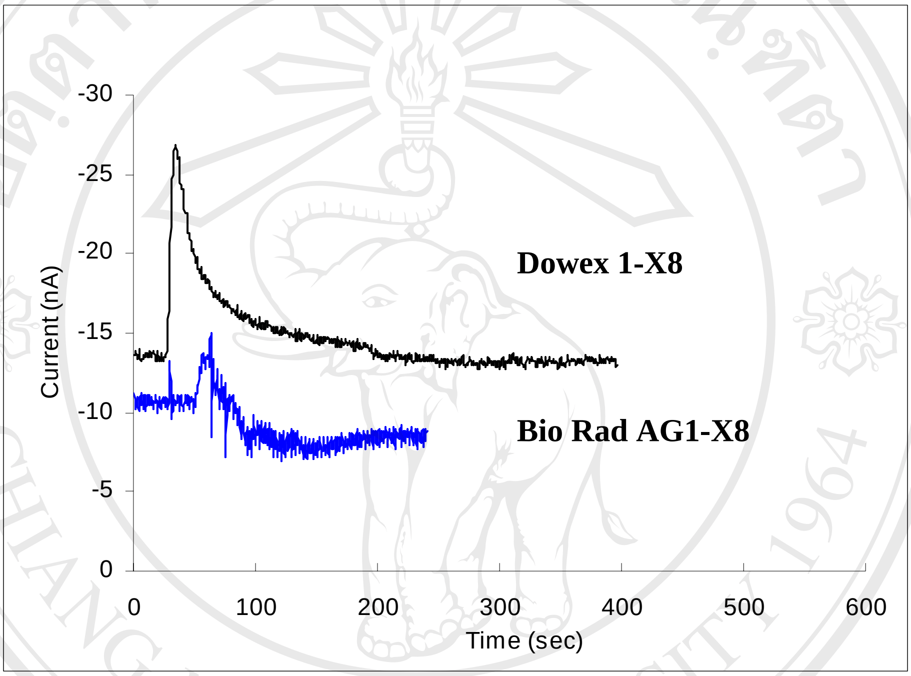

**Figure 3.13** Effect of resin type

### 3.3.4 Effect of time for regenerating column by using acetic acid

Using the conditions in 3.4.3 (Dowex 1-X8), a period after passing the 4 M HOAc though the column was varied 1, 2, 3 and 5 min. A series of phosphate standards (2-10 ppb $PO_4$-P) was tried. Slopes of calibration graph for the above periods, are represented in Figure 3.14 The longer period, the higher slope obtained until the period of 3 min. Slopes due the periods of 3 and 5 min are not different, i.e. sensitivity for the conditions with 3 and 5 min is the same. The period of 3 min for 4 M HOAc solution flowing though the column should be 90 times of the column volume (column volume = 33 µl)

**Table 3.8** Effect of time for regenerating column by using acetic acid

| Concentration of $PO_4$-P (ppb) | Peak area ($x10^{-7}$) (n=1) | | | |
|---|---|---|---|---|
| | 1 min | 2 min | 3 min | 5 min |
| 2 | 1.40 | 1.75 | 2.06 | 2.43 |
| 4 | 2.95 | 3.76 | 4.65 | 5.01 |
| 7 | 4.97 | 5.85 | 7.81 | 7.90 |
| 10 | 6.52 | 8.79 | 11.5 | 12.2 |
| Equation | $y = 0.6x + 0.3$ | $y = 0.9x + 0.1$ | $y = 1x - 0.2$ | $y = 1x + 0.04$ |
| $R^2$ | 0.9930 | 0.9952 | 0.9988 | 0.9940 |

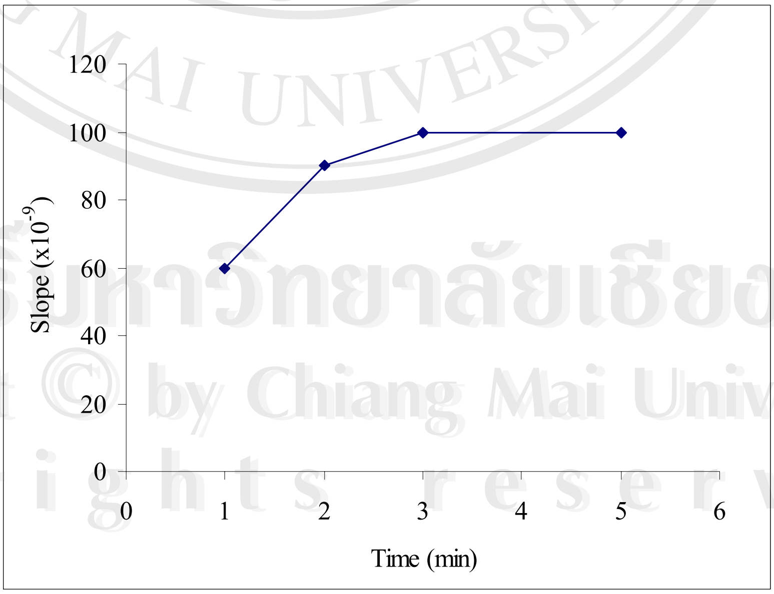

**Figure 3.14** Effect of time for regenerate column

### 3.3.5 Concentration of acetic acid for regenerate column

Using the condition described previously except the concentration of HOAc which was varied for 1-4 M. The results (in Table 3.9 and Figure 3.15) indicate that 4 M HOAc resulted in higher slope of calibration graph than that of the lower concentration and the calibration graph having $R^2$ approaching unity. A concentration of HOAc of higher than 4 M may affect the properties of the resin and it may take longer period to wash away HOAc from the column for the next run cycle. A solution of 4.2 M HOAc was then used for future experiment.

**Table 3.9** Concentration of acetic acid for regenerate column

| Concentration of $PO_4$-P (ppb) | Peak area ($x10^{-7}$) for concentration of HOAc of (n=1) | | | | |
|---|---|---|---|---|---|
| | 1 M | 2 M | 3 M | 4 M | 4.2 M |
| 2 | 4.88 | 5.76 | 8.41 | 8.75 | 8.52 |
| 4 | 5.18 | 5.95 | 8.81 | 13.2 | 12.84 |
| 7 | 5.67 | 7.01 | 9.26 | 20.2 | 19.62 |
| 10 | 6.43 | 7.16 | 12.1 | 25.2 | 26.35 |
| Equation | y = 0.2x + 4 | y = 0.2x + 5 | y = 0.4x + 7 | y = 2x + 5 | y = 2x + 4 |
| $R^2$ | 0.9843 | 0.9108 | 0.8324 | 0.9943 | 0.9999 |

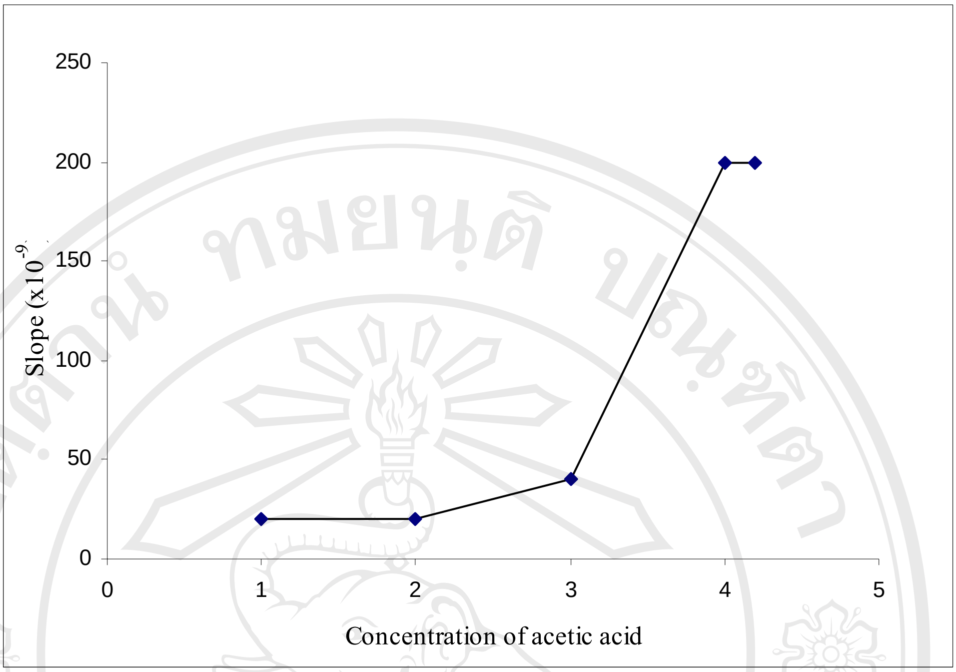

**Figure 3.15** Concentration of acetic acid for regenerate column

### 3.3.6 Summary of the selected conditions for the orthophosphate determination

The conditions for FI orthophosphate determination can be summarized in Table 3.10.

**Table 3.10** Summary of the selected conditions for the orthophosphate determination

| **Parameter** | **Condition** |
|---|---|
| Electrode | Glassy carbon electrode |
| Mixing coil | 50 cm |
| Flow rate | |
| -Std/sample | 1.0 ml/min |
| -HCl (0.1M) | 1.0 ml/min |
| -Acidic molybdate 0.5 % (w/v) in 2.5 % (v/v) $H_2SO_4$ | 0.6 ml/min |
| Potential applied | 220 mV |
| Eluent type | 0.1 M HCl |
| Resin type | Dowex 1-X8 |
| Operation sequence | B |
| Time for column regeneration | 3 min |
| Concentration of acetic acid for column regeneration | 4.2 M |

### 3.3.7 Precision

Employing the conditions in Table 3.10, seven replication of 7 bbp $PO_4$-P standard solution were determined. The results are summarized in Table 3.11, with RSD of 8.7%

**Table 3.11** Precision of 7 ppb of $PO_4$-P

| Inject | Concentration found (ppb) $PO_4$-P |
|---|---|
| 1 | 7.1 |
| 2 | 7.0 |
| 3 | 6.3 |
| 4 | 6.6 |
| 5 | 5.5 |
| 6 | 6.1 |
| 7 | 6.5 |
| Average | 6.4 |
| S.D | 0.6 |
| RSD | 8.7 |

### 3.3.8 Standard calibration graph

Using the condition in Table3.10, a series of standard (2, 5, 10, 20, 40, 60, 100 and 500 ppb $PO_4$-P) was used loaded for 5 min each. Peak profiles are illustrated in Figure 3.18. A calibration graph (a plot of peak area versus concentration and ng of $PO_4$-P) is depicted in Figure 3.16-3.17. From the concentration, the loading time (5 min), the flow-rate (1 ml/min) the amounts of $PO_4$-P can be calculated: 10, 25, 50, 100, 200, 300, 500 and 2500 ng $PO_4$-P respectively. It can be seen that there are breaks for the calibration graph, i.e. 2-20 ppb $PO_4$-P (10-100 ng $PO_4$-P) and 20-500 ppb $PO_4$-P (100-2500 ng $PO_4$-P). A detection limit of 2 ppb $PO_4$-P for 5 min loading time (or 10 ng $PO_4$-P) was obtained. For a solution of lower concentration, a longer loading time should be needed, so that an amount of more than 10 ng $PO_4$-P should be read.

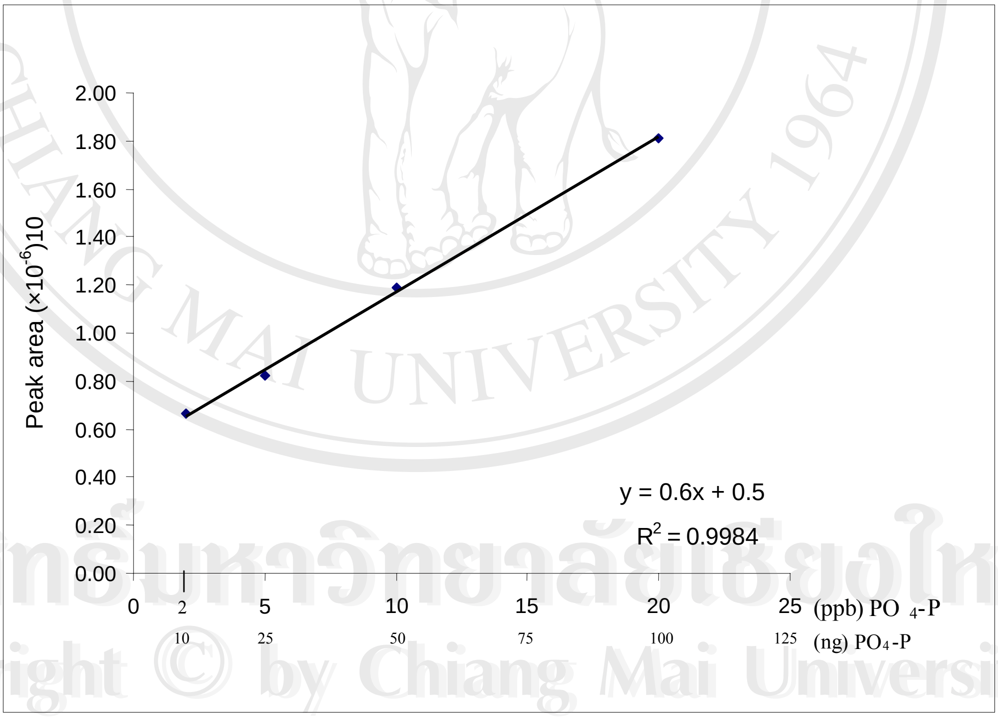

**Figure 3.16** Standard calibration graph of 2, 5, 10 and 20 ppb standard solution $PO_4$-P loading for 5 min

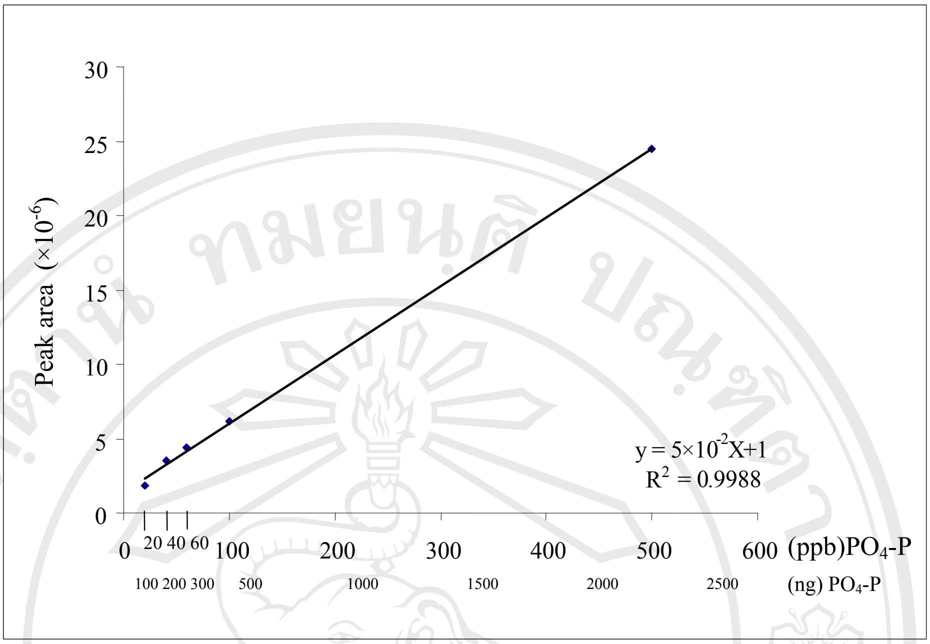

**Figure 3.17** Standard calibration graph of 20, 40, 60, 100 and 500 ppb standard solution PO$_4$-P loading for 5 min

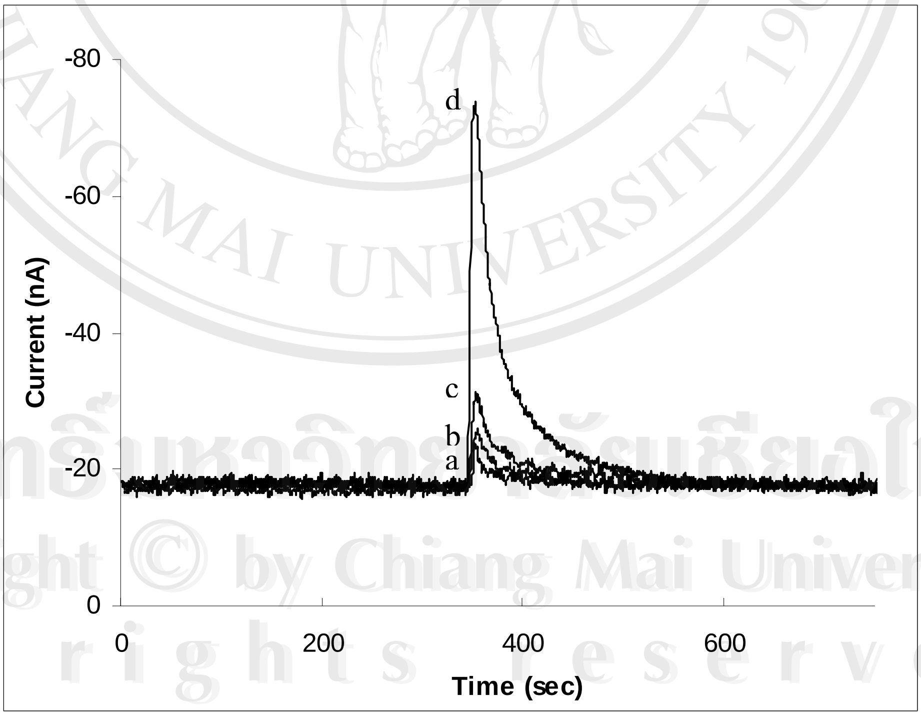

**Figure 3.18** FI gram of phosphate standard concentrations (a) 2, (b) 5, (c)10 and (d) 40 ppb PO$_4$-P; loading for 5 min

### 3.3.9 Single standard calibration graph

Using different concentration of $PO_4$-P solutions, loading time, as summarized in Table 3.12. The amounts of $PO_4$-P can be calculated using the following relationship.

$$\text{ng } PO_4\text{-P} = \underset{(\text{ng } PO_4\text{-P/ml})}{\text{concentration of orthophosphate}} \times \underset{(\text{min})}{\text{loading time}} \times \underset{(\text{ml/min})}{\text{flow rate}}$$

Peak area of each in Table 3.12 was plotted against the amounts of $PO_4$-P as represented in Figure 3.19. It was found that a linear graph was for 10-100 ng $PO_4$-P. A detection limit of 10 ng $PO_4$-P was found. A single standard calibration can be applied.

**Table 3.12** Single standard calibration graph (loading flow rate 1.0 ml/min)

| Concentration $PO_4$-P (ppb) | Loading time (min) | ng $PO_4$-P | Peak area ($10^{-6}$) |
|---|---|---|---|
| 1 | 10 | 10 | 0.53 |
| | 20 | 20 | 1.20 |
| 2 | 20 | 40 | 1.84 |
| | 30 | 60 | 2.83 |
| 5 | 20 | 100 | 4.74 |

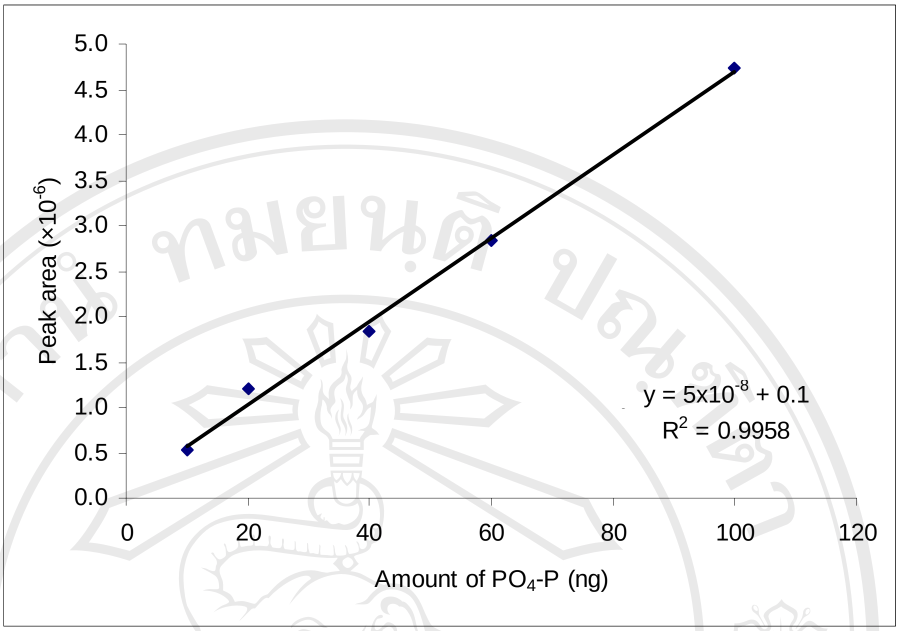

**Figure 3.19** Single standard calibration graph of 10, 20, 40, 60 and 100 ng of $PO_4$-P; 1 ppb loading for 10 and 20 min, 2 ppb loading for 20 and 30 min, 5 ppb loading for 20 min at flow rate 1 ml/min.

### 3.4 Application for determination of orthophosphate $PO_4$-P in samples

The developed procedure was applied to determine trace phosphate in a synthetic sample (see Appendix A), reference rain water (see also Appendix B) and various types of purified water (distilled, deionized, reverse osmosis and filtered) by employing 2 min loading time for synthetic and reference rain water and 30 min purified water. The results are summarized in Table 3.13.

The recoveries of 5 and 8 ppb $PO_4$-P added to synthetic sample were 90% and 94% respectively.

Orthophosphate was not detectable in reference rain water. Recoveries of 5 and 8 ppb $PO_4$-P added to the water were 94-105%.

For purified water the results (0.3-1.5 ppb $PO_4$-P) by using single standard calibration agreed with that obtained by standard addition method. Recoveries of 84-129% were found.

**Table 3.13** Application for determination of $PO_4$-P in samples

| Sample | Single standard calibration | Standard addition | Percent recovery |
|---|---|---|---|
| | Found (ppb) | Found (ppb) | |
| Synthetic sample A[#]<br>(5 ppb added) | 4.48 | NA | 90 |
| Synthetic sample B[#]<br>(8 ppb added) | 7.51 | NA | 94 |
| Reference rain No. 031*<br>(6 ppb added) | 5.99±0.24 | NA | 96-104 |
| Reference rain No. 032*<br>(6 ppb added) | 5.93±0.28 | NA | 94-104 |
| Reference rain No. 051*<br>(8 ppb added) | 7.89±0.25 | NA | 96-102 |
| Reference rain No. 052*<br>(8 ppb added) | 8.09±0.33 | NA | 97-105 |
| Distilled water<br>(Std. ASTM) | 0.60 | 0.50 | 102-109 |
| Deionized water | 0.38 | 0.29 | 96-129 |
| Reverse osmosis water | 0.74 | 0.75 | 84-96 |
| Filtered water | 1.47 | 1.25 | 96-104 |

[#] See appendix A

* See appendix B for the detail of composition in the synthetic rain