CHAPTER II

EXPERIMENTAL

2.1 Instrument and Apparatus

1. Flow-through cell for spectrophotometer, 10 mm, Hellma, Germany
2. Peristaltic pump EYELA model MP-3N, Tokyo Rikakikal Co.,Ltd, Japan.
3. Teflon tubing, inner diameter 1.07 mm, Anachem, UK
4. Tygon tubing, inner diameter 1.52 mm, Cole-parmer, USA
5. Home-made Y-shape connector
6. Spectrophotometer, JENWAY 6400, UK
7. Six port selection valve, Valco instrument, USA
8. Filter paper, 12.5 cm, Whatman No. 542
9. Waterproof pH Tester 10, Eutech Instrument
10. Chromatography column (0.8 cm ID × 30 cm long)

2.2 Chemicals

1. Amberlite XAD-8, Sigma aldrich, Germany.
2. Aluminium nitrate, Extra pure, Merck, Germany.
3. Barium chloride, GR grade Merck, Germany.
4. Coper sulfate, commercial grade, BDH, UK.
5. Cobalt sulfate, Extra pure, Merck, Germany.
6. Cacium chloride, commercial grade, Merck, Germany.
7. Cadmium nitrate, pure, BDH, UK.

8. Chromium nitrate, pure, MERCK, Germany.

9. EDTA di-sodium salt, GR, MERCK, Germany.

10. Ethanol, commercial grade, Merck, Germany.

11. Ferric nitrate, puriss, Carlo Erba, Italy.

12. Ferrous sulfate, commercial grade, Carlo Erba, Italy.

13. Hydrochloric acid, commercial grade, Merck, Germany.

14. Magnesium nitrate, commercial grade, Merck, Germany.

15. Manganese sulfate, Extra pure, MERCK, Germany.

16. Nickel sulfate, GR grade, Carlo Erba, Italy.

17. Potassium iodide, AR grade, Sigma aldrich, Germany.

18. Potassium chloride, Extra pure, MERCK, Germany.

19. Potassium hydroxide, AnalaR, BDH, UK.

20. Sodium selenite, commercial grade, Sigma aldrich, Germany.

21. Sodium acetate, commercial grade, Carlo Erba, Italy.

22. Sodium chloride, puriss, Fluka, Switzerland.

23. Sodium sulfate, AR grade, BDH, UK.

24. Sodium nitrate, AR grade, BDH, UK.

25. Sodium hydrogen carbonate, Carlo Erba, Italy.

26. Sodium bromide, pure, BDH, UK.

27. Sodium iodide, RPE, Carlo Erba, Italy.

28. Sodium fluoride, GR grade Merck, Germany.

29. Sodium phosphate, LAB, M&B Ltd., UK.

30. Sulfamic acid, puriss, Fluka, Switzerland.

31. Zinc sulfate, Extra pure, Carlo Erba, Italy.

2.3 Preparation of Standard Solutions and Reagents

All chemicals used in this work were of analytical reagent grade. All solutions were prepared with de-ionized water.

2.3.1 Preparation of Standard Solutions and Reagents for FIA procedure

2.3.1.1 Selenium stock solution 1000 mg L^{-1}

Selenium stock solution was prepared by dissolving 0.2190 g of sodium selenite in water and adjusting to 100 mL with de-ionized water in volumetric flask, then transferred into PE bottle and kept in refrigerator for further use. Working standard solution of selenium were prepared from stock solution of selenium and diluted with the 0.2 mol L^{-1} hydrochloric acid.

2.3.1.2 Variamine Blue stock solution 500 mg L^{-1}

The stock reagent solution was prepared by dissolving 0.05 g of variamine blue (VB) in 25 mL of 95% of ethanol and diluted with water in a 100 mL volumetric flask. Working reagent solution was prepared from stock solution of variamine blue and diluted with the 0.4 mol L^{-1} sodium acetate.

2.3.1.3 Potassium iodide stock solution 3.0% (w/v)

The stock potassium iodide solution was prepared by dissolving 3.0 g of potassium iodide in water and adjusting to 100 mL in a volumetric flask.

2.3.1.4 Sodium acetate stock solution 0.4 mol L^{-1}

The stock sodium acetate solution was prepared by dissolving 54.432 g of sodium acetate and diluted with water in a 1000 mL in a volumetric flask.

2.3.1.5 Hydrochloric acid stock solution 0.2 mol L^{-1}

The stock hydrochloric acid solution was prepared by adding 8.54 mL of concentrated HCl solution in water and adjusting to 500 mL in a volumetric flask.

2.3.2 Preparation of standard solutions and reagents for SIA procedure

2.3.2.1 Selenium stock solution 1000 mg L^{-1}

Selenium stock solution was prepared by dissolving 0.2190 g of sodium selenite in water and adjusting to 100 mL with de-ionized water in volumetric flask, then transferred into PE bottle and kept in refrigerator for further use.

2.3.2.2 Variamine Blue stock solution 100 mg L^{-1}

The stock reagent solution was prepared by dissolving 0.01 g of variamine blue (VB) in 25 mL of 95% of ethanol and diluted with water in a 100 mL in a volumetric flask.

2.3.2.3 Potassium iodide stock solution 2.0% (w/v)

The stock potassium iodide solution was prepared by dissolving 2.0 g of potassium iodide in water and adjusting to 100 mL in a volumetric flask.

2.3.2.4 Sodium acetate stock solution 0.2 mol L^{-1}

The stock sodium acetate solution was prepared by dissolving 27.216 g of sodium acetate and diluted with water in a 1000 mL in a volumetric flask.

2.3.2.5 Hydrochloric acid stock solution 0.4 mol L^{-1}

The stock hydrochloric acid solution was prepared by adding 17.09 mL of concentrated HCl solution in water and adjusting to 500 mL in a volumetric flask.

2.4 Procedure

2.4.1 Sample collection and pretreatments

Surface water samples were collected from Chiang Mai channel, Suan Dok Gate, Chang Phuak Gate, Thaphae Gate, Chiang mai Gate, Hu Lin corner, Sri Poom corner, Khatum corner, Khu Huang corner and Chiang Mai University reservoir, Ang-Keaw(I) and Ang-Keaw(II) (the sampling site see appendix A), Thailand. The samples were collected in polyethylene bottles subsequently the water samples were filtered through whatman filter paper No. 542 and then the filtrated samples were acidified with concentrated nitric acid (1 mL concentrated nitric acid per a liter of water sample) and then kept in the cold room at 4 ^{o}C for preservation. The sample was transferred into a suitable volume (100 mL to 250 mL) of beaker. Removal of organic interference by resin pretreatment [60]. 0.8-cm-ID column was packed with 5 cm washed resin (Amberlite XAD-8, 16 to 50 mesh). Precodition column with 30 mL pH 12 solution and 20 mL pH 1.6 solution. Using HCl and a pH meter adjust samples to pH 1.6-1.8. Pass samples through preconditioned column at rate of 1 mL/min. Discard first 10 mL and use next 11 to 50 mL. Add 5.0 mL of 0.005% (w/v) sulfamic acid, 2.5 mL of 0.1% (w/v) sodiumfluoride and 1.0 mL of 0.3% (w/v) EDTA, transferred into 50 mL volumetric flask and made up to the mark with deionized distilled water. Finally, it was mixed well and subsequently analysed.

2.4.2 FIA spectrophotometric determination of selenium using VB solution as complexing reagent

Figure 2.1 showed the experimental set up for the FIA spectrophotometric determination of selenium, which was the two channels FIA manifold. Two channels consisted of a Potassium iodide (KI) stream and a stream of Variamine Blue (VB) in sodium acetate solution (as reagent), having the total flow rate of 1.5 mL min^{-1}. A 75 µL selenium(s) in acid solution was manually injected into the flowing carrier stream solution via a six port injection valve. Then this carrier solution was merged with various Se(IV) standard solutions in acidic media at reaction coil (I) (1.07 mm i.d., 40 cm long) (R_1). The mixed solution was going to a three-way connector and merged with Variamine Blue (VB) in sodium acetate, the colored product was formed at the reaction coil (II) (1.07 mm diameter, 175 cm long) (R_2). The resulting colored complex was passed through the flow-through cell, furnished in the spectrophotometer where the absorbance was measured at 546 nm.

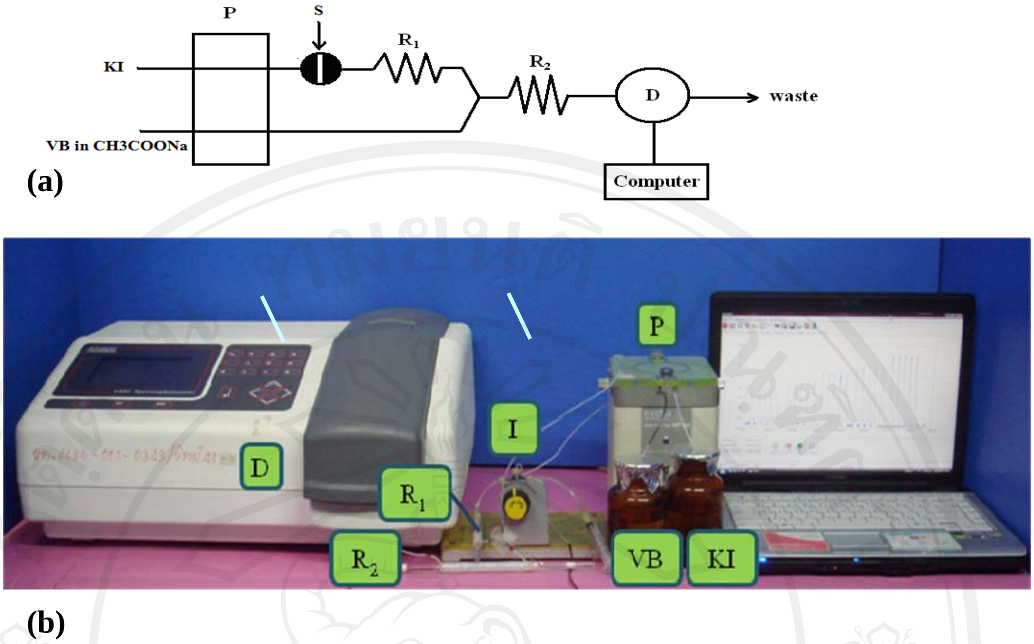


Figure 2.1 Flow injection system for the determination of selenium. (a) schematic diagram and (b) photograph of (a). KI, Potassium iodide; VB, Variamine Blue in sodium acetate solution; R_1, reaction coil (I); R_2, reaction coil (II); I, injection valve; P, pump; D, detector (JENWAY 6400)

2.4.2.1 Optimization of the flow injection system

The studied range for the optimization of developed of flow injection method to determination of selenium was shown in Table 2.1. The univariate optimization was started with the selection of the preliminary experimental conditions. Then, a studied parameter was changed while other parameters were fixed with their constant values. When the studied parameters was undergone changing to the optimized value, another parameter was varied. The other parameters were performed in the same manner through the optimized values. To optimize the conditions of the FIA manifold (Figure 2.1), the preliminary experimental conditions (Table 2.2) were proposed.

Table 2.1 The studied range for the optimization of experimental parameters

Variable	**Studied range**
Wavelength (nm)	537 – 555
Concentration of KI (% w/v)	1.0 – 4.5
Concentration of HCl (mol L^{-1})	0.1 – 0.6
Concentration of ethanol in VB solution (% v/v)	15 – 50
Concentration of VB (mg L^{-1})	6 – 14
Concentration of Sodium acetate (mol L^{-1})	0.1 – 0.9
Flow rate (mL min^{-1})	1.0 – 3.5
Reaction coil (I) length (cm)	0 – 60
Reaction coil (II) length (cm)	75 – 200
Sample injection volume (μL)	50 – 150

Table 2.2 Preliminary experimental conditions of FIA for studying optimum wavelength of Se(IV) with VB

Experimental parameters	**Pretested conditions**
Concentration of KI (% w/v)	2.5
Concentration of HCl (mol L^{-1})	1.0
Concentration of ethanol in VB solution (% v/v)	30
Concentration of VB (mg L^{-1})	50
Concentration of sodium acetate (mol L^{-1})	0.5
Flow rate (mL min^{-1})	2.0
Reaction coil (I) length (cm)	50
Reaction coil (II) length (cm)	75
Sample injection volume (µL)	75
Inner diameter of tubing (mm)	1.07

2.4.2.2 Linearity of calibration graph

Working standard solutions of selenium over the range of 0.05 – 2.50 mg L^{-1} was prepared from the stock solution (1000 mg L^{-1}). The series of selenium standard solutions with different concentrations were flowed into the FIA system (Figure 2.1) by means of five replicate results. Concentrations of selenium were measured by FIA method and recorded as peak heights. A typical calibration graph was obtained by plotting the peak heights against various concentrations of selenium.

2.4.2.3 Precision

The precision of the proposed method was verified by injecting 11 replicates of 0.20 mg L^{-1} selenium standard solution, and calculated % RSD from the equation as follows;

$$\%RSD = \frac{SD \times 100}{\overline{X}} \qquad (2.1)$$

Where $\%RSD$ = percentage relative standard deviation

SD = standard deviation

$\overline{X}$ = mean

2.4.2.4 Detection limit [61]

The detection limit is the minimum concentration of analyte that can be detected and can be calculated from following equation (2.2).

$$S_m = S_{bl} + kSD_{bl} \qquad (2.2)$$

Where Sm is the minimum analytical signal

S_{bl} is the mean blank signal

SD_{bl} is the standard deviation of blank

k is the confidence level of detection : 3

The detection limit or limit of detection (LOD) is the analyte concentration providing the minimum analytical signal. In general, it is also defined as 3σ where σ was standard deviation of the blank signal. The limit of quantitation (LOQ) is defined as 10σ.

2.4.2.5 Accuracy of the proposed method

The accuracy of the proposed method was verified by spiking the treated water samples with various concentrations of selenium standard solutions (0, 0.16, 0.18, 0.20 and 0.22 mg.L^{-1}) respectively using the recommended procedure. Then, selenium concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation as follows;

$$\%\text{Recovery} = \frac{(\text{total Se(VI) concentration- Se(IV) concentration in sample}) \times 100}{\text{Spiked Se(IV) concentration}} \qquad (2.3)$$

2.4.2.6 Interference studies

The interference effects of some possible foreign ions on selenium determination were studied by the proposed FIA procedure under the optimum conditions. A systematic study to check for the effects of some possible foreign ions (Al^{3+}, Ba^{2+}, Cu^{2+}, F^-, Fe^{2+}, Fe^{3+}, K^+, Ni^{2+}, Co^{2+}, Cr^{3+}, Cd^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Na^+, Ca^{2+}, NO_3^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}, Br^-, Cl^-, Na^+) by adding various known amounts of each interference to 1.00 mg L^{-1} of selenium standard solution to obtain the desire weight ratio of interfering ion to Se(IV) ion.

2.4.2.7 Validation method

In order to validate the FIA method for selenium determination, a comparative determination of selenium by the ICP-MS method was carried out. Results obtained by both methods were verified by using student t-test. The calculated t_{cal} value was obtained from the equation as follows [62];

$$t = \frac{\bar{x}_d \sqrt{n}}{S_d} \qquad (2.4)$$

$$S_d = \sqrt{\frac{\sum (x_d - \bar{x}_d)^2}{n - 1}} \qquad (2.5)$$

$$\bar{x}_d = \frac{\sum x_d}{n} \qquad (2.6)$$

Where; x_d the difference between two method

$\bar{x}_d$ the mean difference

S_d the standard deviation

n number of sample

$n-1$ number of degree of freedom

2.4.3 SIA spectrophotometric determination of selenium using VB solution as complexing agent

The SIA system (Figures 2.2 and 2.3) was arranged using the following equipment: FIAlab® 3000 system consists of a syringe pump (syringe reservoir 2.5 mL) and a 6-port selection valve which is connected to a 4-port switching box.

The 6-port selection valve under the following functions:

Valve port 1 was connected to a detector.

Valve port 2 was connected to a sample solution (selenium solution).

Valve port 3 was connected to an oxidant solution (potassium iodide solution).

Valve port 4 was connected to an acid solution (hydrochloric acid solution).

Valve port 5 was connected to a reagent solution (VB in sodium acetate solution).

Valve port 6 was connected to an acid (nitric acid solution).

The 4 ports switching box under the following functions:

Port A was connected to a syringe control (CAVRO XL 3000).

Port C was connected to a valve control unit.

Port B and D weren't required.

A Jenway 6400 spectrophotometer equipped with a 1 cm path length cell over the wavelength range 360 – 800 nm was used as detector. The flow system used Teflon tubes as the liquid channels. The holding coil was constructed by winding the teflon tubing around the small test tubes (1.5 cm o.d.). An absorbance signal can be retrieved directly from a Jenway 6400 spectrophotometer via the RS-232 interface. The absorbance of Se(IV) with VB complex was monitored at 546 nm through a 1 cm path length flow cell.

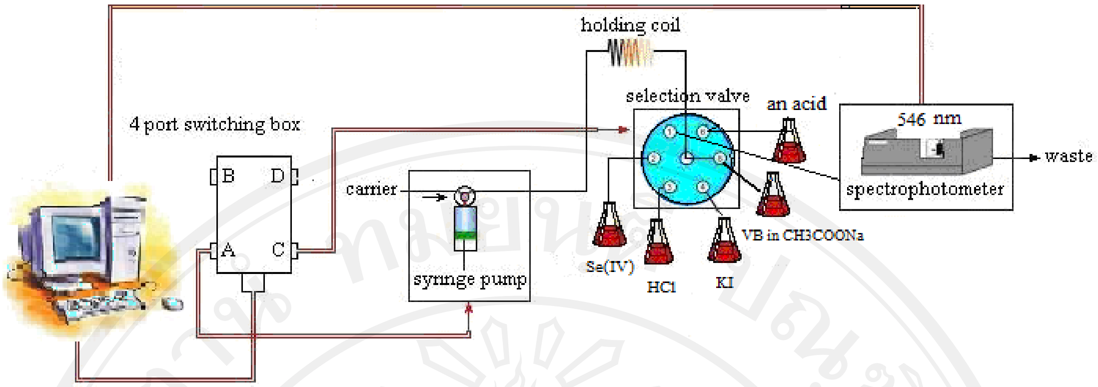


Figure 2.2 SIA manifold for the determination of selenium in water samples

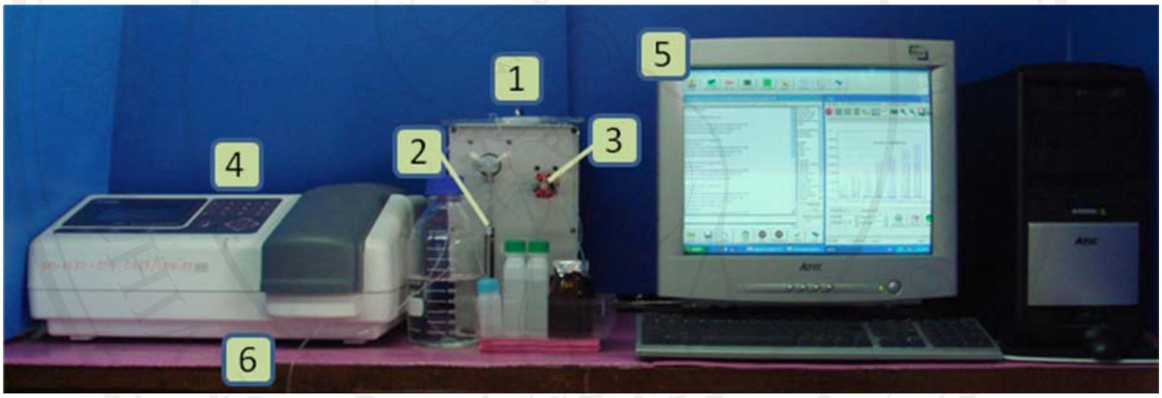


Figure 2.3 The SIA system for determination of selenium. 1, holding coil; 2, syringe pump; 3, selection valve; 4, detector; 5, computer; 6, waste

2.4.3.1 Sequential injection method

The 4 – port RS-232 switching box received an activation command from the computer through master port. When the system was initialized, it activated port a move the piston of the syringe to zero position. It also activated port C to actuate with the valve at position 5. Then, it activated port A to drive the syringe to aspirate the buffer with the desired volume. After that, it activated port C to actuate the valve at position 2 (sample) and it activated port A to drive the syringe to aspirate the desired volume of solution. The method was shown in Table 2.3. Finally, the computer was sending the empty syringe command through port A. It received an absorbance signals from the spectrophotometer and drove the plot module to plot the SIA grams on the developed SIA software (Figure 2.4) [66]. The maximum peak heights were detected at 546 nm and displayed in this process. The time required to analyze one sample was approximately 1.09 min. Table 2.3 lists the steps of the experimental entered to the FIAlab 5.0 for windows software (Figure 2.5).

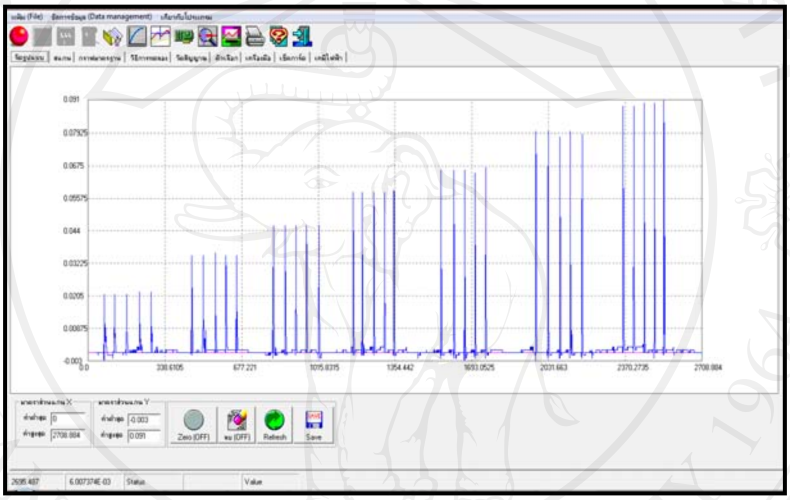
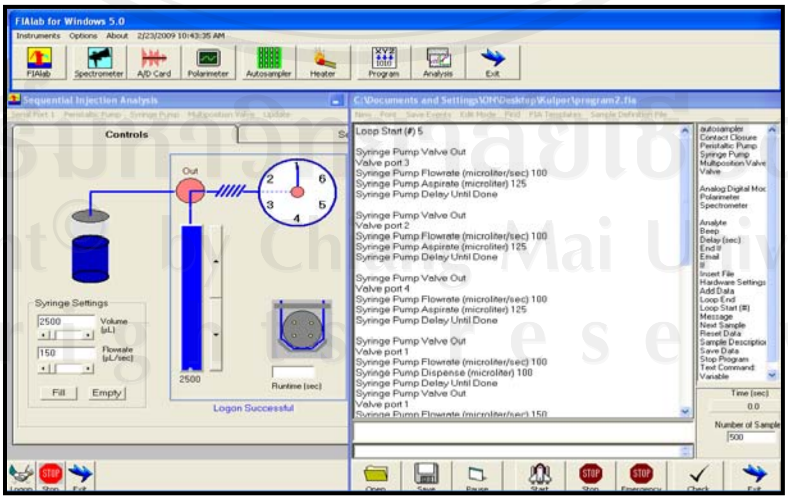


Figure 2.4 The SIA grams obtained by mean of the developed software [66]



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Loop Start (#) 5

Syringe Pump Valve Out
Valve port 3
Syringe Pump Flowrate (microliter/sec) 100
Syringe Pump Aspirate (microliter) 125
Syringe Pump Delay Until Done

Syringe Pump Valve Out
Valve port 2
Syringe Pump Flowrate (microliter/sec) 100
Syringe Pump Aspirate (microliter) 125
Syringe Pump Delay Until Done

Syringe Pump Valve Out
Valve port 4
Syringe Pump Flowrate (microliter/sec) 100
Syringe Pump Aspirate (microliter) 125
Syringe Pump Delay Until Done

Syringe Pump Valve Out
Valve port 1
Syringe Pump Flowrate (microliter/sec) 100
Syringe Pump Dispense (microliter) 100
Syringe Pump Delay Until Done
Syringe Pump Valve Out
Valve port 1
Syringe Pump Flowrate (microliter/sec) 150
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Figure 2.5 FIAlab 5.0 for windows software

Table 2.3 Experimental protocol as shown in the FIAlab for windows software

Command	**Description**
Loop Start (#) 5	The experimental was done 5 repeat.
Syringe Pump Valve Out Valve port 3 Syringe Pump Flowrate (microliter/sec) 100 Syringe Pump Aspirate (microliter) 125 Syringe Pump Delay Untill Done	125 μL of HCl solution was aspirated into holding coil by using flow rate 100 μL s^{-1}.
Syringe Pump Valve Out Valve port 2 Syringe Pump Flowrate (microliter/sec) 100 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	100 μL of selenium solution was aspirated into holding coil by using flow rate 100 μL s^{-1}.
Syringe Pump Valve Out Valve port 4 Syringe Pump Flowrate (microliter/sec) 100 Syringe Pump Aspirate (microliter) 125 Syringe Pump Delay Untill Done	125 μL of KI solution was aspirated into holding coil by using flow rate 100 μL s^{-1}.

Table 2.3 (Continued)

Command	Description
Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Dispense (microliter) 100 Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	HCl, selenium and KI were mixed well by using flow rate 125 μL s^{-1}.
Syringe Pump Valve Out Valve port 5 Syringe Pump Flowrate (microliter/sec) 100 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	100 μL of VB in sodium acetate solution was aspirated into holding coil by using flow rate 100 μL s^{-1}.

Table 2.3 (Continued)

Command	**Description**
Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Dispense (microliter) 100 Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 125 Syringe Pump Aspirate (microliter) 100 Syringe Pump Delay Untill Done	VB was mixed in the solution (Se-KI-HCl).
Syringe Pump Valve In Syringe Pump Flowrate (microliter/sec) 100 Syringe Pump Fill Syringe Pump Delay Untill Done Syringe Pump Valve Out Valve port 1 Syringe Pump Flowrate (microliter/sec) 250 Syringe Pump Empty Syringe Pump Delay Untill Done	The syringe pump to aspirate filled a carrier solution. Then, it pushed a carrier in syringe pump to holding coil and the complex solution moved to a detector by using flow rate 250 µL s^{-1}.
Loop End	

2.4.3.2 Optimization of the sequential injection system

The studied range for the optimization of development of sequential injection to determination of selenium was shown in Table 2.4. The optimization was started with the selection of the preliminary experimental conditions. Then, a studied parameter was varied, while others parameters were fixed with their constant values. When the studied parameter was undergone changing to the optimized value, another parameter was varied. The other parameters were performed in the same manner through the optimized values. To optimize the conditions of the SIA system (Figures 2.2 and 2.3), the preliminary experimental conditions (Table 2.5) were proposed.

Table 2.4 The studied range for the optimization of experimental parameters

Variable	**Studied range**
Concentration of KI (% w/v)	1.0 – 4.0
Concentration of HCl (mol L^{-1})	0.2 – 1.0
Concentration of ethanol in VB solution (% v/v)	15 – 40
Concentration of VB solution (mg L^{-1})	50 – 200
Concentration of CH_3COONa (mol L^{-1})	0.1 – 0.6
Aspiration volume of sample (μL)	50 – 175
Aspiration volume of KI (μL)	75 – 200
Aspiration volume of HCl (μL)	75 – 175
Aspiration volume of VB in CH_3COONa (μL)	75 – 200
Flow rate (μL s^{-1})	50 – 175

Table 2.5 Preliminary experimental conditions of SIA for studying optimum concentration of VB

Experimental parameters	Pretested conditions
Wavelength (nm)	546
Concentration of KI (% w/v)	1.5
Concentration of HCl (mol L^{-1})	0.5
Concentration of ethanol in VB solution (% v/v)	30
Concentration of CH_3COONa (mol L^{-1})	0.4
Aspiration volume of sample (µL)	125
Aspiration volume of KI (µL)	125
Aspiration volume of HCl (µL)	125
Aspiration volume of VB in CH_3COONa (µL)	100
Length of holding coil (cm)	125
Inner diameter of tube (mm)	1.07
Flow rate (µL s^{-1})	125

2.4.3.3 Linearity of calibration graph

Working standard solutions of selenium over the ranges of 0.01 – 2.5 mg L^{-1} was prepared from the stock solution (1000 mg L^{-1}). A series of selenium standard solutions with different concentrations were injected into the finally proposed SIA manifold by means of a syringe pump in five replicates. The resulting peak heights were measured. A typical calibration graph was obtained by plotting the peak heights against various concentrations of selenium.

2.4.3.4 Precision

The precision of the proposed method was verified by injecting 11 replicates of 0.05, 0.10, 0.60 and 2.00 mg L^{-1} selenium standard solutions in the same day and after a week, and calculated %RSD using equation 2.1.

2.4.3.5 Detection limit

Detection limit of the proposed method for selenium determination was studied using the same procedure as described in section 2.4.2.4.

2.4.3.6 Accuracy of the proposed method

The accuracy of the proposed method was verified by spiking the treated water samples with various concentrations of selenium standard solutions (0, 0.4, 0.5, 0.6 and 0.7 mg L^{-1}) respectively using the recommended procedure. Then, the results were plotted standard addition curve. Se(IV) concentration in sample was calculated from y = mx + c (y is signal of spiking the treated water samples with concentrations of selenium standard solutions 0 mg L^{-1}). Total Se(IV) concentration was calculated from y = mx + c (y is signal of spiking the treated water samples with various concentrations of selenium standard solutions 0.3, 0.5, 0.7 and 0.9 mg L^{-1}). Finally, the percentage recovery was calculated from equation 2.3.

2.4.3.7 Interference studies

The interference effects of some possible foreign ions in the SIA system for selenium determination were studied using the same procedures as described in 2.4.2.6. for FIA.

2.4.3.8 Validation method

The proposed SIA instrumentation has been tested to the determination of selenium in natural surface water samples collected in Chiang Mai city. The results obtained by SIA were confirmed by comparison with those obtained by ICP-MS using the student t-test as described earlier.