CHAPTER 2

EXPERIMENTAL

2.1 Instruments and Apparatus

Instruments and Apparatus	**Model**	**Company**	**Country**
1. UV-VIS spectrophotometer	6305	Jenway Ltd.	UK
2. UV-VIS spectrophotometer	6400	Jenway Ltd.	UK
3. UV-VIS spectrophotometer	Lambda 25	Perkin Elmer	USA
4. Sequential Injection Analysis (SIA)	FIAlab-3000	FIAlab instrument	USA
5. ICP-MS	7500 C	Agilent	Japan
6. Flow-through cell	-	Hellma	Germany
7. Peristaltic pump	EYELA MP-3N	Rikakikal Co., Ltd	Japan
8. Waterproof pH Tester 10	-	Eutech Instrument	USA
9. Micropipette	5000	Nichiryo	Japan
10. Hotplate	-	Baird and Tatkock	England
11. Tygon tube	-	Cloe-Parmer	USA
12. PTFE tube	-	Cloe-Parmer	USA
13. Home-made connector T-shape	-	-	-
14. Six-port selection valve	-	Valco instrument	USA

2.2 Chemicals

Chemicals	Molecular Formula	Company	Country
1. Cadmium standard 1000 mg L^{-1} (single element solution)	Cd	Fisher Scientific	UK
2. Rhodamine B	$C_{28}H_{31}ClN_2O_3$	Merck	Germany
3. Potassium iodide	KI	Merck	Germany
4. Hydroxylamine HCl	H_3NOHCl	Merck	Germany
5. Phosphoric acid	H_3PO_4	Carlo Erba	Italy
6. Sodium dihydrogen phosphate	NaH_2PO_4	Merck	Germany
7. Nitric acid	HNO_3	Merck	Germany
8. Hydrochloric acid	HCl	Merck	Germany
9. Acetic acid	CH_3COOH	Merck	Germany
10. Sodium acetate	CH_3COONa	Fluka	Switzerland
11. Aluminium sulphate	$Al_2(SO_4)_3.18H_2O$	Merck	Germany
12. Potassium cyanide	KCN	Merck	Germany
13. Sodium chloride	NaCl	Merck	Germany
14. Magnesium sulphate	$MgSO_4.7H_2O$	BDH	England
15. Sodium nitrile	$NaNO_2$	Fluka	Switzerland
16. Manganese(II) nitrate 4-hydrate	$Mn(NO_3)_2.4H_2O$	Merck	Germany

Chemicals (Continued)

Chemicals	**Molecular Formula**	**Company**	**Country**
17. Potassium dihydrogen phosphate	KH_2PO_4	Fluka	Switzerland
18. Nickel(II) nitrate	$Ni(NO_3)_2.6H_2O$	BDH	England
19. Barium chloride	$BaCl_2.2H_2O$	Merck	Germany
20. Aluminium chloride	$AlCl_3.6H_2O$	Merck	Germany
21. Cobalt(II) nitrate	$Co(NO_3)_2.6H_2O$	Merck	Germany
22. Potassium sulphate	K_2SO_4	BDH	England
23. Sodium bromide	$NaBr$	RDH	UK
24. Zinc nitrate	$Zn(NO_3)_2.6H_2O$	Carlo Erba	Italy
25. Potassium chloride	KCl	Merck	Germany
26. Copper(II) nitrate	$Cu(NO_3)_2.\ 3H_2O$	Carlo Erba	Italy
27. Chromium(III) nitrate	$Cr(NO_3)_3.9H_2O$	Merck	Germany
28. Iron(III) nitrate	$Fe(NO_3)_3.9H_2O$	Carlo Erba	Italy
29. Ferric sulphate	$Fe(SO_4)_3$	Fluka	Switzerland
30. Sodium nitrate	$NaNO_3$	BDH	England

All chemicals used in this study were of analytical-reagent grade or higher purity and used without further purification.

2.3 Preparation of Standard and Reagent Solutions

All reagents were of analytical-reagent grade. Deionized water was used to prepare some solutions, 0.00318 mol L^{-1} hydrochloric acid (HCl) for prepare potassium iodide (KI) solution and 1.0% nitric acid (HNO_3) for prepare cadmium(II) standard solution. All labs were used for handling solutions was cleaned with detergent solution, rinsed with tab water, soaked for least 24 h in 1:2 (v/v) nitric acid/water, rinsed with deionized water and finally dried before use.

2.3.1 Preparation of standard and reagent solutions for FIA and SIA systems

2.3.1.1 Cadmium(II) stock standard solution 100 mg L^{-1}

Cadmium(II) stock solution was prepared by transferring 10 mL of cadmium(II) standard 1000 mg L^{-1} (single element) into a 100 mL volumetric flask and adjusting to 100 mL with 1.0% nitric acid there transferred into PE bottle and kept in refrigerator for further use. Solution of lower concentration was contained by accurate dilution of this solution.

2.3.1.2 Rhodamine B stock solution 0.01 mol L^{-1}

The rhodamine B stock standard solution 0.01 mol L^{-1} was prepared by dissolving 0.47902 g of rhodamine B in deionized water and diluted in a 100 mL volumetric flask. Solution of lower concentration was contained by accurate dilution of this solution. The solution was kept in the dark place.

2.3.1.3 Potassium iodide (KI) stock solution 1.0 mol L^{-1}

The potassium iodide stock standard solution 1.0 mol L^{-1} was prepared by dissolving 16.6010 g of potassium iodide in 0.00318 mol L^{-1} hydrochloric acid and diluted in a 100 mL volumetric flask. Solution of lower concentration was contained by accurate dilution of this solution.

2.3.1.4 Hydroxylamine HCl stock solution 0.01 mol L^{-1}

The hydroxylamine HCl stock standard solution 0.01 mol L^{-1} was prepared by dissolving 0.0695 g of hydroxylamine HCl in deionized water and diluted in a 100 mL volumetric flask. Solution of lower concentration was contained by accurate dilution of this solution.

2.3.1.5 Phosphate buffer solution 0.10 mol L^{-1} pH 3.0

The buffer solution was prepared by dissolving 0.6884 g of sodium dihydrogen phosphate (NaH_2PO_4) in 500 mL of deionized distilled water in a 1000 mL volumetric flask and adjusted pH to 3.0 by using phosphoric acid and diluted with water in a 1000 mL volumetric flask.

2.3.1.6 Phosphate buffer solution 0.15 mol L^{-1} pH 3.0

The buffer solution was prepared by dissolving 1.0326 g of sodium dihydrogen phosphate (NaH_2PO_4) in 500 mL of deionized distilled water in a 1000 mL volumetric flask and adjusted pH to 3.0 by using phosphoric acid and diluted with water in a 1000 mL volumetric flask.

2.4 Sample Preparation

In this investigation, wastewater samples were collected from different locations in Chiang Mai Province in 1000 mL polyethylene (PE) bottles. Subsequently the water samples were filtered through wet quantitative filter paper, and then the filtrated water samples were acidified with 5 mL of concentrated nitric acid, and then kept in 4 °C for preservation.

2.5 Preliminary Studies of Spectrophotometric Determination of Cadmium(II) by Using Rhodamine B as A Complexing Agent

2.5.1 Absorption Spectra

(A) Rhodamine B

Five millilitres of 1.0×10^{-4} mol L^{-1} Rhodamine reagent solution was transferred into a 25 mL volumetric flask

(B) Rhodamine B-KI complex

Five millilitres of 1.0×10^{-4} mol L^{-1} rhodamine B reagent solution was transferred into a 25 mL volumetric flask. Then, added 5 mL of 0.1 mol L^{-1} KI reagent solution and mixed well.

(C) Cd(II)-Rhodamine B complex

One millilitre of 0.2 mg L^{-1} Cd(II) standard solution was transferred into a 25 mL volumetric flask. Then, added 5 mL of 1.0×10^{-4} mol L^{-1} rhodamine B reagent solution and mixed well.

(D) Cd(II)-Rhodamine B-KI complex

One millilitre of 0.2 mg L^{-1} Cd(II) standard solution was transferred into a 25 mL volumetric flask. After that, 5 mL of 1.0 $\times 10^{-4}$ mol L^{-1} rhodamine B and 5 mL of 1.0 mol L^{-1} KI reagent solutions were added and mixed well.

The contents were diluted to final volume with deionized water and mixed thoroughly. Finally, the absorption spectra of complexes were scanned from 400-700 nm with JENWAY 6400 and the signals were recorded with computer.

2.6 Study of the Composition of the Cd(II)-Iodide-Rhodamine B Complex by the Mole-Ratio Method

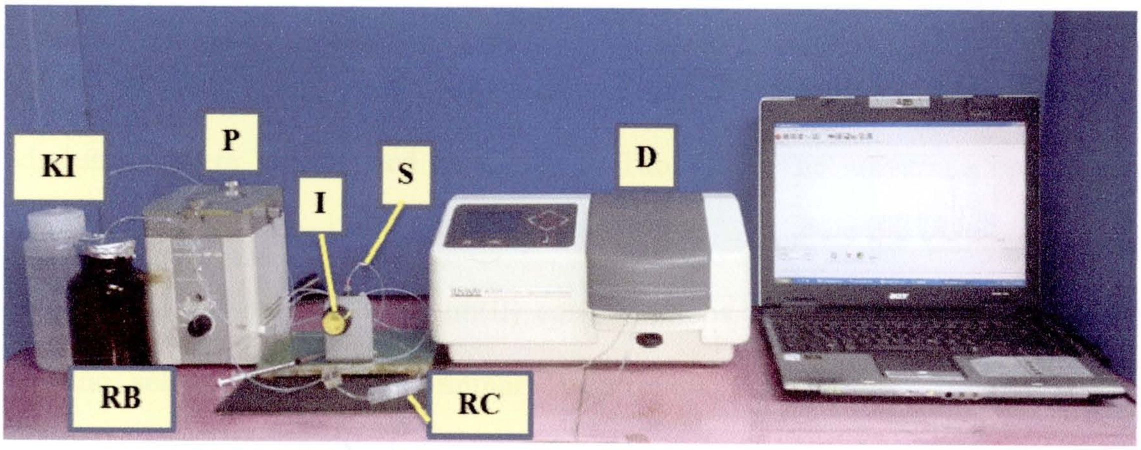
The mole-ratio method of Cd(II)-Iodide-Rhodamine B complex was defined as 2 series of solutions were prepared in which cadmium(II) concentration was fixed while the rhodamine B concentration was varied. Another one is prepared in which cadmium(II) and rhodamine B concentrations were fixed while the KI concentration was varied.

2.7 Procedure

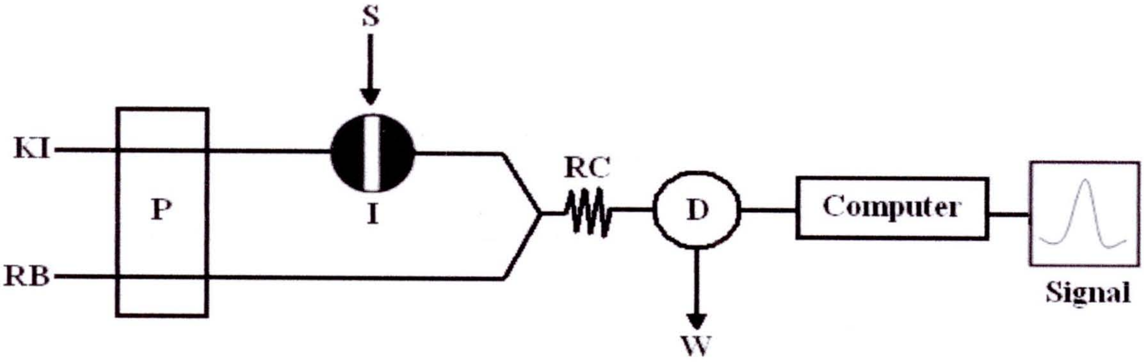
2.7.1 FIA spectrophotometric determination of cadmium(II) using rhodamine B as a complexing agent

The FIA system was designed and constructed as shown in Figure 2.1. The FIA manifold for the determination of cadmium(II) was of tygon and teflon tubing. The FI system consisted of a six-port injection valve with an external loop for reagent injection; the tubing connecting the ports was made from PTFE (0.107 cm i.d.), two individually two-channel peristaltic pumps used to sample and propel the sample and/or standard and reagent solutions, mixing reactors made of tygon tubing and PTFE tubing, spectrophotometer equipped with a flow-through cell for measurement and chart recorder.

Two channels consisted of a potassium iodide (KI), and a rhodamine B (RB) as reagent stream, having the total flow rate of 3.5 mL min^{-1}. A 200 μL standard or sample solution was injected into the reagent stream. The injected reagent was merged with the iodide-rhodamine B stream. Then the solution mixture was mixed in a reaction coil (RC, 0.086 cm inner diameter, 75 cm in length), where Cd(II)-Iodide-Rhodamine B complex was formed. The resulting violet colored complex was reached through the flow-through cell of the spectrophotometer where the absorbance was measured at 612 nm.



(a)



(B)

Figure 2.1 Flow injection system for the determination of cadmium(II)

(a) Photograph of the FIA manifold and

(b) Schematic diagram of (a). KI=potassium iodide; RB=rhodamine B; P=pump; S=sample; I=injection valve; RC=reaction coil; D=detector; W= waste

2.7.1.1 Optimization of the flow injection (FI) system

The various analytical parameters in the FI system for determination of cadmium(II) were investigated as shown in Table 2.1. The univariate optimization method to achieve the best sensitivity obtained by slope of the calibration graph between peak height and cadmium(II) concentrations. The 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 parameter was undergone changing to the optimized value, another parameter was varied. To optimize the conditions of the FIA system (Figure 2.1), the preliminary experimental conditions (Table 2.2) were proposed.

Table 2.1 The studied range for the optimization of FIA parameters

Parameter	Studied range
Wavelength (nm)	605-620
pH	1.5-4.0
Concentration of KI ((mol L^{-1})	0.05-0.11
Concentration of Hydroxylamine HCl (x10^{-4} mol L^{-1})	0.5-3.0
Concentration of Rhodamine B (x10^{-4} mol L^{-1})	1.0-5.0
Flow rate (mL min^{-1})	1.5-6.0
Reaction coil inner diameter (cm)	0.051-0.132
Reaction coil length (cm)	25-150
Sample volume (µL)	75-300

Table 2.2 Preliminary experimental conditions of FIA for studying optimum wavelength of Cd(II)-Iodide-Rhodamine B

parameter	**Fixed Value**
pH	2.5
Concentration of Phosphate buffer (mol L^{-1})	1.0
Concentration of KI ((mol L^{-1})	0.09
Concentration of Hydroxylamine HCl (x10^{-4} mol L^{-1})	1.0
Concentration of Rhodamine B (x10^{-4} mol L^{-1})	4.0
Flow rate (mL min^{-1})	4.0
Reaction coil inner diameter (cm)	0.086
Reaction coil length (cm)	100
Sample volume (µL)	100

2.7.1.2 Analytical characteristics of FIA method for determination of cadmium(II)

2.7.1.2.1 Linearity

In order to study the linear range, using the FIA manifold (Figure 2.1) under the optimum conditions (Table 3.12). Working standard solutions of cadmium(II) over the ranges of 0.00-4.00 mg L^{-1} were prepared from the intermediate cadmium(II) stock solution (10 mg L^{-1}). Concentrations of cadmium(II) were measured by FIA method and recorded as peak heights. The linear range was obtained and used for plotting the calibration curve.

2.7.1.2.2 Calibration curve

The calibration curve for determination of cadmium(II) was constructed by analyzing various cadmium(II) standard solutions over the range of 0.00-1.00 mg L^{-1} and the curve was plotted between Δpeak heights and cadmium(II) concentration.

2.7.1.2.3 Precision

The precision of the proposed FIA method was verified by repeating injection of 0.2 mg L^{-1} cadmium(II) standard solution for 11 replicates, and calculated % RSD from the equation as follows;

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

When %RSD = percentage relative standard

SD = standard deviation

$\overline{X}$ = mean

2.7.1.2.4 Detection limit [82]

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 S_m is the minimum analyte 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 is the analyte concentration providing the minimum analytical signal.

2.7.1.2.5 Accuracy of the proposed FIA method

The accuracy of the proposed FIA method was verified by spiking the treated wastewater samples with various concentrations of cadmium(II) standard solutions (0.1, 0.2, 0.3, 0.4 and 0.5 mg L^{-1}) respectively using the recommended procedure. Then, cadmium(II) concentrations were calculated from linear regression equation obtained from the calibration graph. Finally, the percentage recovery was calculated from the equation as follows;

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

2.7.1.3 Interference studies

The effect of interfering ion including cations and anions were studied by using the proposed FIA procedure under optimum conditions. A systematic study to check for the effects of some possible foreign ions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Na^{+}, Ca^{2+}, NO_2^-, NO_3^-, SO_4^{2-}, HCO_3^-, Br^-, I^-, Cl^-, F^-, Ba^{2+}, K^+, PO_4^{3-}) by adding known amounts of each interference to 0.2 mg L^{-1} of cadmium(II) standard solution and injecting into FIA system.

2.7.1.4 Determination of cadmium(II) in wastewater samples using standard addition method by FIA method

Application of the FIA method for determination of cadmium(II) in wastewater samples was carried out. After the optimum experimental conditions were obtained, the proposed FIA method was applied to ten wastewater samples collected from different areas in Chiang Mai Province. The sample was pumped into the continuous FIA manifold for the determination of cadmium(II). When the analysis was completed, the concentrations of the cadmium(II) was evaluated from the peak height signal by using standard addition method.

2.7.1.5 Comparison with ICP-MS method and validation method

A comparative determination of cadmium(II) in wastewater samples was carried out by using the proposed FIA method and inductively coupled plasma mass spectrophotometric (ICP-MS) method. Results obtained by both methods were verified by using student *t*-test. The calculated t_{cal} value was obtained from the equation as follows [83];

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

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

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

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

2.7.2 SIA spectrophotometric determination of cadmium(II) using rhodamine B as a complexing agent

The SIA system 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 (cadmium(II) solution).

Valve port 3 was connected to a reagent solution (Rhodamine B solution).

Valve port 4 was connected to a surfactant solution (KI solution).

Valve port 5 was connected to a buffer solution (Phosphate buffer pH 3.0).

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 available.

A Jenway 6400 spectrophotometer equipped with a 1 cm path length cell over the wavelength range 360-800 nm. 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 Cd(II)-Iodide-Rhodamine complex was monitored at 612 nm through a 1 cm path length flow cell. The SIA system and SI manifold were shown in Figure 2.2 and 2.3 respectively.

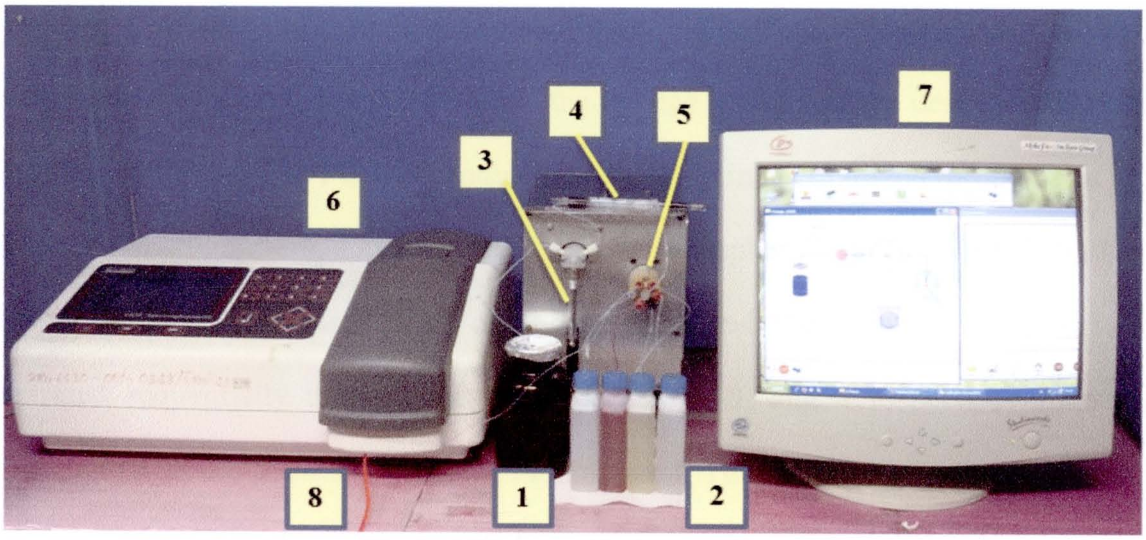


Figure 2.2 The SIA system for determination of cadmium(II): 1=carrier; 2=sample/reagent; 3= syringe pump; 4=holding coil; 5=selection valve; 6=detector; 7=computer; 8=waste.

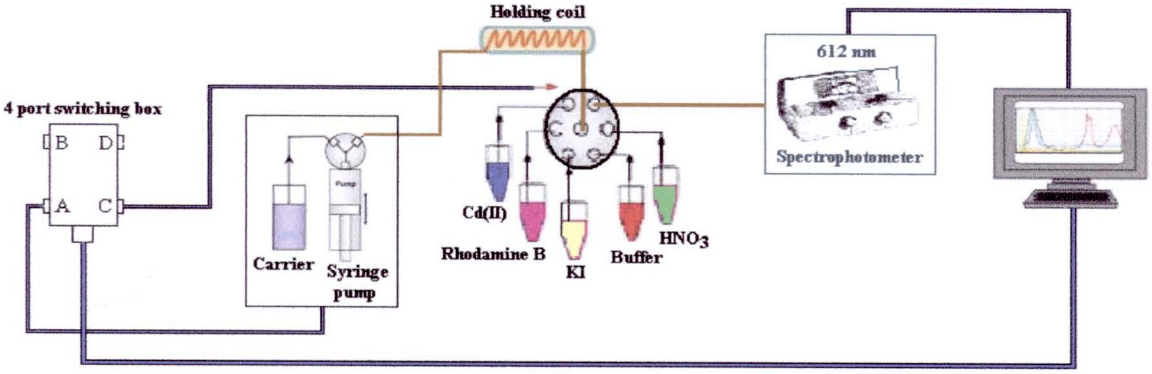
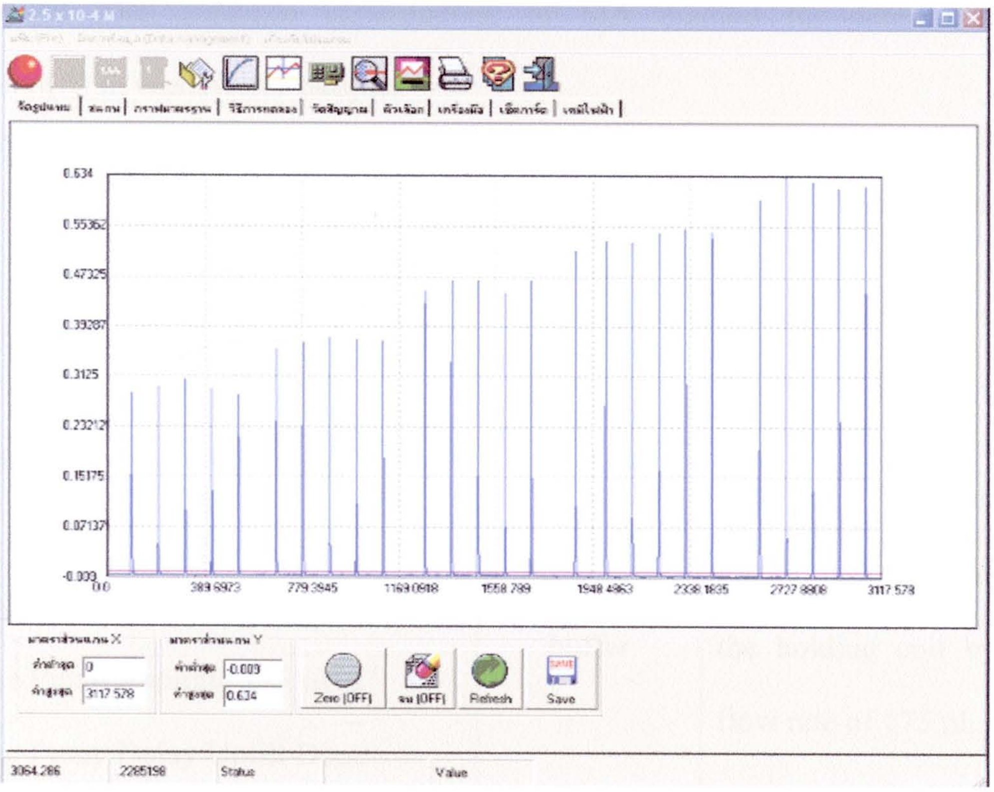


Figure 2.3 SIA manifold for the determination of cadmium(II) using rhodamine B

2.7.2.1 Sequential injection (SI) procedure

The 4 – port RS-232 switching box received an activation command from the PC through master port. When the system was initialized, it activated port to 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. After that, the PC was sending the empty syringe command through port A. In the automatic system, 125 μL of 0.15 mol L^{-1} phosphate buffer pH 3.0, 200 μL of standard/sample solutions, 150 μL of Rhodamine B and 150 μL of KI solutions were aspirated sequentially through the valve port 5, 2, 3, and 4 respectively into the holding coil at the same flow rate of 175 μL sec^{-1}. The absorbance of Cd(II)-Iodide-Rhodamine B complex was detected as peak heights with spectrophotometer at λ_{max} 612 nm. The time required to analyze one sample was approximately 1.5 min. The plot module to plot the SIA grams on Senee SIA software (Figure 2.4). Table 2.3 demonstrated the steps of the experimental entered to the FIAlab for windows 5.0 software (Figure 2.5).



มาตราส่วนแกน X
ค่าต่ำสุด 0
ค่าสูงสุด 3117.578

มาตราส่วนแกน Y
ค่าต่ำสุด -0.003
ค่าสูงสุด 0.634

3064.286 2285198 Status Value

Figure 2.4 Senee SIA software for plot the SIA grams

Figure 2.5 FIAlab for windows 5.0 software [84]

Table 2.3 The sequence experimental of SIA method for determination of cadmium(II) in wastewater samples

Procedure	Sample/ Reagent	Description
Loop Start (#) 5	-	The experiment carried out 5 replicate determinations.
Syringe Pump Valve Out Valve port 5 Syringe Pump Flowrate 175 μL s^{-1} Syringe Pump Aspirate 125 μL s^{-1} Syringe Pump Delay Untill Done	Phosphate buffer	125 μL of phosphate buffer was aspirated into the holding coil by using flow rate of 175 μL s^{-1}.
Syringe Pump Valve Out Valve port 2 Syringe Pump Flowrate 175 μL s^{-1} Syringe Pump Aspirate 200 μL s^{-1} Syringe Pump Delay Untill Done	Cadmium(II)	200 μL of cadmium(II) solution was aspirated into the holding coil by using flow rate of 175 μL s^{-1}.
Syringe Pump Valve Out Valve port 3 Syringe Pump Flowrate 175 μL s^{-1} Syringe Pump Aspirate 150 μL s^{-1} Syringe Pump Delay Untill Done	Rhodamine B	150 μL of rhodamine B solution was aspirated into the holding coil by using flow rate of 175 μL s^{-1}.

Table 2.3 (Continued)

Procedure	**Sample/ Reagent**	**Description**
Syringe Pump Valve Out Valve port 4 Syringe Pump Flowrate 175 μL s^{-1} Syringe Pump Aspirate 150 μL s^{-1} Syringe Pump Delay Untill Done	KI	150 μL of KI solution was aspirated into the holding coil by using flow rate of 175 μL s^{-1}.
Loop Start (#) 3 SyringePump Valve Out Valve port 1 SyringePump Flowrate 175 μL s^{-1} SyringePump Aspirate 125 μL SyringePump Delay Until Done Loop End	Mixing step	Sample and reagents were mixed and formed complexed (Cd(II)-Rhodamine B-KI)
Loop Start (#) 2 SyringePump Valve Out Valve port 1 SyringePump Flowrate 175 μL s^{-1} SyringePump Dispense 100 μL SyringePump Delay Until Done SyringePump Valve Out Valve port 1 SyringePump Flowrate 175 μL s^{-1} SyringePump Aspirate 125 μL SyringePump Delay Until Done	Mixing step	Sample and reagents were mixed and formed complexed (Cd(II)-Rhodamine B-KI)

Table 2.3 (Continued)

Procedure	**Sample/ Reagent**	**Description**
Loop End		
Delay (sec) 20	Cd(II)-Iodide-Rhodamine B complex	Wait for 40 s to complete the reaction
SyringePump Valve In SyringePump Flowrate 175 μL s^{-1} SyringePump Fill SyringePump Delay Until Done SyringePump Valve Out Valve port 1 SyringePump Flowrate 175 μL s^{-1} SyringePump Empty SyringePump Delay Until Done	Cd(II)-Iodide-Rhodamine B complex	The carrier solution in syringe pump was pushed into holding coil and the complex solution moved to a detector by using flow of rate 175 μL s^{-1}.
Loop End	-	Pump stop, valve turns to position 5 for the next replication.

2.7.2.2 Optimization of the SI system

The studied range for the optimization of all parameters of SIA method for determination of cadmium(II) 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 other 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 experimental conditions of the SI system for determining cadmium(II), the preliminary experimental conditions were proposed (Table 2.5).

Table 2.4 The studied range for the optimization of all parameters of SIA method

Variable	**Studied range**
pH	2.0-4.0
Concentration of phosphate buffer (mol L^{-1})	0.05-0.25
Concentration of KI (mol L^{-1})	0.05-0.15
Concentration of hydroxylamine HCl (x 10^{-4} mol L^{-1})	0.5-2.5
Concentration of rhodamine B (x 10^{-4} mol L^{-1})	1.5-4.0
Aspiration volume of phosphate buffer (μL)	50-175
Aspiration volume of KI (μL)	75-200
Aspiration volume of rhodamine B (μL)	75-200
Aspiration volume of sample (μL)	100-250
Flow rate (μL s^{-1})	100-225
Holding time (s)	0-50

Table 2.5 Preliminary experimental conditions of SIA method for studying optimum conditions for determination of cadmium(II)

Experimental parameters	**Pretested conditions**
Wavelength (nm)	612
pH	3.0
Concentration of phosphate buffer (mol L^{-1})	0.10
Concentration of rhodamine B (x 10^{-4} mol L^{-1})	2.5
Concentration of KI (mol L^{-1})	0.07
Concentration of hydroxylamine HCl (x 10^{-4} mol L^{-1})	1.5
Aspiration volume of phosphate buffer (µL)	125
Aspiration volume of rhodamine B (µL)	125
Aspiration volume of KI (µL)	125
Aspiration volume of sample (µL)	150
Length of holding coil (cm)	125
Inner diameter of tube (cm)	0.086
Flow rate (µL s^{-1})	150
Holding time (s)	30

2.7.2.3 Analytical characteristics of SIA method for determination of cadmium(II)

2.7.2.3.1 Linearity

In order to study the linear range, using the SIA manifold (Figure 2.2 and 2.3) under the optimum conditions (Table 3.34). Working standard solutions of cadmium(II) over the range of 0.00-5.00 mg L^{-1} were prepared from the intermediate cadmium(II) stock solution (10 mg L^{-1}). Concentrations of cadmium(II) were measured by SIA method and recorded as peak heights. The linear range was obtained and used for plotting the calibration curve.

2.7.2.3.2 Calibration curve

The calibration curve for determination of cadmium(II) was constructed by analyzing various cadmium(II) standard solutions over the range of 0.00-1.00 mg L^{-1} and the curve was plotted between Δpeak heights and cadmium(II) concentration.

2.7.2.3.3 Precision

The precision of the proposed SIA method was verified by injecting 11 replicate of 0.2 mg L^{-1} cadmium(II) standard solutions, and the %RSD was calculated from equation 2.1 (in section 2.7.1.2.3).

2.7.2.3.4 Detection limit

The detection limit of the proposed SIA method for determination of cadmium(II) was studied using the same procedures as described in section 2.7.1.2.4.

2.7.2.3.5 Accuracy of the proposed SIA method

The accuracy of the proposed SIA method was verified by spiking the treated wastewater samples with various known concentrations of cadmium(II) standard solutions (0.1, 0.2, 0.3. 0.4 and 0.5 mg L^{-1}) respectively using the recommended procedure. The standard addition curve was obtained by plotting peak heights against various cadmium(II) concentrations added. Cadmium(II) concentration in sample was calculated from $y = mx+c$ (y is signal of spiking the treated water samples with concentrations of cadmium(II) standard solutions 0 mg L^{-1}).

2.7.2.4 Interferences studied

The interference effects of some possible foreign ions in the SIA system for cadmium(II) determination were studied using the same procedures as described in section 2.7.1.3.

2.7.2.5 Determination of cadmium(II) in wastewater samples using standard addition method by SIA method

Application of the SIA method for determination of cadmium(II) in wastewater samples was carried out. After the optimum experimental conditions were obtained, the proposed SIA method was applied to ten wastewater samples collected from different areas in Chiang Mai Province. The sample was aspirated into the continuous SIA manifold for the determination of cadmium(II). When the analysis was completed, the concentrations of the cadmium(II) was evaluated from the peak height signal by using standard addition method.

2.7.2.6 Comparison with ICP-MS method and validation method

The proposed SIA instrumentation has been tested to the determination of cadmium(II). The results obtained by SIA method were confirmed by comparison with those obtained by ICP-MS using the student *t*-test as described earlier (section 2.7.1.5).