

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals, apparatus and instruments

2.1.1 Chemicals

All chemicals used are analytical reagent grade and all standard and reagent solutions were prepared with ultrapure water (Milli Q water, resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$) obtained from a Millipore water purification system (Millipore, Sweden).

Chemicals used in this research are listed below:

1. Acetic acid : CH_3COOH (Lab Scan, Thailand)
2. Bismuth nitrate pentahydrate: $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (Carlo Erba, Italy)
3. Cadmium chloride dihydrate : $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ (Fluka, Switzerland)
4. 99.5%(v/v) Ethanol : $\text{C}_2\text{H}_5\text{OH}$ (Merck, Germany)
5. Lead(II) nitrate : $\text{Pb}(\text{NO}_3)_2$ (Merck, Germany)
6. 65%(w/v) Nitric acid : HNO_3 (Merck, Germany)
7. Sodium acetate trihydrate: $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ (Carlo Erba, Italy)
8. Sodium hydroxide : (NaOH) (Merck, Germany)

2.1.2 Software

1. Microsoft Excel 2007 (Microsoft, USA)
2. Software program written in-house for control of solenoid valves
3. GPES software for control of the voltammograph (VA-757, Metrohm, Switzerland)

2.1.3 Materials and instruments

1. Peristaltic pump (4 channel) (Ismatec, Switzerland)
2. Tygon pump tubing (1.14 mm ID) (Ismatec, Switzerland)
3. Three way solenoid valves (Biochemvalve, USA)
4. Three way valves (Ismatec, Switzerland)
5. Flow through electrochemical cell (Cross-flow cell, Model MF – 1093, BAS, USA) composes of a 6 mm diameter glassy carbon working electrode (GCE), a Ag/AgCl reference electrode (RE) and a stainless steel auxiliary electrode (AE)
6. Voltammetric analyzer (VA-757, Metrohm, Switzerland)
7. pH meter model 744 (Metrohm, Switzerland)
8. Micropipette (Eppendorf, Germany)
9. Mettler Toledo balance (Mettler Toledo, Switzerland)
10. Polishing kit PK-4 MF-2060 (BASi, USA)
11. 1 μm dimond polish (BASi, USA)

2.2 Preparation of reagents

2.2.1 Acetate buffer solution

Sodium acetate 3-hydrate 2.41 g was dissolved with water. Acetic acid 2 mL was added into the sodium acetate solution. The pH of solution was measured, and adjusted to 4.5 by adding 1M NaOH. Finally, the solution was made up to 500 mL with water to obtain acetate buffer 0.1 M and pH 4.5.

2.2.2 Metal standard solution

Stock standard solution of Cd(II) 1000 mg/L was prepared by dissolving 0.2032 g of cadmium chloride (Fluka, Switzerland) in 0.1 M hydrochloric acid and the volume was made up to 100 mL in a volumetric flask.

Stock standard solution of Pb(II) 1000 mg/L was prepared by dissolving 0.3128 g of lead(II) nitrate (Merck, Germany) in 0.1 M nitric acid and the volume was made up to 100 mL in a volumetric flask.

Stock standard solution of Bi(III) 1000 mg/L was prepared by dissolving 0.2356 g of bismuth(III) nitrate 5-hydrate (Carlo Erba, Italy) in 0.5 M nitric acid and the volume was made up to 100 mL in a volumetric flask.

2.2.3 Working standard solution of Cd(II) and Pb(II)

The working standard solution of Cd(II) and Pb(II) were prepared daily by diluting the stock standard solution of each metal with 0.01 M nitric acid.

2.2.4 Nitric acid solution

Nitric acid (Merck, Germany) 63.8 mL was added into water and the solution was made up to 1000 mL to obtain 1 M nitric acid solution.

Nitric acid (Merck, Germany) 6.4 mL was added into water and the solution was made up to 1000 mL to obtain 0.01 M nitric acid solution.

2.2.5 Bi(III) solution

A Bi(III) solution 0.6 mg/L was daily prepared by diluting the Bi(III) 1000 mg/L with 0.1 M acetate buffer solution.

2.3 Preparation of electrode

2.3.1 Preparation of electrode before analysis

The glassy carbon electrode was polished with a water slurry of 1 μm alumina polish (Al_2O_3) on a polishing pad and polished about 2 min to obtain a fresh surface. Then it was immersed three times into water and into ethanol alternatively to eliminate the remaining Al_2O_3 particles (1 μm) from its surface. Procedure of analysis were checked by recording about 10 voltammograms at the 50 $\mu\text{g/L}$ of each Cd(II) and Pb(II) to achieve a good stabilization of the background current.

2.3.2 Storage of electrode

The glassy carbon electrode was kept in 0.1 M NaOH solution when was not in use. By this procedure the cleaned surface of the glassy carbon electrode was maintained.

2.4 Manifold of flow based anodic stripping voltammetric system for determination of cadmium and lead

Flow based voltammetric analysis system with bismuth film electrode (VA-BiFE) is shown in Figure 2.1. The system consisted a of peristaltic pump (Ismatec, Switzerland) with pump tubing, two 3-way solenoid valves (Biochemvalve, USA), a mixing coil (PTFE, i.d. 0.05 mm), and a voltammograph (VA 757, Metrohm, Switzerland) with an electrochemical flow-cell. The electrochemical flow-cell (cross-flow cell, Model MF- 1093, BAS, USA) composed of a 6 mm diameter glassy carbon working electrode (GCE), a Ag/AgCl reference electrode (RE) and a stainless steel auxiliary electrode (AE). The system was controlled by a personal computer using a software program written in-house.

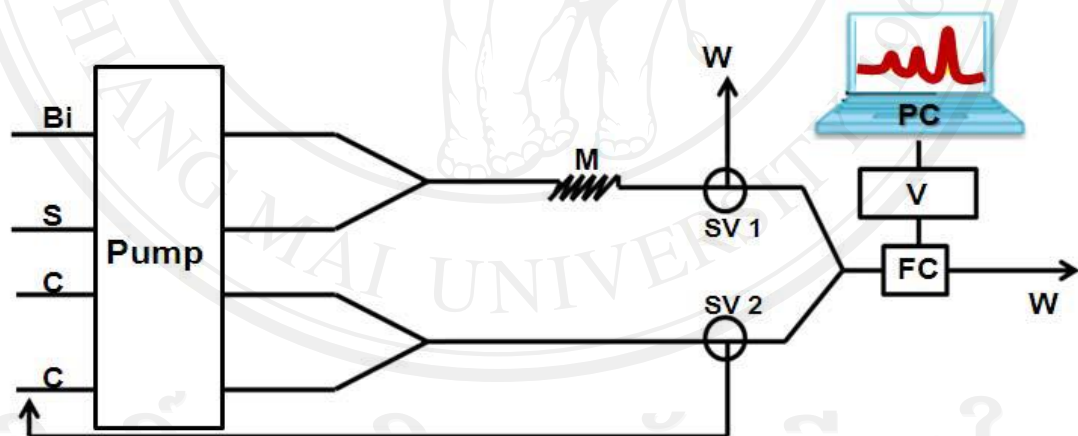


Figure 2.1 Manifold of flow base anodic stripping voltammetric system for determination of cadmium and lead; S = standard/sample, Bi = Bi(III) plating solution, C = cleaning solution(1 M nitric acid), P = peristaltic pump, M = mixing coil, SV 1-2 = solenoid valves, FC = electrochemical flow cell, V = voltammetric analyzer, PC = personal computer, W = waste.

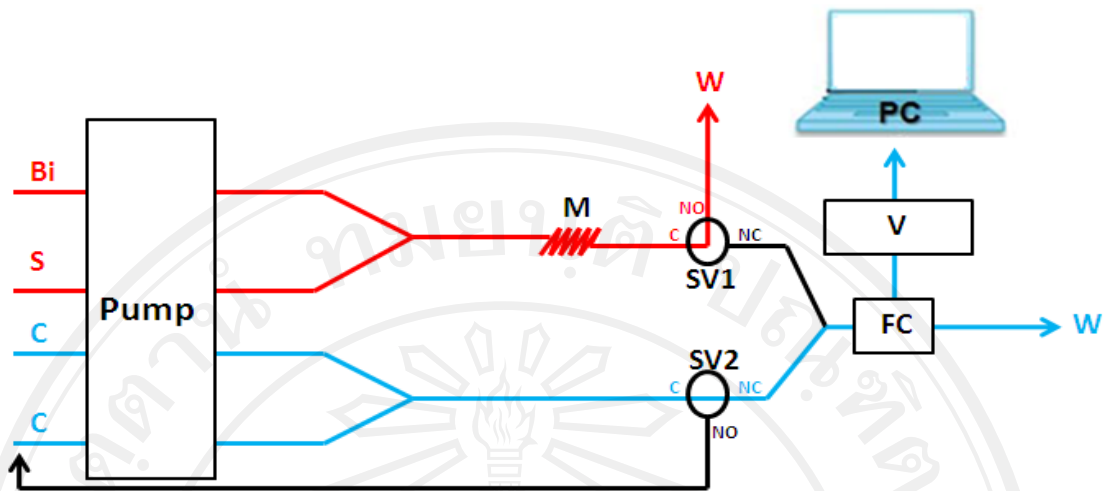
2.5 Operational procedure of the system

In the flow system, three-way solenoid valves (Biochemvalve, USA) was acted as a switching valve to allow sample or cleaning solution flow to the flow cell. In the first step, the electrode was electrochemically cleaned by applying a potential of 0.6 V to the working electrode while 1 M nitric acid was flowed through the flow cell. The solenoid valves positions were set as shown in the Figure 2.2 (a). The second step, the solenoid valves were switched to the positions as shown in the Figure 2.2 (b). Standard or sample solution was mixed on-line with Bi(III) plating solution before entering a voltammetric cell. When the analyte metals reached the flow cell, the potential of -1.50 V versus Ag/AgCl was applied to the glassy carbon electrode for a specified time. Then the flow was briefly stopped in order to perform the anodic stripping. In this step, the square wave anodic stripping voltammogram was recorded between -1.50 and 0.60 V. Then, peak potential and peak current of each metal were determined. A linear calibration graph of each metal was obtained by plotting between concentration of metal and peak current.

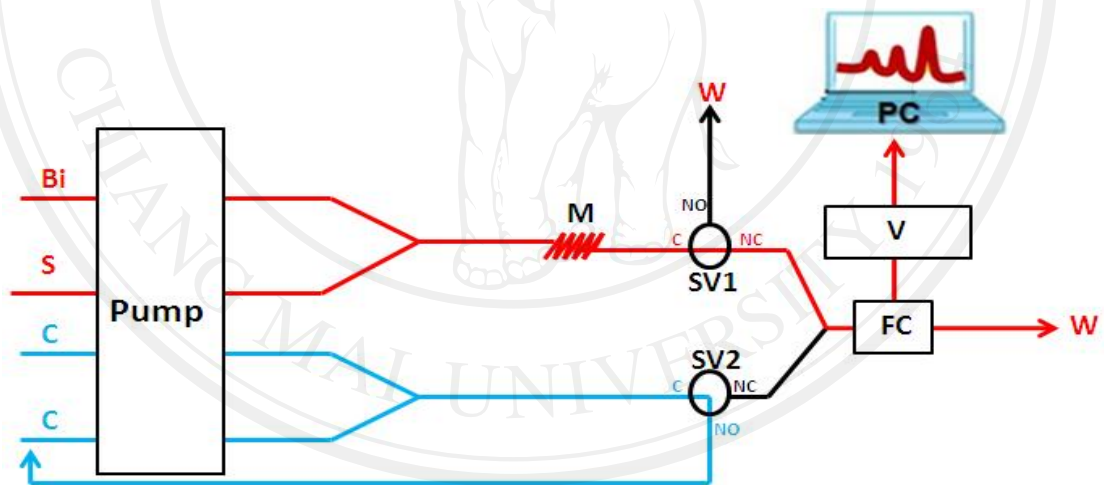
The operation of the system consisted of 4 steps which were cleaning step, deposition step, equilibration step and stripping step. The detail for each step are described and illustrated in Table 2.1 and Figure 2.2 (a) and (b), respectively. Instrumentation set up of the system is illustrated in Figure 2.3.

Table 2.1 Description of each operation step for determination of cadmium and lead

Step	Operation	Duration (s)	Flow rate (mL/min)	Solenoid valve status		Potential (V)	Description
				SV1	SV2		
1	Cleaning step	60	1.0	off	on	0.6	Load 1 M nitric acid for cleaning surface electrode
2	Deposition step	150	1.0	on	off	-1.5	Load mixed standard or sample solution with Bi(III) plating solution for deposition preconcentration on surface electrode
3	Equilibration step	30	0	off	off		Stop flow solution before Stripping
4	Stripping step	4.2	0	off	off	Scan -1.50 to 0.60	Stripping and recording of voltammogram



(a) Cleaning step: 1 M nitric acid (C) was propelled to the flow cell



(b) Deposition step or preconcentration step: mixed standard or sample solution with Bi(III) plating solution was pushed to the flow cell

Figure 2.2 Flow direction of each operation step; solenoid valve position: NC = normal close, NO = normal open and C = common (a) cleaning step (b) deposition step.

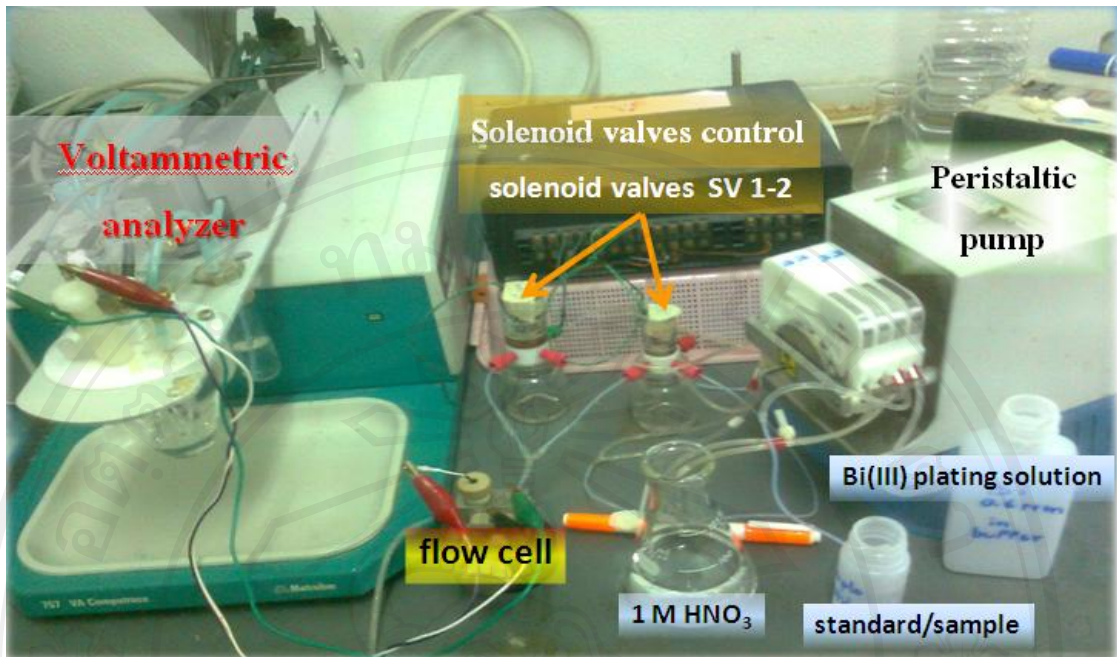


Figure 2.3 Instrumentation set up for determination of cadmium and lead

2.6 Optimization of flow based – ASV system using bismuth film electrode as working electrode

The optimization of flow based ASV system using bismuth film electrode as working electrode was performed by univariate method. One parameter was varied while the other were kept constant. The condition that provided high sensitivity, low consumption and convenient operation for determination of cadmium and lead at trace level should be considered. Almost of the results were compared in term of sensitivity, or the slope of the calibration graphs study of some parameters are described below.

2.6.1 Concentration of cleaning solution

Concentrations of nitric acid solution has a crucial role on the efficient cleaning the working electrode surface. Therefore, effect of nitric acid concentration was studied at 0.2, 0.4, 0.6, 0.8 and 1.0 M by considering the percentage relative standard deviation of peak current of the analyte metals. The conditions for the study are summarized in Table 2.2.

Table 2.2 The conditions for the study of effect of nitric acid

Parameter	Value
Standard solutions of Cd(II) and Pb(II)	100 $\mu\text{g/L}$
Concentration of acetate buffer solution	0.1 M
Concentration of Bi(III) in acetate buffer solution	0.5 mg/L
Flow rate of the sample or standard solution	0.5 mL/min
Pre-mixing coil length	100 cm
Deposition time	90 s
Deposition potential	-1.5 V
Cleaning time	60 s
Cleaning potential	0.6 V
equilibration time	30 s
Scanning potential	-1.5 to 0.6 V

2.6.2 Effect of times for polishing and storage of GCE

The procedure for storage of GCE and times for polishing of GCE were investigated in order to obtain high peak current of the metal ions. Two methods for storage of GCE was investigated.

1. The normal storage. The GCE was to kept in dry air at room temperature when was not in use. The GCE was polished with a water slurry of 1 μm alumina powder (Al_2O_3) on a polishing pad for about 2 min. Then it was immersed and stirred into water to remove the remaining Al_2O_3 particles before analysis.

2. The special storage was. The GCE kept in 0.1 M NaOH solution when was not in use. It was polished with a water slurry of 1 μm alumina powder (Al_2O_3) on a polishing pad and for 2 min to obtain a fresh surface. Then it was immersed three times into water and into ethanol alternatively to eliminate the remaining Al_2O_3 particles from its surface before analysis.

Procedure of analysis was performed by recording of peak current at the 50 $\mu\text{g/L}$ of each Cd(II) and Pb(II) at deposition time 60 s and cleaning time 60 s, while other conditions were the same as in section 2.6.1.

2.6.3 Concentration of acetate buffer pH 4.5

Effect of concentration of acetate buffer was investigated by varying concentration of acetate buffer pH 4.5 at 0.01, 0.03, 0.05, 0.10, 0.20 and 0.40 M. The optimal concentration was chosen by considering slope of calibration graphs of standard solutions of Cd(II) and Pb(II) in the range of 10 to 30 $\mu\text{g/L}$. Most of the conditions used were similar to those in Table 2.2 and 1.0 M nitric acid was used as cleaning solution.

2.6.4 Effect of pH of acetate buffer solution

The influence of the pH of buffer solution was examined by varying the pH at 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0, while other condition was the same as in section 2.6.3 and 0.1 M acetate buffer solution was used. The optimal concentration was chosen by considering on metal stripping signals of Cd(II) and Pb(II) at 20 $\mu\text{g/L}$ each of Cd(II) and Pb(II).

2.6.5 Effect of concentration Bi(III) plating solution

Effect of concentration Bi(III) plating solution was investigated by varying concentration of Bi(III) at 0.00, 0.10, 0.40, 0.60, 0.80 and 1.00 mg/L. The optimal concentration was chosen by considering on metal stripping signals of Cd(II) and Pb(II) at 10 and 30 $\mu\text{g/L}$ each of Cd(II) and Pb(II). The conditions for the study are summarized in Table 2.3. The optimal concentration Bi(III) plating solution was selected for further experiments.

Table 2.3 The conditions for the study of effect of concentration Bi(III) plating solution.

Parameter	Value
Concentration of nitric acid for cleaning solution	1.0 M
Standard solutions of Cd(II) and Pb(II)	10 and 30 $\mu\text{g/L}$
Concentration of acetate buffer solution	0.1 M
pH of acetate buffer solution	4.5
Flow rate of the sample or standard solution	0.5 mL/ min

Table 2.3 (Continued)

Parameter	Value
Pre-mixing coil length	100 cm
Deposition time	90 s
Deposition potential	-1.5 V
Cleaning time	60 s
Cleaning potential	0.6 V
Equilibration time	30 s
Scanning potential	-1.5 to 0.6 V

2.6.6 Flow rate

The influence of the flow rate on stripping peak currents was investigated at 0.20, 0.32, 0.50, 1.00, 2.00, 3.00 and 4.00 ml/min, with a fixed sample volume of metal standard solution at 20 $\mu\text{g/L}$ each of Cd(II) and Pb(II) at 1.60 mL. The conditions for the study were the same as in the Table 2.3, with Bi(III) concentration of 0.6 mg/L.

2.6.7 Stripping sweep mode

Effect of square wave parameters including of: frequency (Hz), amplitude (V) and voltage step (V) on metal stripping signals was investigated with 20 $\mu\text{g/L}$ each of Cd(II) and Pb(II). The effect of the frequency (Hz) was investigated in the range of 5 to 90 Hz, the voltage step was investigated in the range of 0.001 to 0.020 V, and the effect of the amplitude was investigated in the range of 0.010 to 0.125 V. The conditions for the study were the same in section 2.6.5.

2.6.8 Deposition potential

The effect of deposition potential was studied with standard solutions of Cd(II) and Pb(II) at 20 $\mu\text{g/L}$ each and deposition potential varying at -1.1, -1.2, -1.3, -1.4, -1.5 and -1.6 V. The conditions for the study are summarized in Table 2.4.

Table 2.4 The conditions for the study of effect of deposition potential

Parameter	Value
Concentration of nitric acid for cleaning solution	1.0 M
Standard solutions of Cd(II) and Pb(II)	20 $\mu\text{g/L}$
Concentration of acetate buffer solution	0.1 M
pH of acetate buffer solution	4.5
Concentration of Bi(III) in acetate buffer solution	0.6 mg/L
Flow rate of the sample or standard solution	0.5 mL/min
Pre-mixing coil length	100 cm
Deposition time	90 s
Cleaning time	60 s
Cleaning potential	0.6 V
Equilibration time	30 s
Scanning potential	-1.5 to 0.6 V

2.6.9 Deposition time

The deposition time was studied with standard solutions of Cd(II) and Pb(II) at 10 and 30 $\mu\text{g/L}$ each and deposition potential varying at 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300s, while other conditions were the same as in section 2.6.7. The optimal concentration was chosen by considering on metal stripping signals of Cd(II) and Pb(II).

2.6.10 Stability of sensitivity of the calibration graphs

Sensitivity of the Cd(II) and Pb(II) analysis was examined from slopes of the calibration graphs. The stability of sensitivity was studied by constructing calibration graphs for 2 times and 2 days, using concentration range of 1-30 $\mu\text{g/L}$ of Cd(II) and Pb(II) while other conditions were the same as in section 2.6.9.

2.7 Analytical characteristics of the procedure

2.7.1 Calibration curves and limit of detection

Under the optimum conditions of this system, the calibration graphs of Cd(II) and Pb(II) were constructed by using the standard solutions of Cd(II) and Pb(II) in the range of 2 - 40 µg/L. From the voltammograms obtained, peak potentials and peak currents of each metal were determined. A linear calibration graph of each metal was obtained by plotting between concentration of metal and peak current.

$$\frac{S_y}{x} = \left(\frac{\sum(Y_i - \hat{Y}_i)^2}{n - 2} \right)^{\frac{1}{2}} \quad (2.1)$$

$$C_L = 3 \times \frac{S_y/x}{b} \quad (2.2)$$

When: Y_i = response value from the instrument corresponding to the individual x- values

\hat{Y}_i = value of y on the calculated regression line corresponding to the individual x- values

n = number of points on the calibration line

b = slope of the straight line

2.7.2 Precision study

The precision of the system was examined by analysis of standard solutions of Cd(II) and Pb(II) at low, medium and high concentration levels ca. 5.0, 20.0 and 40.0 µg/L, respectively for 11 replicates. The percentage of relative standard deviation (%RSD) values was used for evaluating the precision and can be calculated from the equation (2.3).

$$\%RSD = \frac{SD \times 100}{\bar{x}} \quad (2.3)$$

When: $\%RSD$ = percentage relative standard

SD = standard deviation

\bar{x} = mean

2.7.3 Accuracy of the system

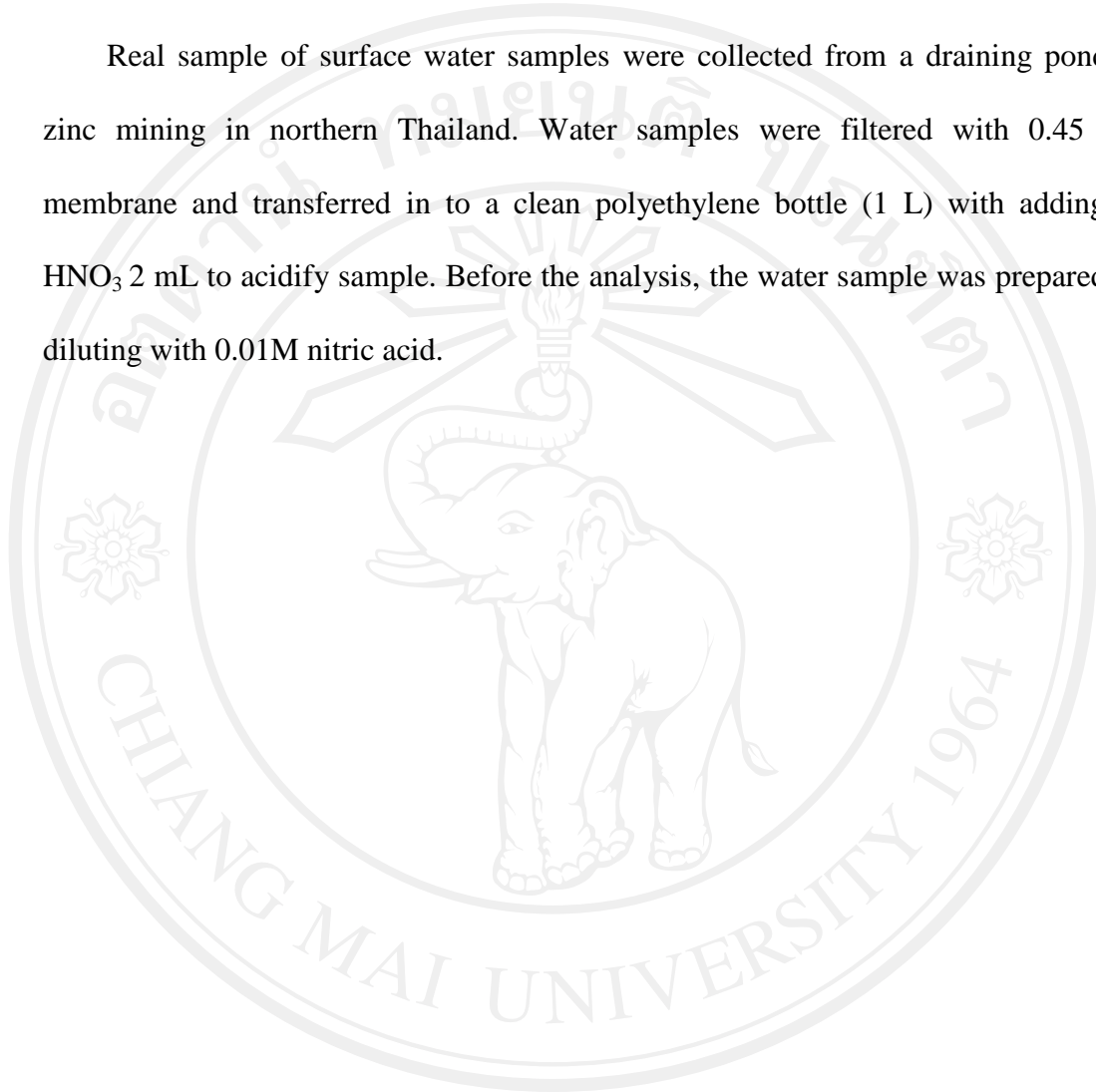
The accuracy of the system was studied by examining the percent recoveries of Cd(II) and Pb(II) at spiked concentration of Cd(II) and Pb(II) standard solution of 5, 10, 15, 20 and 25 µg/L in water samples. The recovery percentage for each sample was calculated from the equation (2.4).

$$\%Recovery = \frac{(\text{total concentration} - \text{concentration in sample})}{\text{concentration of add standard}} \times 100 \quad (2.4)$$

2.8 Preparation of sample for analysis

2.8.1 Water sample

Real sample of surface water samples were collected from a draining pond of zinc mining in northern Thailand. Water samples were filtered with 0.45 μm membrane and transferred in to a clean polyethylene bottle (1 L) with adding of HNO_3 2 mL to acidify sample. Before the analysis, the water sample was prepared by diluting with 0.01M nitric acid.



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