

CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and materials

2.1.1 Standards chemicals

- Stock solution (1000 mg L⁻¹) of copper (Carlo Erba, USA)
- Stock solution (1000 mg L⁻¹) of cadmium (Carlo Erba, USA)
- Stock solution (1000 mg L⁻¹) of lead (Merck, Germany)
- Stock magnesium nitrate (10,000 mg L⁻¹) (Perkin-Elmer, USA)
- Stock ammonium hydrogen phosphate (10%) (Perkin-Elmer, USA)
- Stock palladium (10,000 mg L⁻¹) (Perkin-Elmer, USA)
- Lanthanum (III) nitrate, pour analyse (Panreac quimicasa, EU)

2.1.2 General chemicals and solvents

- Supra pur nitric acid 65 % (Merck, Germany)
- Nitric acid 65 %, AR grade (Merck, Germany)
- Ammonium hydroxide 28-30 %, A.C.S reagent (J.T. Baker, USA)
- Glacial acetic acid, AR grade (B.D.H, England)
- Ultra pure water (18.2 MΩ) (Maxima, ELGA, England)

2.1.3 Samples

Rainwater samples were collected from nine sites in Hat Yai City Municipality, Songkhla Province (15 October 2004 – 11 December 2004) (Figure 2-2).

2.2 Instruments and apparatus

2.2.1 Graphite furnace atomic absorption spectrometer (GFAAS)

- Atomic furnace spectrometer Model AAnalyst 800 (GFAAS)
(Perkin-Elmer, USA)

- THGA graphite with pyrolytic graphite coated tubes (Perkin-Elmer, USA)
- An autosampler AS 800 (Perkin-Elmer, USA)
- Argon gas, high purity 99.999 % (TIG, Thailand)
- Computer system
- Hollow cathode lamp copper, cadmium and lead (Perkin-Elmer, USA)
- Zeeman background correction (Perkin-Elmer, USA)
- Winlab 32 AA Furnace (Perkin-Elmer, USA)

2.2.2 Apparatus

- pH meter model 15 (Denver Instrument, USA)
- Conductivity meter model Xmate 300 (MELTTER TOLEDO, Germany)
- Syringe filter 0.45 μm (Cole-Parmer, USA)
- Polyethylene bottle 250.0 mL
- Polyethylene bottle 60.0 mL
- Polyethylene bottle 30.0 mL
- Microlitre pipette model: P20, P100, P 1000 (Gilson, France)
- General glassware such as volumetric 10, 25, 50, 100, 250, 500 mL; beaker 50, 100, 500 mL
- Polyethylene buckets 20 \times 20 cm.
- Polyethylene bag

2.2.3 Solid phase extraction

- Amberlite IRC-748 chelating resin (Supelco, USA)
- Syringe 3.0 mL (Terumo, Philippines)
- Syringe 50.0 mL (Becton Dickinson, Singapore)
- Syringe adaptor (Alltech, USA)
- Stopcock one-way stopcock (Alltech, USA)
- Cotton
- Stand and clamp

2.3 Methodology

2.3.1 Preparation of working standard solutions

Standard working solutions of the metals were prepared in 1 % (v/v) nitric acid by daily dilution of the corresponding stock solutions.

2.3.2 Ammonium acetate buffer (1 M)

Ammonium acetate buffer solution was prepared by mixing 77.0 mL of 28 % ammonium hydroxide with 57.0 mL of glacial acetic acid and diluting to 1 L. The buffer pH adjusted to 9.5 by ammonium hydroxide or nitric acid (Sturgeon *et al.*, 1979).

2.3.3 Optimization of graphite furnace atomic absorption spectrometer (GFAAS)

A standard furnace program usually includes the following steps:

- Drying: The solvent is vaporized during the drying steps.
- Pyrolysis: The pyrolysis step is used to remove as many matrix components as possible. A matrix modifier can be used to stabilize the analyte or aid in removal of matrix components.
- Atomization: The sample is atomized to form ground state atoms in the path of the radiation beam during this step.
- Clean-out: A high temperature clean-out step after atomization prepared the furnace for subsequent samples (AAAnalyst 800, Perkin-Elmer).

The optimum conditions of GFAAS system were studied for pyrolysis temperature, atomization temperature, to compare the use of with matrix modifier and without matrix modifier and types of matrix modifier.

2.3.3.1 Pyrolysis temperature

The standard solutions of Cu 40.0 $\mu\text{g L}^{-1}$, Cd 4.0 $\mu\text{g L}^{-1}$ and Pb 80.0 $\mu\text{g L}^{-1}$ were determined by GFAAS. Other parameters were set as the recommended conditions by AAAnalyst 800 instrument manual. Optimum pyrolysis temperature

were investigated by varied temperature at 1000 - 1500 °C, 300 - 1100 °C and 300-1200 °C for Cu, Cd and Pb, respectively. The integrated absorbance of these metals was obtained from the peak areas. Pyrolysis temperature was obtained from at least 100 °C below the temperature where the first analyte losses are measured (Schlemmer and Radziuk, 1999).

2.3.3.2 Atomization temperature

The standard solutions of Cu 40.0 $\mu\text{g L}^{-1}$, Cd 4.0 $\mu\text{g L}^{-1}$ and Pb 80.0 $\mu\text{g L}^{-1}$ were determined by GFAAS. Pyrolysis temperature was set at optimum condition obtained from 2.3.3.1 and other parameters were set as the recommended conditions by AAnalyst 800 instrument manual. Optimum atomization temperature were investigated by varied temperature at 1700-2200 °C, 1200-1800 °C and 1200-1800 °C for Cu, Cd and Pb, respectively. The integrated absorbances of these metals were obtained from the peak areas. The atomization temperature was obtained from good peak shape i. e. minimizing peak tailing and keeping integrated time to a reasonable length (Schlemmer and Radziuk, 1999).

2.3.3.3 The effect of utilizing a matrix modifier and without a matrix modifier for determination of Cu, Cd and Pb

The standard solutions of Cu 40.0 $\mu\text{g L}^{-1}$, Cd 4.0 $\mu\text{g L}^{-1}$ and Pb 80.0 $\mu\text{g L}^{-1}$ were determined by GFAAS between with and without matrix modifier. Pyrolysis temperature and atomization temperature were set at optimum condition obtained from 2.3.3.1 and 2.3.3.2. The integrated absorbance of these metals was obtained from the peak areas. The result was evaluated from good peak shape and good peak area.

2.3.3.4 Type of matrix modifiers

The standard solutions of Cu 40.0 $\mu\text{g L}^{-1}$, Cd 4.0 $\mu\text{g L}^{-1}$ and Pb 80.0 $\mu\text{g L}^{-1}$ were determined by GFAAS with modifiers. Pyrolysis temperature and atomization temperature were set at optimum condition obtained from 2.3.3.1 -

2.3.3.2. The optimum type of modifier were investigated by varied type of modifiers as follow: $\text{Mg}(\text{NO}_3)_2$, $\text{Pd}(\text{NO}_3)_2$, $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{La}(\text{NO}_3)_2$. The optimum type of matrix modifiers was obtained from good peak area and peak shape.

2.3.3.5 Linear range

The standard stock solutions of Cu, Cd and Pb were diluted with 1 % (v/v) nitric acid to various concentrations in the range of 2.0-200.0 $\mu\text{g L}^{-1}$, 0.2-10.0 $\mu\text{g L}^{-1}$ and 5.0-200.0 $\mu\text{g L}^{-1}$, respectively. A 20.0 μL of each concentration were analyzed by GFAAS at the optimum conditions from 2.3.3.1-2.3.3.4. The linear dynamic range obtained from plotting the absorbance versus the concentration. The linearity of response was considered by the correlative coefficient of the linear curve.

2.3.3.6 Detection limit (DL)

The detection limit is defined by the IUPAC as the concentration which will give an absorbance signal three times the magnitude of the baseline noise. The baseline noise may be statistically quantitated typically by making 10 or more replicate measurements of the baseline absorbance signal observed for an analytical blank, and determining the standard deviation of the measurements. The detection limit is then defined as the concentration which produces an absorbance signal three times the standard deviation of the blank (Beaty and Kerber, 1993).

The calibration curve of Cu, Cd and Pb were determined by GFAAS. The absorption of blank (10 replicates) was measured. The detection limit was defined by equation (2.1) (Ingle and Crouch, 1988).

$$\text{Detection limit} = (3 \cdot \text{SD})/m \quad \dots\dots(2.1)$$

When, SD = standard deviation of blank
 m = slope of calibration graph

2.3.3.7 Accuracy and precision

The accuracy and precision of analytical method can be evaluated from percent recovery and % RSD, respectively (EPA method 7010). The percent recovery was performed by spiking Cu, Cd and Pb in 1 % (v/v) nitric acid and the concentration was adjusted to 40.0, 8.0 and 80.0 $\mu\text{g L}^{-1}$, respectively. The

concentration of spiking solution was evaluated by using calibration graph and then % RSD and % recovery were calculated.

2.4 Sample preparation using solid phase extraction

2.4.1 Preparation of Amberlite IRC-748 resin columns

The columns were prepared by placed a small piece of cotton at the bottom of 3.0 mL syringe as shown in Figure 2-1. Then 2.0 mL of resin in ultra pure water was added in the column. Second cotton was placed above the resin. The flow rate was set to 2.0 mL min⁻¹ by gravity. Then columns were conditioned by passing 10.0 mL of ammonium acetate buffer (1 M). The flow was stopped when the liquid level reaches the top of the resin bed (Malla *et al.*, 2002).



Figure 2-1 Preparation of Amberlite IRC-748 resin column

2.4.2 Effect of eluent concentration on desorption of Cu, Cd and Pb on Amberlite IRC-748 resin

This experiment was adapted from Malla *et al.*, 2002. The columns were prepared as described in 2.4.1. A total of 250.0 mL mixed standard solution containing 5.0 µgL⁻¹ of Cu, 1.0 µgL⁻¹ of Cd and 10.0 µgL⁻¹ of Pb was pass through the

column at flow rate 2.0 mL min^{-1} through triplicate 2.0 mL of resin columns. Before elution step the column was washed with 10.0 mL of buffer solution. The retained metals ions were eluted with 25.0 mL of 1.0 M , 3.0 M and 5.0 M nitric acid and diluted to 50.0 mL with ultra pure water and then analyzed in duplicate by GFAAS. Column blanks were obtained by the same procedure with no analyte added.

2.4.3 Effect of pH of sample solution on adsorption of Cu, Cd and Pb on Amberlite IRC-748 resin

The optimum eluent concentration of 1 M nitric acid found in the previous experiment was then used to study the effect of pH solution on adsorption of Cu, Cd and Pb on Amberlite IRC-748 resin. A mixed standard solution pH of 2.5, 3.5, 6, 8.5 and 9.5 were used and the procedure used in 2.4.1 and 2.4.2 was repeated.

2.4.4 Effect of column volume on adsorption of Cu, Cd and Pb on Amberlite IRC-748 resin

The optimum eluent concentration and pH of 3.5 found in 2.4.2-2.4.3 was then used to study the effect of column volume. The column volume was set to 0.5 , 1.0 , 1.5 , 2.0 and 2.5 mL and the procedure used in section 2.4.1-2.4.3 was repeated.

2.4.5 Effect of flow rate on adsorption of Cu, Cd and Pb on Amberlite IRC-748 resin

The optimum eluent concentration, solution pH and column volume of 2.0 mL found in the previous experiment was then used to study the effect of different flow rates during the adsorption step and desorption step. The flow rate of solutions was set to 1.0 , 2.0 and 4.0 mL min^{-1} and the procedure used in section 2.4.1-2.4.4 was repeated.

2.4.6 Effect of eluent volume on desorption of Cu, Cd and Pb on Amberlite IRC-748 resin

The optimum eluent concentration, solution pH, column volume and flow rate of solutions of 4.0 mL min^{-1} found in the previous experiment was then used to study the effect of eluent volume on desorption of Cu, Cd and Pb on Amberlite IRC-748 resin. The eluent volumes was set to 10.0, 25.0, 30.0, 40.0 and 50.0 mL and the procedure used in section 2.4.1-2.4.5 was repeated.

2.4.7 The comparison the calibration and standard addition method for determination of Cu, Cd and Pb in rainwater samples

The aim of this experiment was to study the effect of matrix for determination of Cu, Cd and Pb in rainwater samples. Rainwater were preconcentrated by the previous optimum conditions that obtained from 2.4.1-2.4.6 and then Cu, Cd and Pb was determined by using calibration and standard addition method.

2.4.8 The study of percent recovery of Cu, Cd and Pb in rainwater, drinking water and deionized water

The aim of this experiment was to establish the validity of this method and then applied for determination of Cu, Cd and Pb in rainwater samples. The % recovery was assessed by spiking Cu at concentration 1.0 and $10.0 \mu\text{g L}^{-1}$, Cd at concentration 1.0 and $5.0 \mu\text{g L}^{-1}$ and Pb at concentration 1.0 and $10.0 \mu\text{g L}^{-1}$ in rainwater samples, drinking water and deionized water and the calibration graph was established. Consequently, the metal concentration added and the % recovery of Cd was calculated from calibration graph furthermore standard addition method was applied for Cu and Pb.

2.4.9 Accuracy and precision

The section 2.4.8 was selected to study the accuracy and precision of this method. The accuracy was evaluated from % recovery and the precision of this

method was carried out by repeating measurement of Cu, Cd and Pb at 10, 5 and 10 $\mu\text{g L}^{-1}$ three times and then the precision of this method was evaluated from % RSD.

2.5 Determination of Cu, Cd and Pb in rainwater samples

2.5.1 Sampling

Rainwater samples were collected from nine sites in Hat Yai City Municipality, Songkhla Province is shown in Figure 2-2. Polyethylene bottles and polyethylene buckets were washed and soaked in 10 % nitric acid for 2 days and rinsed with ultra pure water. All cleaned containers should be sealed in clean polyethylene bags until required. A sample of rainwater was collected in a polypropylene bucket on the chair at sites. The height of the collection bucket should be 0.5 m from the ground (EANET, 2000). The sample collection at sites is shown in Figure 2-3. As soon as the sample collection was completed, one small portions in a polyethylene bottles for measurements of conductivity and pH. The measurement of conductivity and pH of rainwater samples are shown in Figure 2-4 and 2-5. The remaining rainwater samples were filtered through with 0.45 μm membrane syringe filter and acidification of the sample with 2.0 mL of 10 % nitric acid for preservation and storage at $-20\text{ }^{\circ}\text{C}$ (Ward, 1995). The sample filtration is shown in Figure 2-6. The information of rainwater samples is shown in Table 2-1.



Figure 2-2 Sampling sites in Hat Yai City Municipality



Figure 2-3 Sample collections at fountain circus

Table 2-1 The information of rainwater samples

Location	Date of collection	Time	pH	EC ($\mu\text{s cm}^{-1}$)
1. Department of chemistry, Prince of Songkla University	15/10/2004	03.45 p.m.-04.05 p.m.	3.85	7.14
2. Makro Supermarket	7/12/2004	07.00 a.m.-08.30 a.m.	3.95	36.30
3. Srinakorn School	8/12/2004	01.30 p.m.-02.30 p.m.	4.66	5.67
4. Hat Yai Municipality Park	8/12/2004	08.30 a.m.(7/12/2004)- 10.00 a.m.(8/12/2004)	4.45	12.29
5. Kuanlung Cross-road	9/12/2004	04.30 p.m.(8/12/2004)- 01.00 p.m.(9/12/2004)	5.97	11.62
6. Fountain Circus	9/12/2004	02.00 p.m.(8/12/2004)- 02.00 p.m.(9/12/2004)	6.06	12.55
7. Hat Yai Wittayalai Somboonkulan ya School	9/12/2004	05.00 p.m.(8/12/2004)- 01.00 p.m.(9/12/2004)	5.49	9.23
8. Jiranakorn Stadium	11/12/2004	07.10 a.m.-05.30 p.m.	4.19	73.76
9. Hat Yai Railway Station	11/12/2004	07.10 a.m.-05.30 p.m.	5.35	119.67



Figure 2-4 Conductivity measurements (EC, $\mu\text{S cm}^{-1}$)



Figure 2-5 pH measurements

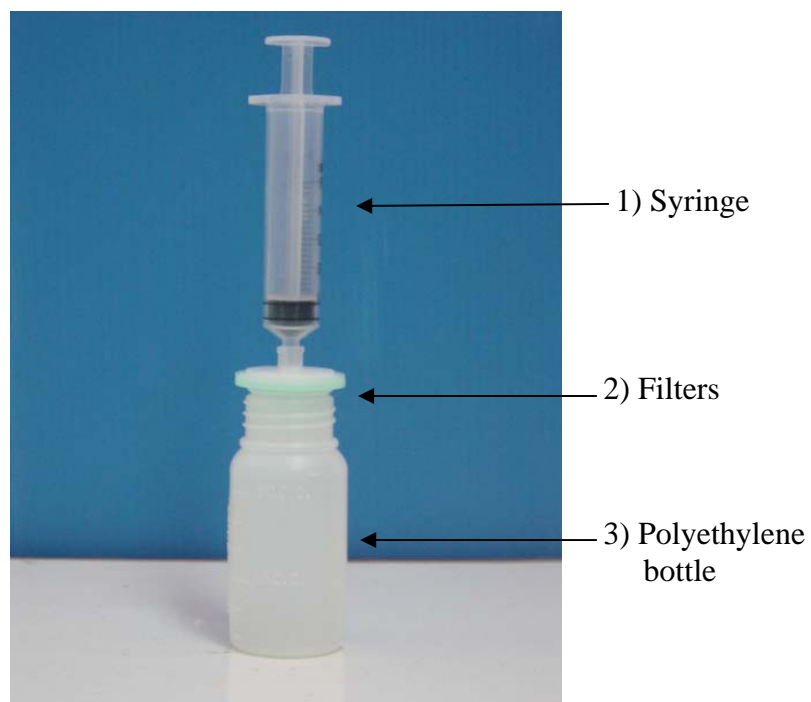


Figure 2-6 Sample filtration 1) Syringe 2) Filters and 3) Polyethylene bottle

2.5.2 Determination of Cd, Cu and Pb in rainwater samples

A 250.0 mL of rainwater were preconcentrated by the previous optimum conditions that obtained from 2.4.1-2.4.6. The concentration of Cd was calculated by calibration method. The concentrations of Cu and Pb were calculated by standard addition method.