CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and apparatus

2.1.1 Chemicals

1. 1-(2-pyridylazo)-2-naphthol (PAN) :$C_{15}H_{11}N_3O$ (Carlo Erba, Italy)
2. Octylphenoxypolyethoxyethanol (Triton X-114) :

   $C_8H_{16}C_6H_4(CH_2CH_2O)_{10}H$ (Fluka, Buches, Switzerland)
3. Nitric acid, 65% (AR grade, Merck, Germany)
4. Methanol, 99.9% (AR grade, Merck, Germany
5. Hydrogen peroxide, 30% (AR grade, Merck, Germany)
6. Glacial acetic acid (Qrec, New Zealand)
7. Cadmium standard solution, 1000 ppm (Fluka, Buches, Switzerland)
8. Lead standard solution, 1000 ppm (Fluka, Buches, Switzerland)
9. Deionized water

2.1.2 Apparatus

1. Flame atomic absorption spectrophotometer, model Analyst 680 (Shimadzu, Tokyo, Japan)
2. Hollow cathode lamp (Shimadzu, Tokyo, Japan)
3. Microwave digestion system, model Ethos Sel (Milestone Ethos, Italy)
4. Oven (Memmert, Denmark)
5. Centrifuge Model PLC-012E (Gemmy industrial corporation, Taiwan)

6. Hot plate Model C-MAC HS7 (IKA, Germany)
7. Micropipettes (Gilson Medical Electronics, France)
8. pH/Ion 510 bench meter (Oakton instruments, USA)
9. Magnetic bar (Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
10. Thermometer
11. Stand and clamp
12. Ice bath
13. Stopwatch
14. stainless steel scissors

2.2 Preparation standard solutions and reagents

Deionized water was used to prepare all solutions. All labware used for handling solution was cleaned with detergent solution, rinsed with tap water, soaked for at least 24 h in 10% nitric acid and then rinsed with deionized water before used.

2.2.1 Nitric acid solution

Stock solution of 1% HNO_3 was prepared daily by adding 3.80 ml of concentrated HNO_3 solution in deionized water and adjusting to 250 ml with deionized water in volumetric flask.

2.2.2 Acetic acid solution

A 0.50 mol l^{-1} acetic acid solution was prepared by adding 2.86 ml of acetic acid glacial in deionized water and adjusting to 100 ml with deionized water in volumetric flask.

2.2.3 Acetate buffer solution

Acetate buffer solution pH 3 was prepared by dissolving 0.0650 g of trihydrate sodium acetate ($CH_3COONa \cdot 3H_2O$) in 0.50 mol l^{-1} acetic acid solution and adjusting to 50 ml with 0.50 mol l^{-1} CH_3COOH in volumetric flask.

2.2.4 Methanol in 0.1 mol l^{-1} HNO_3

Methanol in 0.1 mol l^{-1} HNO_3 was prepared by adding 0.35 ml of concentrated HNO_3 in 99.9% methanol and adjusting to 50 ml with 99.9% methanol in volumetric flask.

2.2.5 Preparation of 100 μg ml^{-1} standard solution for cadmium

A concentration of 100 μg ml^{-1} standard solution was prepared by transferring a portion of 5.00 ml of 1000 μg ml^{-1} standard solution in to 50 ml volumetric flask and adjusting to volume with 1% HNO_3.

2.2.6 Preparation of 50 μg ml^{-1} standard solution for cadmium

A concentration of 50 μg ml^{-1} standard solution was prepared by transferring a portion of 25.00 ml of 100 μg ml^{-1} standard solution in to 50 ml volumetric flask and adjusting to volume with 1% HNO_3.

2.2.7 Preparation of 100 μg ml^{-1} standard solution for lead

A concentration of 100 μg ml^{-1} standard solution was prepared by transferring a portion of 5.00 ml of 1000 μg ml^{-1} standard solution in to 50 ml volumetric flask and adjusting to volume with 1% HNO_3.

2.2.8 Preparation of the working standard solutions for cadmium

Working standard solutions for calibration curve at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 μg ml^{-1} were prepared daily by transferring portions of 0.25, 0.50, 0.75, 1.00, 1.25 ml of 50 μg ml^{-1} standard solution in to 25 ml volumetric flasks and adjusting to volume with 1% HNO_3.

2.2.9 Preparation of the working standard solutions for lead

Working standard solutions for calibration curve at concentrations of 5, 10, 15, 20, 25 μg ml^{-1} were prepared daily by transferring portions of 1.25, 2.50, 3.75, 5.00, 6.25 ml of 100 μg ml^{-1} standard solution in to 25 ml volumetric flasks and adjusting to volume with 1% HNO_3.

2.2.10 1-(2-pyridylazo)-2-naphthol (PAN) solution

A $4x10^{-3}$ mol l^{-1} of PAN solution was prepared by dissolving 0.0519 g of PAN with methanol and adjusting to 50 ml with methanol in volumetric flask.

2.2.11 Triton X-114 solution

A 1% v/v of Triton X-114 solution was prepared by dissolving 0.50 ml of Triton X-114 with deionized water and adjusting to 50 ml with deionized water in volumetric flask.

2.3 Analytical characteristics of the method

2.3.1 Precision

The precision of the FAAS instrument was studied by analyzing 1 μg ml^{-1} standard solution of Cd for ten times and analyzing 10 μg ml^{-1} standard solution of Pb for ten times. The relative standard deviation was calculated.

2.3.2 Detection limit

The detection limits of the method for determination of cadmium and lead were studied after sample measurement by analyzing blank ten times**.**

2.3.3 Accuracy

Accuracy was calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample. The extracted sample solutions were prepared by spiking 1 μg ml^{-1} and 2 μg ml^{-1} of cadmium standard solutions and 10 μg ml^{-1} and 20 μg ml^{-1} of lead standard solutions, relatively to 5 ml of extracted sample solutions prior to cloud point extraction. The recovery was replicated three times and the results were calculated from the following equation.

$$\% \text{ recovery} = \frac{\textit{spike sample result} - \textit{sample result}}{\textit{spike amount added}} \times 100$$

2.4 Sample preparation

2.4.1 Preparation of human hair samples

Prior to analysis, the human hair samples were cut into 2 cm with a stainless steel scissors for preventing contaminants. The hair samples were first washed with acetone (human hair (g) : acetone (ml) = 0.50 : 25.00), after that the hair samples were washed triply with deionized water (human hair (g) : deionized water (ml) = 0.50 : 25.00), and finally, they were again washed with acetone one more time. The hair samples were then oven-dried at 110 ºC for 16 hrs. This optimum condition was followed by the International Atomic Energy Agency (IAEA) [4].

Figure 2.1 The human hair samples (2 cm)

2.4.2 Human hair samples decomposition

In order to decompose the human hair samples, 0.50 g of hair sample was put into the PTFE digestion vessel and treated with 10.00 ml of concentrated HNO_3. The hair samples were then decomposed by microwave digestion system.

Figure 2.2 The microwave digestion system [48]

Table 2.1 Heating program for the hair sample digestion procedure

Step	Power (watts)	Time (min)
1	250	6
2	350	6
3	500	10
4	250	6
5	0	2

After cooling to room temperature, the solutions were transferred into beakers. To each sample, 0.50 ml of 30% H_2O_2 was added dropwise and heated for some minutes. The solutions obtained were made up to 50.00 ml with deionized water.

2.5 Cloud point extraction (CPE) procedure

For cloud point extraction of cadmium and lead was used 1-(2-pyridylazo)-2-naphthol (PAN) as chelating agent to form hydrophobic chelates and used octylphenoxypolyethoxyethanol (Triton X-114) as non-ionic surfactant. The aliquots of 10.00 ml of the solution containing the analyte, PAN, Triton X-114 and acetate buffer solution pH 3. Then, the volume made up to 10.00 ml with deionized water. The mixtures were heated at 40ºC. Separation of the supernatant aqueous phase and surfactant rich-phase was achieved by centrifugation at 3400 rpm for 10 minutes. After phase separation by centrifugation the mixtures were cooled in an ice–acetone bath, the surfactant-rich phase became viscous and retained at the bottom of the test tube. The supernatant aqueous phase was separated by a dropper. The surfactant-rich phase was treated with methanol in 0.1 mol l^{-1} HNO_3 in order to reduce its viscosity and facilitate sample handling. The final solution was introduced into the flame atomic absorption spectrophotometer. The flow chart of CPE procedure is shown in Figure 2.3.

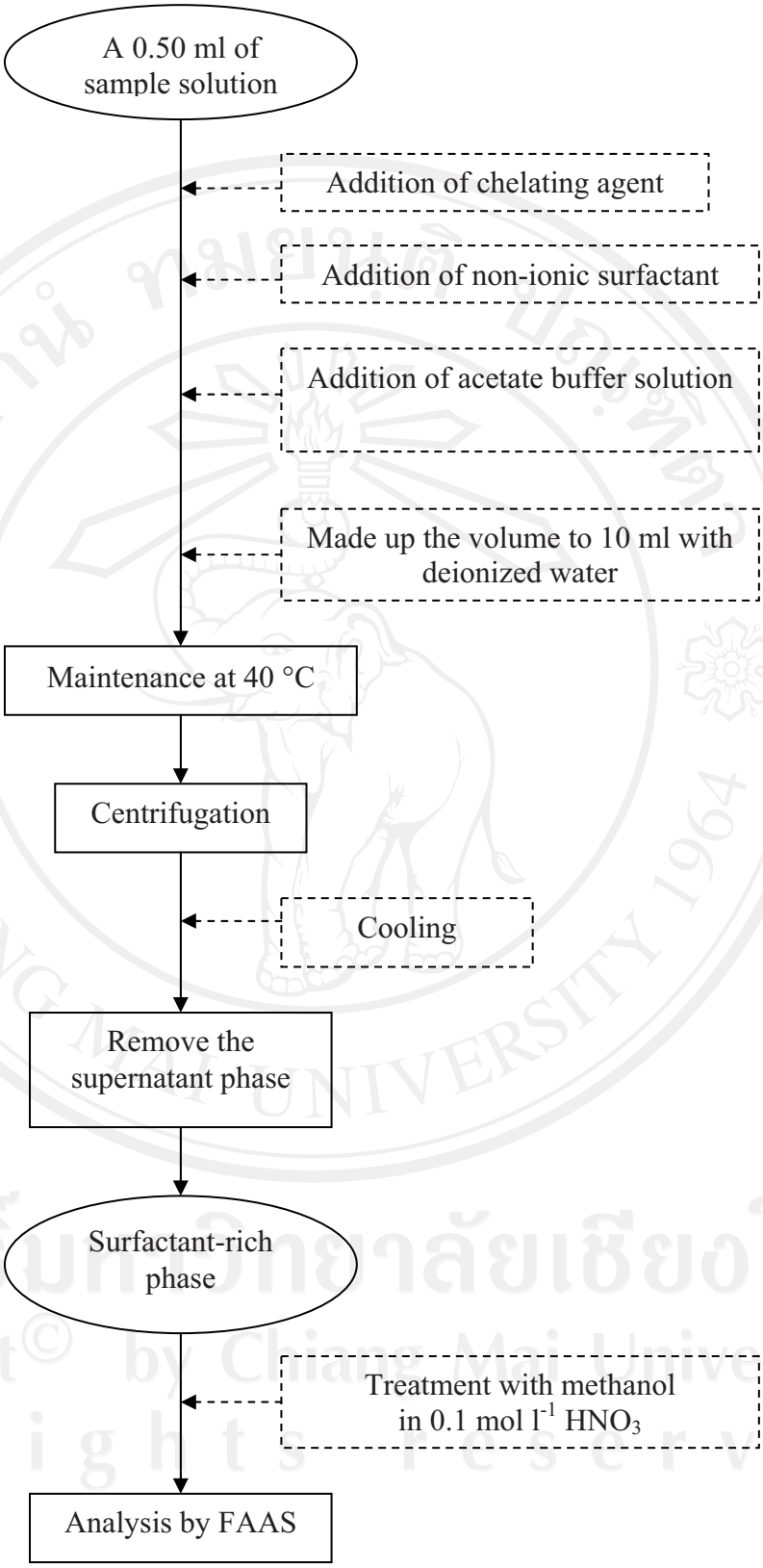

Figure 2.3 The flow chart of cloud point extraction procedure

2.6 Optimization of cloud point extraction (CPE)

There were several chemical parameters that influence on the extraction efficiency should be studied and optimized. The factors including the concentration of PAN and Triton X-114, complexing time, pH of the sample solution and the viscosity affecting the detection process were investigated. The extracted solutions were determined by flame atomic absorption spectrophotometry. The optimum conditions in this study were obtained by the maximum analytical signal of cadmium and lead condition.

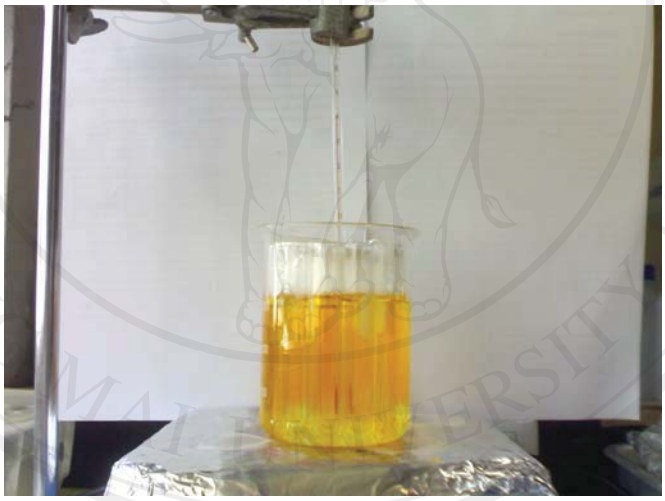

Figure 2.4 Cloud point extraction experiment

Optimization procedure of CPE was examined as described in **section 2.5**. The variations in extraction efficiency of chemical parameters were studied. And the conditions of chemical parameters are shown in **Table 2.2**.

Table 2.2 The conditions for optimization of cloud point extraction

Parameters	Studied range
Complexing time (min)	15 - 60
PAN concentration (mol l^{-1})	$2x10^{-5}$ - $1x10^{-3}$
Triton X-114 concentration (%v/v)	0.02 - 0.20
pH	1 - 6
Buffer solution volume (ml)	0.20 - 3.00
MeOH in 0.1 mol l^{-1} HNO_3 volume (µl)	100 - 1000

2.7 The interference effect study

The experimental procedure was examined as described in **section 2.5**. The effect of interferences to Cd in cloud point extraction was studied by adding Cd and interfering elements with various ratios into the studied samples. And for the effect of interferences to Pb was studied by adding Pb and interfering elements with various ratios into the studied samples. The interfering elements including Fe, Zn, Mn and Cu were investigated. The conditions of interference effects are shown in **Table 2.3** and **Table 2.4**.

Table 2.3 The conditions of interference effects for Cd

Parameters	Conditions
1. Sample (ml)	5.00
2. Cd : interfering element (μg ml^{-1})	1:100, 1:500, 1:1000, 1:1500, 1:2000
3. PAN (mol l^{-1})	$2x10^{-4}$
4. Triton X-114 (%v/v)	0.04
5. Acetate buffer solution pH 3 (ml)	0.40
6. Heat (ºC), time (min)	40, 30
7. Centrifugation (rpm), time (min)	3400, 10
8. Cooling time (min)	10
9. Volume of MeOH in 0.1 mol l^{-1} HNO_3 (μl)	500

Table 2.4 The conditions of interference effects for Pb

Parameters	Conditions
1. Sample (ml)	5.00
2. Pb : interfering element ($\mu g\ ml^{-1}$)	1:100, 1:500, 1:1000, 1:1500, 1:2000
3. PAN (mol l^{-1})	$2x10^{-4}$
4. Triton X-114 (%v/v)	0.04
5. Acetate buffer solution pH 3 (ml)	0.40
6. Heat (ºC), time (min)	40, 30
7. Centrifugation (rpm), time (min)	3400, 10
8. Cooling time (min)	10
9. Volume of MeOH in 0.1 mol l^{-1} HNO_3 (μl)	500

2.8 Determination of cadmium and lead in human hair samples

2.8.1 Cloud point extraction condition

The conditions of cloud point extraction used for preconcentration of Cd and Pb in human hair samples are shown in **Table 2.5**.

Table 2.5 The conditions for preconcentration of Cd and Pb in human hair samples

Parameters	Conditions
1. Sample (ml)	5.00
2. PAN (mol l^{-1})	$2x10^{-4}$
3. Triton X-114 (%v/v)	0.04
4. Acetate buffer solution pH 3 (ml)	0.40
5. Heat (ºC), time (min)	40, 30
6. Centrifugation (rpm), time (min)	3400, 10
7. Cooling time (min)	10
8. Volume of MeOH in 0.1 mol l^{-1} HNO_3 (µl)	500

2.8.2 Flame atomic absorption spectrophotometry

The flame atomic absorption spectrophotometer (Analyst 680, Shimadzu, Tokyo, Japan) was used for cadmium and lead determination. The cadmium and lead hollow cathode lamps (Shimadzu) were used as light source. The operating parameters of Cd and Pb were set as recommended by the manufacturer in **Table 2.6**.

Table 2.6 Instrument settings and analytical conditions of Cd and Pb in FAAS

Parameters	Cd	Pb
Wavelength (nm)	228.8	283.3
Slit width (nm)	0.3	1.0
Lamp current (mA)	4	10
Fuel gas	Air/acetylene	Air/acetylene
Flow rate of air (l/min)	8	8
Flow rate of acetylene (l/min)	1.8	2.0
Burner height (mm)	7	7

2.9 Human hair samples

The human hair samples were collected from different cases. The human hair samples including untreated hair sample, hair sample of a person worked in heavy metals laboratory, hair samples of workers who worked in electronic industry and dyed hair samples were studied. The representation of human hair samples are shown in **Table 2.7**.

Table 2.7 Human hair samples from various cases

Samples	Details
A-1	Untreated hair sample
B-1	Hair sample from the person who worked in laboratory of heavy metals for 4 years
C-1	Hair sample from the worker who worked in electronic industry for 5 years
C-2	Hair sample from the worker who worked in electronic industry for 8 years
C-3	Hair sample from the worker who worked in electronic industry for 15 years
D-1	Dyed hair sample for 6 months
D-2	Dyed hair sample for 1 year
D-3	Dyed hair sample for 2 years

Table 2.7 Human hair samples from various cases (continued)

Samples	Details
D-4	Dyed hair sample for 4 years
D-5	Dyed hair sample for 7 years
D-6	Dyed hair sample for 8 years
D-7	Dyed hair sample for 9 years
D-8	Dyed hair sample for 10 years