CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and apparatus

2.1.1 Chemicals

1. Ammonium-pyrrolidine dithio carbamate (APDC): $C_5H_9NS_2.NH_4$ (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. Chromium standard solution, 1000 ppm (Fluka, Buches, Switzerland)
8. Lead standard solution, 1000 ppm (Fluka, Buches, Switzerland)
9. Nickel standard solution, 1000 ppm (Fluka, Buches, Switzerland)
10. Deionized water

2.1.2 Apparatus

1. Flame atomic absorption spectrophotometer, model AAnalyst 800 (Perkin Elmer, Norwalk, USA)
2. Hollow cathode lamp (Shimadzu, Tokyo, Japan)

3. Ultrasonic cleaner, ultrasonic power 250W; frequency 53 kHz (KUDOS-SK250H, China)
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. Thermometer
10. Ice bath

2.2 Preparation standard solutions and reagents

2.2.1 Nitric acid solution

Stock solution of 1% HNO_3 was prepared daily by adding 15.40 ml of concentrated HNO_3 solution in deionized water and adjusting to 1000 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 glacial acetic acid in deionized water and adjusting to 100 ml with deionized water in volumetric flask.

2.2.3 Acetate buffer solution

Acetate buffer solution pH 4 was prepared by dissolving 0.0650 g of trihydrate sodium acetate ($CH_3COONa.3H_2O$) 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 Ammonium-pyrrolidine dithio carbamate (APDC) solution

A $6x10^{-3}$ mol l^{-1} of APDC solution was prepared by dissolving 0.0493 g of APDC with methanol and adjusting to 50 ml with methanol in volumetric flask.

2.2.6 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.

2.2.7 Standard solution preparation

- Preparation of 100 μg ml^{-1} standard solution for Pb, Cr and Ni

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

- Preparation of 50 ppm standard solution for Cr and Ni

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.

- Preparation of the working standard solutions for Pb, Cr and Ni

The five concentrations of Pb, Cr and Ni standard solutions were prepared from 100 μg ml^{-1} Pb and 50 μg ml^{-1} Cr and Ni standard solutions. The five concentrations of Pb, Cr and Ni standard solutions for FAAS determination are shown in **Table 2.1.**

Table 2.1 The concentrations of lead, chromium and nickel standard solutions for FAAS determination

Standard solution	**Standard solution concentration (μg ml^{-1})**
Pb	1, 5, 10, 15, 20
Cr	0.5, 1, 2, 3, 4
Ni	0.5, 1, 2, 3, 4

2.3 Analytical characteristics of the method

2.3.1 Precision [45]

The precision of the FAAS instrument was studied by analyzing the 3, 2 and 10 μg ml^{-1} standard solution of Ni, Cr and Pb, respectively, for ten times. The relative standard deviation of the results will be indicated the repeatability of the FAAS instrument. Relative standard deviation, RSD, may be expressed as a fraction less than 1 or as a percentage.

$$RSD = SD/X$$

$$\%\ RSD = (SD/X) \times 100$$

Where RSD is the relative standard deviation

SD is the standard deviation

X is the mean of data

2.3.2 Limit of Detection (LOD) and Limit of quantitation (LOQ) [46]

Limit of Detection (LOD) is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence). The LOD is typically determined to be in the region where the signal to noise ratio is greater than 5.

Limit of quantitation (LOQ) is the level above which quantitative results may be obtained with a specified degree of confidence. The LOQ is mathematically defined as equal to 10 times the standard deviation of the results for a series of replicates used to determine a justifiable limit of detection.

The LOD and LOQ of the method for determination of Ni, Cr and Pb were studied after sample measurement by analyzing blank for ten times.

The detection limits = (3 x SD) / Slope

Where SD is standard deviation of bank

Slope is slope of calibration curve

3 is Z-value at 99% confidence limit level

The limits of quantitation = (10 x SD) / Slope

Where SD is standard deviation of bank

Slope is slope of calibration curve

10 is Z-value at 95% confidence limit level

2.3.3 Accuracy [46]

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 3 and 5 μg ml^{-1} of nickel standard solutions, 1 and 2 μg ml^{-1} of chromium standard solutions and 10 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 the percentage of recovery. The recovery was replicated three times and the results were calculated from the following equation.

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

2.4 Preparation of human hair samples

Prior to analysis, all hair samples were cut into 1-2 cm with stainless steel scissor and washed according to the procedure described by the International Atomic Energy Agency(IAEA) [54]. Humanhair samples were first washed with acetone, and then washed three times with DI water and were again washed with acetone once more time. Finally, samples were dried in an oven at 110 ºC for 16 hrs and kept in a desiccators.

Figure 2.1 The human hair samples

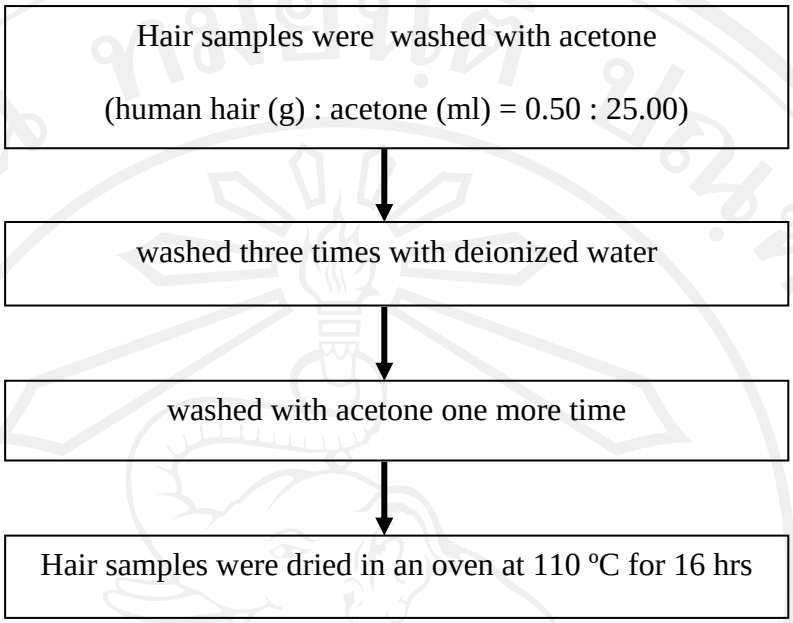

Figure 2.2 The flow chart of hair washing

2.5 Ultrasonic acid digestion (UAD) method

The 200 mg of human hair sample was weighed and put into test tube before adding mixed standard solution of Ni, Cr and Pb. Then, the concentrated HNO_3 and 30 % H_2O_2 were added. The solution was kept at room temperature, marked as presonication time (PST). After each time intervals of the PST, test tubes were placed inside the ultrasonic bath and were sonicated (ultrasound energy remaining at 53 kHz), termed as sonication time (ST). The final volume was made up to 10 ml in volumetric flasks with deionized water and stored in polyethylene tubes at 4 ºC for analyses. Blanks were also treated in the same way without samples for each experiment.

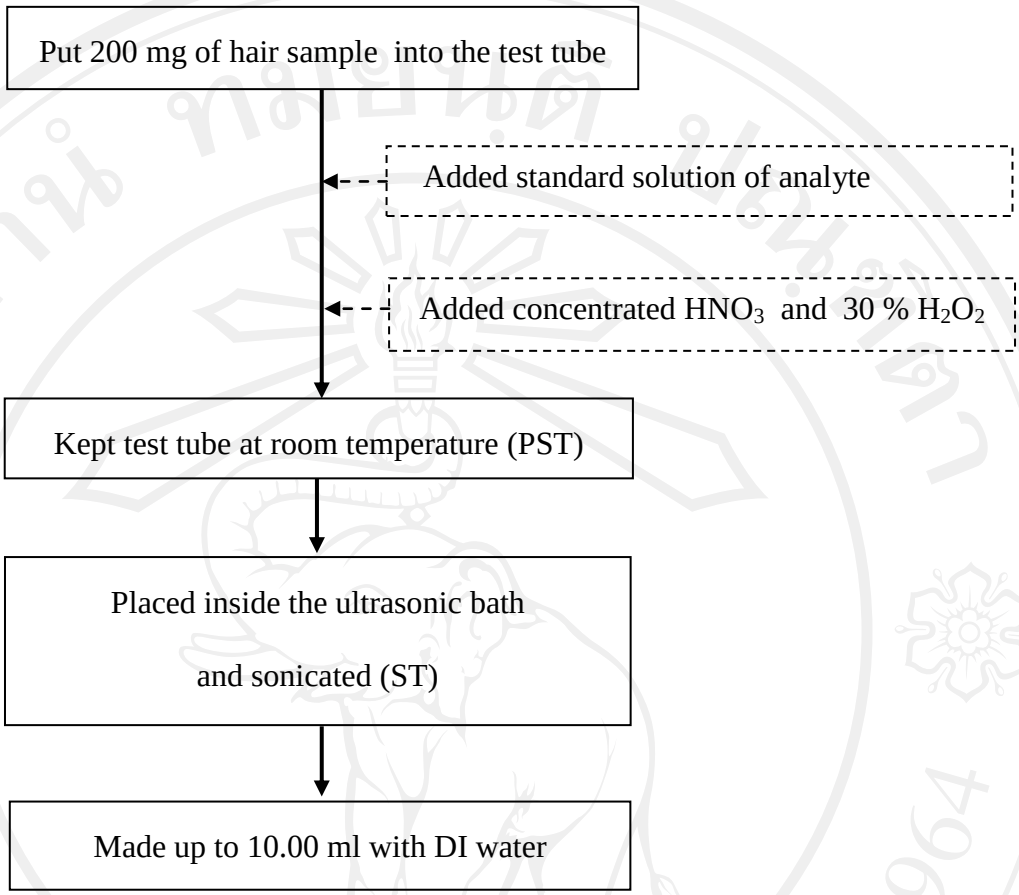

Figure 2.3 The flow chart of ultrasonic acid digestion

2.5.1 Optimization of ultrasonic acid digestion

Variables influencing the ultrasonic acid digestion were optimized within the intervals are shown in **Table 2.2**. The digest solutions were determined by flame atomic absorption spectrometry. The optimum conditions in this study were obtained by the maximum analytical signal of chromium, nickel and lead condition.

Table 2.2 The conditions for optimization of ultrasonic acid digestion

Parameters	Studied rang
1. Sample (mg)	200
2. Analyte Cr ($\mu g\ ml^{-1}$)	2
3. Analyte Ni ($\mu g\ ml^{-1}$)	3
4. Analyte Pb ($\mu g\ ml^{-1}$)	10
5. Solvent systems $HNO_3 : H_2O_2$ (ml)	2:0, 2:0.5, 2: 1, 2:1.5, 2: 2
6. Presonication time (min)	0-60
7. Sonication time (min)	5-60
8. Temperature of ultrasonic bath (ºC)	30-80

2.6 Cloud point extraction (CPE) procedure

For cloud point extraction of chromium, nikel and lead was used ammonium-pyrrolidine dithio carbamate (APDC) 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, APDC, Triton X-114 and acetate buffer solution pH 4. Then, the volume made up to be 10.00 ml with deionized water. The mixtures were heated at 40 ºC. Separation of the supernatant aqueous phase and surfactant rich-phase was achieved by centrifugation at 3400 rpm

for 10 minutes. The surfactant-rich phase became viscous and the supernatant aqueous phase was separated. To decrease the viscosity of surfactant-rich phase and facilitate both sample handling and introduction to FAAS, the methanol containing 0.1 mol l^{-1} HNO_3 was added to make the final volume of 3.5 ml. The solution was introduced by conventional aspiration into a flame atomic absorption spectrometer. The flow chart of CPE procedure is shown in **Figure 2.4**.

2.6.1 Optimization of cloud point extraction (CPE)

There were several parameters that influence on the extraction efficiency should be studied and optimized are shown in **Table 2.3**. The extracted solutions were determined by flame atomic absorption spectrometry. The optimum conditions in this study were obtained by the maximum analytical signal of chromium, nickel and lead condition.

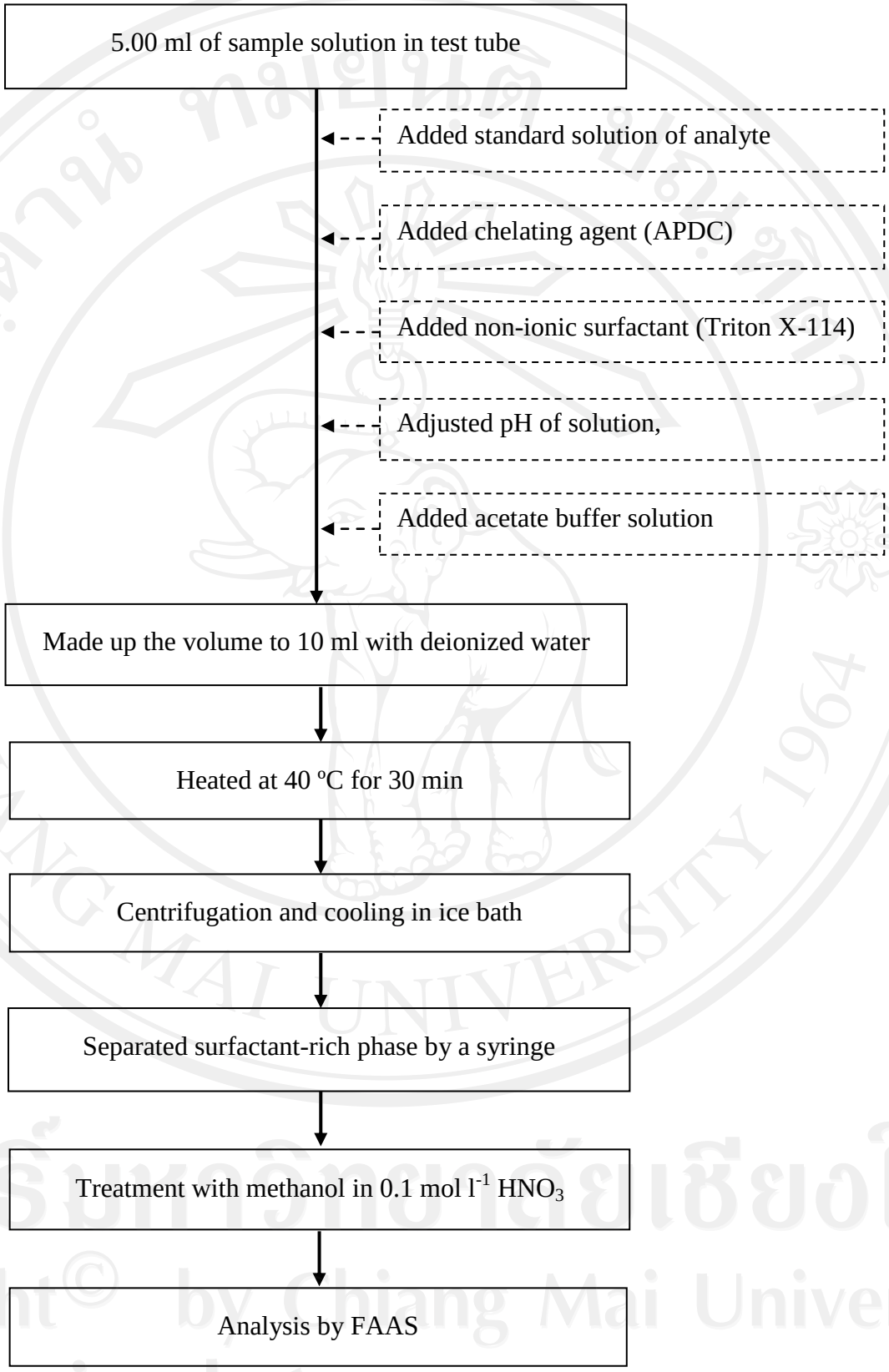

Figure 2.4 The flow chart of cloud point extraction procedure

Table 2.3 The conditions for optimization of cloud point extraction

Parameters	Studied range
1. Sample solution (ml)	5
2. Analyte Cr ($\mu g\ ml^{-1}$)	2
3. Analyte Ni ($\mu g\ ml^{-1}$)	3
4. Analyte Pb ($\mu g\ ml^{-1}$)	10
5. APDC concentration ($mol\ l^{-1}$)	$3x10^{-5}$ – $1.5x10^{-3}$
6. Triton X-114 concentration (%v/v)	0.01 - 0.30
7. Complexing time (min)	10 – 60
8. Temperature (^oC)	30 - 50
9. pH of solution	2 - 8
10. Acetate buffer solution pH 4 (ml)	0.4
11. MeOH in 0.1 $mol\ l^{-1}$ HNO_3 volume (μl)	500

2.7 Determination of chromium,nickel and lead in human hair samples

The conditions of ultrasonic acid digestion used for digest human hair samples are shown in **Table 2.4**.

Table 2.4 Operating conditions for ultrasonic acid digestion of Cr, Ni and Pb from human hair samples

Parameters	**Conditions**
1. Sample (mg)	200
2. Solvent systems HNO_3 : H_2O_2 (ml)	2 : 1
3. Presonication time (min)	10
4. Sonication time (min)	30
5. Temperature of ultrasonic bath (ºC)	60

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

Table 2.5 The conditions for preconcentration of Cr, Ni and Pb in human hair samples

Parameters	**Conditions**
1. Sample (ml)	5.00
2. APDC (mol l^{-1})	$6x10^{-4}$
3. Triton X-114 (%v/v)	0.10
4. Acetate buffer solution pH 4 (ml)	0.50
5. Complexing time (min)	30
6. Temperature (^{o}C)	40
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

The flame atomic absorption spectrometers (AAS Analyst 800, PerkinElmer, Germany) was used for chromium, nickel and lead determination. The hollow cathode lamp (PerkinElmer) was the light source. The operating parameters of Cr, Ni and Pb were set as recommended by the manufacturer in **Table 2.6** [48].

Table 2.6 Instrument settings and analytical conditions of Cr, Ni and Pb in FAAS

Parameters \ Metal	Cr	Pb	Ni
1. Wavelength (nm)	357.9	283.3	232
2. Slit width (nm)	0.7	1.0	0.2
3. Lamp current (mA)	4	10	20
4. Flow rate of air/ acetylene (l/min)	8/1.8	8/1.8	8/1.8

The human hair samples were collected from different cases described are shown in **Table 2.7**.

Table 2.7 Human hair samples from various cases

Samples	Details
A	Untreated hair sample
B	Hair sample from the person who drug abuse for 1 years
C-1	Hair sample from the person who dyed color hair for 3 years
C-2	Hair sample from the person who dyed color hair for 5 years
D-1	Hair sample from the person who smoking for 1 years
D-2	Hair sample from the person who smoking for 3 years
E-1	Hair sample from the worker who worked in food industry for 2 years
E-2	Hair sample from the worker who worked in lens industry for 2 years
E-3	Hair sample from the worker who worked in electronic industry for 4 years