# CHAPTER 2

# EXPERIMENTAL

## 2.1 Instruments and Apparatus

2.1.1 Analytical balance, BP121S model, Sartϕrius, Germany

2.1.2 Atomic Absorption Spectrophotometer, AAS 670 model, Shimudzu, Japan

2.1.3 Balance, Precisa junior 500 C model, Swiss Quality, Switzerland

2.1.4 Basic orbital incubator, IOX400.XX2.C model, Gallenkamp, England

2.1.5 Horizontal shaker, 7400 Tubigen (KL2) model, Edmund Buhler, Germany

2.1.6 Centrifuge, Allegra 64 R model, Beckman Coulter, USA

2.1.7 Centrifuge tube, 50 mL Polycarbonate material, Nalgene, USA

2.1.8 Hot plate/stirrer, 4658 model, Cole-Parmer Instrument, USA

2.1.9 pH meter, Precisa pH 900 model, Swissmade, Switzerland

2.1.10 Oven, BD/ED/FD with R3-Controller model, Binder, Germany

2.1.11 Sieve 0.1 mm, Maruto Testing Machine MFG.Co.Ltd., Japan

## 2.2 Chemicals and Reagents

2.2.1 Ammonium acetate ($CH_3COONH_4$, 96% w/w), G.R., Merck, Germany

2.2.2 Ammonia solution ($NH_3$, 25% w/w), AnalaR, BDH, England

2.2.3 Glacial acetic acid ($CH_3COOH$, 100% w/w), AnalaR, BDH, England

2.2.4 Hydroxylamine hydrochloride ($NH_2OH \bullet HCl$, 99% w/w), A.R. grade, Ajax Chemicals, Australia.

2.2.5 Hydrogen peroxide ($H_2O_2$, 30% w/w), A.R. grade, Carlo Erba, Italy.

2.2.6 Hydrochloric acid ($HCl$, 37% w/v), A.R. grade, J.T. Baker, USA.

2.2.7 Magnesium chloride hexahydrate ($MgCl_2 \bullet 6H_2O$, 99% w/w), A.R. grade, Fluka, Switzerland.

2.2.8 Nitric acid ($HNO_3$, 65% w/v), G.R., Merck, Germany.

2.2.9 Stock standard solution of copper 1000 mg/L, AAS grade, Fluka, Switzerland.

2.2.10 Stock standard solution of zinc 1000 mg/L, AAS grade, Fluka, Switzerland.

2.2.11 Stock standard solution of manganese 1000 mg/L, AAS grade, Fluka, Switzerland.

2.2.12 Stock standard solution of cadmium 1000 mg/L, AAS grade, Fluka, Switzerland.

2.2.13 Stock standard solution of lead 1000 mg/L, AAS grade, Fluka, Switzerland.

2.2.14 Stock standard solution of chromium 1000 mg/L, AAS grade, Fluka, Switzerland.

2.2.15 Sodium acetate ($CH_3COONa$, 99% w/w), AnalaR, BDH, England.

## 2.3 The Preparation of Chemical Solutions

All standard and chemical solutions were prepared by using Milli-Q (deionized water) obtained from a Millipore Milli-Q system (18.2 MΩ/cm). All used glasswares and containers were soaked in 10% v/v nitric acid for at least 24 h and then rinsed with distilled and deionized waters before use.

2.3.1 Magnesium chloride 1 M, pH 7

A 203.31 g magnesium chloride hexahydrate was dissolved in 500 mL of deionized water. The pH of solution was adjusted to pH 7 by adding a few drops of 1 M ammonium hydroxide and then made up to 1000 mL with deionized water.

2.3.2 Sodium acetate 1 M, pH 5

A 82.03 g sodium acetate was dissolved in 500 mL of deionized water. The solution was adjusted to pH 5 with concentrated acetic acid and then made up to 1000 mL with deionized water.

2.3.3 Hydroxylamine hydrochloride 0.04 M in 25% v/v acetic acid

A 2.7801 g of hydroxylamine hydrochloride was dissolved and then diluted to 1000 mL with 25% v/v acetic acid.

2.3.4 Hydrogen peroxide solution 30% w/w, pH 2

The hydrogen peroxide solution 30% w/w was freshly prepared by acidifying to pH 2 with nitric acid before use.

2.3.5 Ammonium acetate 3.2 M in 20% v/v nitric acid

A 246.62 g of ammonium acetate was dissolved and then diluted to 1000 mL with 20% v/v nitric acid.

### 2.3.6 Standard solution

A standard solution of 100 mg/L was initially prepared by diluting 10 mL of 1000 mg/L of a stock a standard solution of each element with 1% v/v nitric acid in a 100 mL volumetric flask. Working standard solutions of selected elements were fleshy prepared by diluting 100 mg/L to desired concentration ranges before use.

### 2.3.7 25 % v/v Glacial acetic acid

A 250 mL of glacial acetic acid was mixed with 750 mL of deionized water.

### 2.3.8 20% v/v Nitric acid

A 200 mL of concentrated nitric acid was mixed with 800 mL of with deionized water.

## 2.4 Sampling and Sample Preparation

In this work, sediment samples were collected at 12 sampling sites from the Kwai Noi River in Amphoe Muang at Kanchanaburi Province as shown in Figure 2.1. Only the top portions (about 3-5 cm) of sediments were taken by using hand-grab and then stored in sealed polyethylene plastic bags. At each sampling site, sediments were sampled about 1 Kg. Sediment samples were collected for two times as follow.

i) The first sampling: 18 August 2002 (Lot-I)

ii) The second sampling: 16 March 2003 (Lot-II)

After sampling, samples were dried overnight at 60-70 °C, subsequently ground and homogenized using a ceramic mortar and then sieved through a 0.1 mm sieve. Samples were stored in plastic bottles and kept at room temperature till required for extraction procedure.

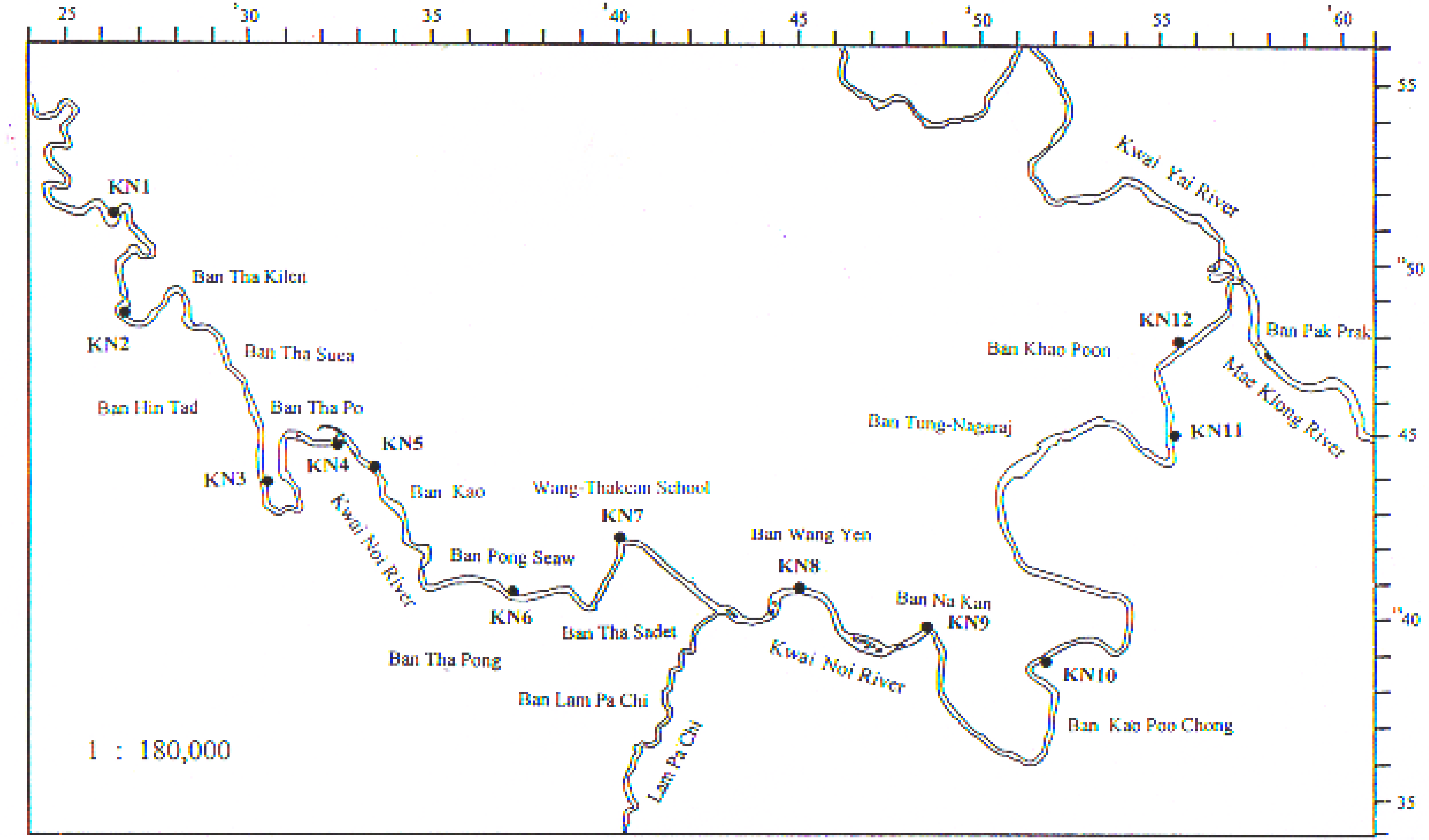


**Figure 2.1** Sampling sites of sediment samples collected from the Kwai Noi River at Kanchanaburi Province.

## 2.5 Optimization of Extraction Parameters

Tessier's sequential extraction procedure [20] was designed for sediments, which has often been modified by other investigators. This procedure was developed for metal partitioning into five geochemical fractions, namely exchangeable (F-I), bound to carbonate (F-II), bound to Fe and Mn oxide (F-III), bound to organic matter (F-IV), and residual fractions (F-V). In this work, Tessier's method was also modified for metal extraction from sediment samples. Within a sequential extraction procedure, the extraction efficiency for each fraction has been variable due to differentiation among geochemical phase of sediment. Before this method applied to samples, some extraction parameters such as the ratio of extracting solution volume per sediment weight (V/m), extraction times ($T_E$), and repetitive extractions ($R_E$) were particularly optimized. To study the effects of these extraction parameters on the extractable metals, experimental procedures for sequential extraction were performed as described below.

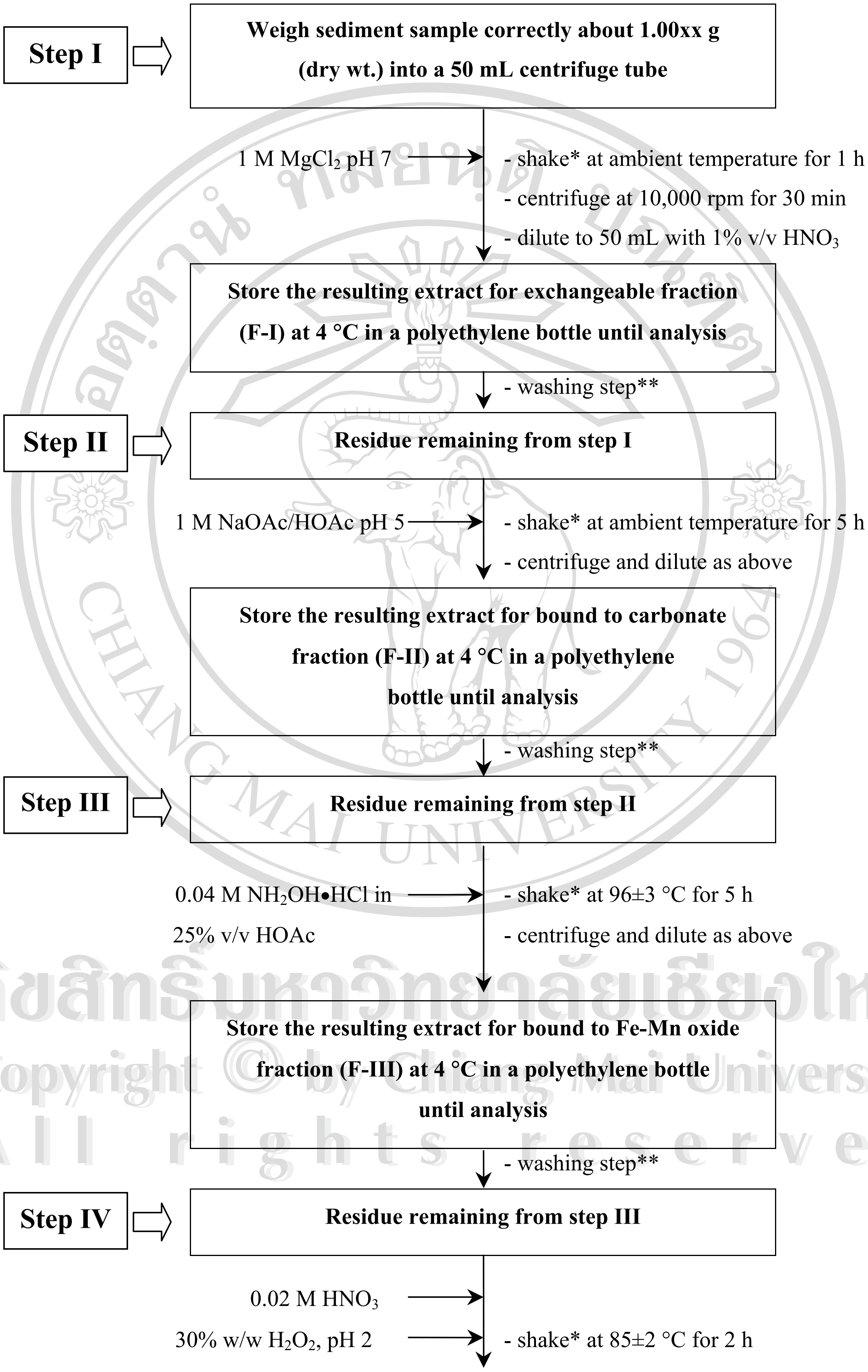
### 2.5.1 Effect of Ratio of Extracting Solution Volume per Sediment Weight

In this work, optimum V/m ratios for each extraction step were investigated prior to metal analysis in each extract. The effect of V/m ratio was studied by varying an extracting solution volume used for each extraction step of F-I to F-IV for two sediment samples collected for Lot-I (S2 and S5). Each extraction step of F-I to F-IV was practically performed in the same Tessier's method [20]. Using extraction conditions shown in Table 2.1, experimental procedures employed for each extraction step were performed by using the sequential extraction scheme as shown in Figure 2.2. In practice, V/m ratios were varied in the range of 8-64, 8-64, 10-80, and 10-80 mL/g for F-I, F-II, F-III, and F-IV, respectively. To study the effect of V/m ratios, extraction time of each extraction step was controlled at 1, 5, 6, and 5 h, whereas extraction temperature was fixed at RT, RT, 96±3, and 85±2°C for F-I, F-II, F-III, and F-IV, respectively. The blank was performed using the same extraction procedure for each extraction step, but without sediment samples.

**Table 2.1** The range of varied extracting solution volumes for study of the effect of V/m ratios for sequential extraction method.

| **Fraction** | **Extracting solution** | **Range of V/m ratio (mL/g)** | **Extraction condition** |
|---|---|---|---|
| Exchangeable (F-I) | 1 M $MgCl_2$, pH 7 | 8 – 64 | 1 h at room temp. |
| Bound to carbonate (F-II) | 1 M NaOAc, pH 5 (HOAc) | 8 – 64 | 5 h at room temp. |
| Bound to Fe-Mn oxide (F-III) | 0.04 M $NH_2OH$•HCl in 25% v/v HOAc | 10 – 80 | 6 h at 96±3 °C |
| Bound to organic matter (F-IV)** | 30% w/w $H_2O_2$, pH 2 ($HNO_3$) | 10 – 80 | 2 h at 85±2 °C<br>3 h at 85±2 °C |

*After extraction with 30% w/w $H_2O_2$, sample was continuously extracted with 5 mL of 3.2 M $NH_4OAc$ prepared in 20% v/v $HNO_3$ at room temperature for 30 min.


Step I
Weigh sediment sample correctly about 1.00xx g (dry wt.) into a 50 mL centrifuge tube
1 M $MgCl_2$ pH 7
- shake* at ambient temperature for 1 h
- centrifuge at 10,000 rpm for 30 min
- dilute to 50 mL with 1% v/v $HNO_3$
Store the resulting extract for exchangeable fraction (F-I) at 4 °C in a polyethylene bottle until analysis
- washing step**
Step II
Residue remaining from step I
1 M NaOAc/HOAc pH 5
- shake* at ambient temperature for 5 h
- centrifuge and dilute as above
Store the resulting extract for bound to carbonate fraction (F-II) at 4 °C in a polyethylene bottle until analysis
- washing step**
Step III
Residue remaining from step II
0.04 M $NH_2OH \bullet HCl$ in 25% v/v HOAc
- shake* at 96±3 °C for 5 h
- centrifuge and dilute as above
Store the resulting extract for bound to Fe-Mn oxide fraction (F-III) at 4 °C in a polyethylene bottle until analysis
- washing step**
Step IV
Residue remaining from step III
0.02 M $HNO_3$
30% w/w $H_2O_2$, pH 2
- shake* at 85±2 °C for 2 h

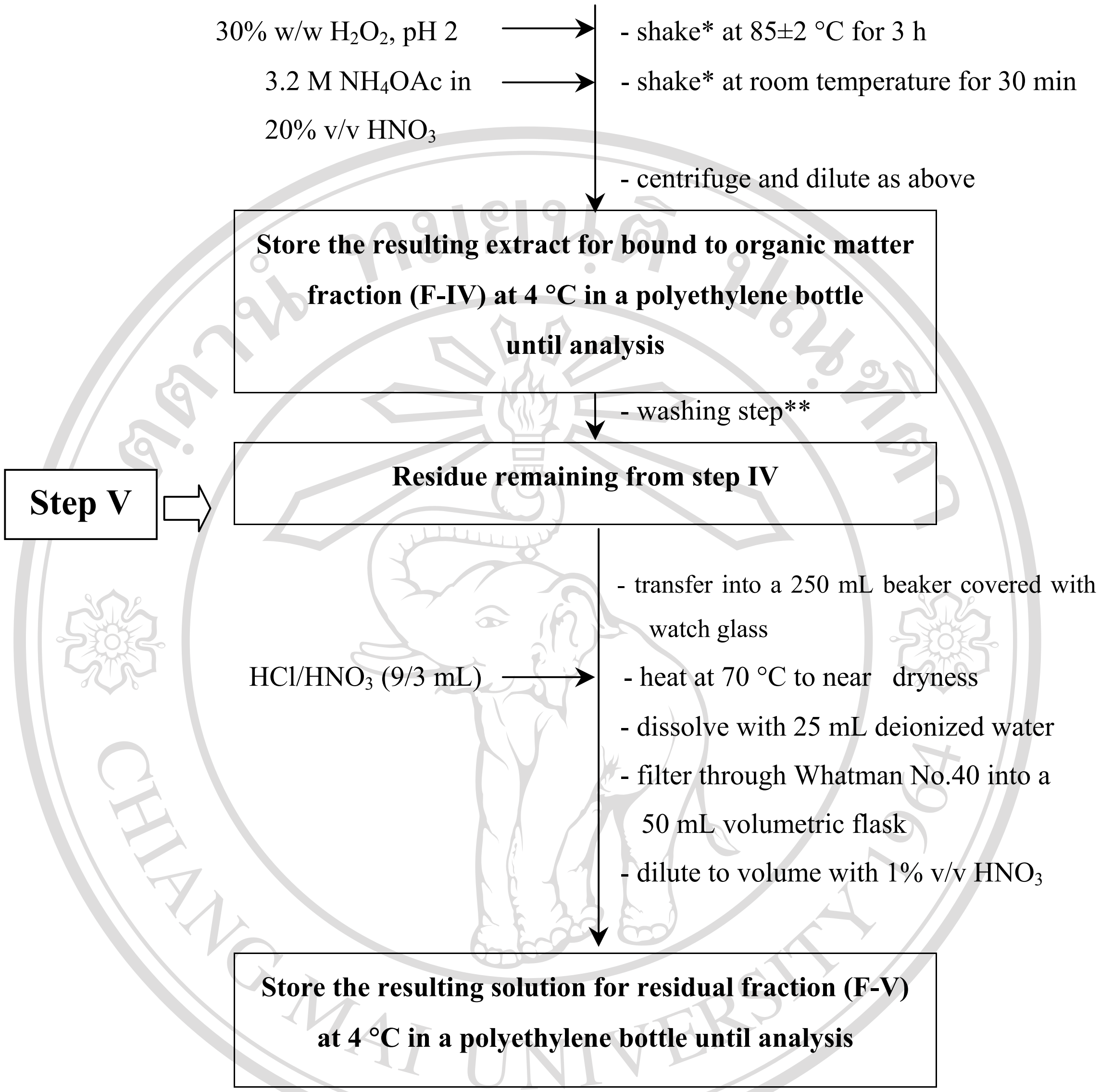


**Figure 2.2** The five-stage sequential extraction procedure employed for sediment samples (modified from Teesier's method [20]).

*Experiment for each extraction step was performed using an end-over-end mechanical shaker.

**Solid residue remaining from each extraction step was added with 8 mL of deionized water and then shaken at room temperature for 15 min. After centrifugation at 10,000 rpm for 15 min, the supernatant was discharged. The remained residue was kept for the next extraction step.

### 2.5.2 Effect of Extraction Times

In this work, optimum extraction time for each extraction step was investigated for determination of metals extracted from each fraction. For evaluating the suitable extraction time, two sediment samples collected for Lot-I (S2 and S5) were used. In practice, experimental procedures for each extraction step of F-I to F-IV were performed by using the sequential extraction scheme as shown in Figure 2.2, but extraction times for each fraction were varied in the range of 1-6, 1-6, 1.8, and 1.8 h for F-I, F-II, F-III, and F-IV, respectively. To study the effect of extraction time for each extraction step, V/m ratios was used at 32, 32, 40, and 10 mL/g, whereas extraction temperature was fixed at RT, RT, 96±3, and 85±2°C for F-I, F-II, F-III, and F-IV, respectively. The blank was performed using the same extraction procedure for each extraction step, but without sediment samples.

**Table 2.2** The range of varied leaching times for study of the effect of extraction times for sequential extraction method.

| **Fraction** | **Extracting solution** | **V/m ratio (mL/g)** | **Range of extraction time (h)** | **Extraction Temp.** |
|---|---|---|---|---|
| Exchangeable (F-I) | 1 M $MgCl_2$, pH 7 | 32 | 1 - 6 | Room temp. |
| Bound to carbonate (F-II) | 1 M NaOAc, pH 5 (HOAc) | 32 | 1 - 6 | Room temp. |
| Bound to Fe-Mn oxide (F-III) | 0.04 M $NH_2OH{\bullet}HCl$ in 25% v/v HOAc | 40 | 1 - 8 | 96±3 °C |
| Bound to organic matter (F-IV)* | 30% w/w $H_2O_2$, pH 2 ($HNO_3$) | 10 | 1 - 8 | 85±2 °C |

*After extraction with 30% w/w $H_2O_2$, sample was continuously extracted with 5 mL of 3.2 M $NH_4OAc$ prepared in 20% v/v $HNO_3$ at room temperature for 30 min.

### 2.5.3 Effect of Repetitive Extractions

In this work, repetitive extractions were investigated in order to evaluate extraction efficiencies of each extraction step of F-I to F-IV for the optimized sequential extraction method. For each extraction step, optimum extraction conditions such as V/m ratios, extraction times, temperature, and shaking method are summarized in Table 2.3. For investigating the effect of repetitive extractions, two sediment samples collected for Lot-II (S2 and S5) were applied. Using the optimum extraction conditions shown in Table 2.3, experimental procedures for four extraction steps (F-I to F-IV) were performed by using the sequential extraction scheme shown in Figure 2.2, but an individual extraction step was successively extracted four times with specific reagents for each fraction.

**Table 2.3** Optimum extraction condition for an individual extraction step of sequential extraction method.

| Fraction | Extracting solution | Extraction condition |
|---|---|---|
| Exchangeable (F-I) | 32 mL 1 M $MgCl_2$, pH 7 | 1 h at room temp. |
| Bound to carbonate (F-II) | 32 mL 1 M NaOAc, pH 5 (HOAc) | 3 h at room temp. |
| Bound to Fe-Mn oxide (F-III) | 40 mL 0.04 M $NH_2OH$•HCl in 25% v/v HOAc | 5 h at 96±3 °C |
| Bound to organic matter (F-IV) | 3 mL 0.02 M $HNO_3$+5 mL 30% w/w $H_2O_2$<br>5 mL 30% w/w $H_2O_2$<br>5 mL 3.2 M $NH_4OAc$ in 20% v/v $HNO_3$ | 2 h at 85±2 °C<br>2 h at 85±2 °C<br>30 min at room temp. |
| Residual (F-V) | 9 mL HCl/ 3 mL $HNO_3$ | Heat at 70 °C to near dryness using sand-bath |

## 2.6 Comparison between Tessier's and the Optimized Sequential Extraction Methods

Extraction conditions obtained from Tessier's and the optimized sequential extraction methods are shown in Table 2.4. Both Tessier's and the optimized sequential extraction methods were then employed to extract heavy metals from two samples (S2 and S5, Lot-I) in order to determine metal concentration and evaluate metal distribution in different sediment phases. Using the optimum extraction conditions for both methods, experimental procedures for four extraction steps (F-I to F-IV) were performed by using the sequential extraction scheme as shown in Figure 2.2. The blank was performed using the same extraction procedure for each extraction step, but without sediment samples.

**Table 2.4** Comparison of extraction conditions between Tessier's and the optimized sequential extraction methods.

| **Fraction** | **Extracting solution** | **Extraction condition** | | | | |
|---|---|---|---|---|---|---|
| | | **V/m ratio (mL/g)** | | **Time (h)** | | **Temp. (°C)** |
| | | **Ts**[a] | **Op**[b] | **Ts**[a] | **Op**[b] | |
| Exchangeable (F-I) | 1 M $MgCl_2$, pH 7 | 8 | 32 | 1 | 1 | Room temp. |
| Bound to carbonate (F-II) | 1 M NaOAc, pH 5 (HOAc) | 8 | 32 | 5 | 3 | Room temp. |
| Bound to Fe-Mn oxide (F-III) | 0.04 M $NH_2OH$•HCl in 25% v/v HOAc | 20 | 40 | 6 | 5 | 96±3 °C |
| Bound to organic matter (F-IV) | $HNO_3$+ $H_2O_2$*<br>30% w/w $H_2O_2$<br>3.2 M $NH_4OAc$** | 3+5<br>5<br>5 | 3+5<br>5<br>5 | 2<br>3<br>30 min | 2<br>2<br>30 min | 85±2 °C<br>85±2 °C<br>Room temp. |
| Residual (F-V) | HCl/$HNO_3$ (9/3 mL) | Heated at 70 °C to near dryness using sand-bath | | | | |

[a] Tessier's method, [b] The optimized sequential extraction method.

*3 mL of 0.02 M $HNO_3$ + 5 mL of 30% w/w $H_2O_2$ pH 2 ($HNO_3$).

**prepared in 20% v/v $HNO_3$.

## 2.7 Accuracy of the Optimized Sequential Extraction Method

In this work, the accuracy of the sequential extraction method was evaluated by comparing the sum of metal concentrations found in all fractions with total metal contents obtained using hot-acid digestion. For evaluating the accuracy of the optimized sequential extraction method, two sediment samples collected for Lot-I (S2 and S5) were employed. Using the optimum extraction conditions shown in Table 2.3, all experimental procedures for each extraction step were performed by using the sequential extraction scheme shown in Figure 2.2. For obtaining total metal concentrations, hot-acid digestion method (aqua regia) was performed as described in residual step (Figure 2.2). The blank was performed using the same extraction procedure for each extraction step, but without sediment samples.

## 2.8 Accuracy of Hot-Acid Digestion

In this work, standard addition method was employed to estimate the accuracy of hot-acid digestion method. In practice, two sediment samples collected for Lot-I (S7 and S8) were correctly weighed about 1.00xx g into a 250 mL beaker covered with watch glasses. A 1.00 mL aliquot of 100.0 mg/L of selected element standard solutions was spiked into the beaker containing the sample. A mixture was stand at room temperature for 24 h prior to hot-acid digestion procedure that also performed as described for the digestion of the residual step (Figure 2.2).

## 2.9 Precision of the Optimized Sequential Extraction Method

In this work, the repeatability of the optimized sequential extraction method was investigated by using repeatable extraction for six replicates for each fraction.

Two sediment samples collected for Lot-I (KN3 and KN4) were mixed and then homogenized prior to the determination of heavy metals using the optimized sequential extraction method. For investigating the precision of sequential extraction, mixed sample was performed for six replicates. Using the optimum extraction conditions described in Table 2.3, all experimental procedures for each extraction step were performed by using the sequential extraction scheme shown in Figure 2.2.

## 2.10 Instrumental Parameters

The extracted metal concentrations from sediment samples for an individual fraction were analyzed by using flame atomic absorption spectrophotometer (FAAS). The optimal parameters recommended by the manufacturer shown in Table 2.5 were used throughout this work. To determine metal concentrations after extraction and digestion procedures, the resulting extracts were directly analyzed or performed by diluting to desire concentration ranges.

**Table 2.5** Instrumental parameters of FAAS for the determination of heavy metals extracted form sediment samples.

| **Parameters** | **Elements** | | | | | |
|---|---|---|---|---|---|---|
| | **Zn** | **Mn** | **Cu** | **Pb** | **Cd** | **Cr** |
| Wavelength (nm) | 213.9 | 279.5 | 324.8 | 283.3 | 228.8 | 357.9 |
| Slit width (nm) | 0.5 | 0.4 | 0.5 | 1.0 | 0.3 | 0.5 |
| Air flow-rate (L/min) | 8 | 8 | 8 | 8 | 8 | 8 |
| Acetylene flow-rate (L/min) | 2.0 | 1.9 | 1.8 | 2.0 | 1.8 | 2.6 |
| Lamp current (mA) | 4 | 5 | 3 | 5 | 4 | 5 |