2. EXPERIMENTAL

2.1 Chemicals and Reagents

All chemicals are analytical reagent grade and used without further purified, otherwise stated, as follows:

1) Acetic acid (CH_3COOH, glacial), 99.8% w/v (BDH, Poole, England)
2) Ammonia solution (NH_3 or NH_4OH), 44% v/v (Merck, Darmstadt, Germany)
3) Ferric chloride ($FeCl_3$) (Merck, Darmstadt, Germany)
4) Humic acid (Sigma-Aldrich, Steinheim, Germany)
5) Hydrochloric acid (HCl), 37% v/v (Merck, Darmstadt, Germany)
6) Hydrogen peroxide (H_2O_2), 30% v/v (BDH, Poole, England)
7) Mercury (II) nitrate ($Hg(NO_3)_2 \bullet H_2O$) (UNIVAR, AJAX, Australia)
8) Metal standard solutions (1000 mg/l each: zinc, cadmium, lead and copper) (for AAS, Merck, Darmstadt, Germany)
9) Nitric acid (HNO_3), 60% v/v (Merck, Darmstadt, Germany)
10) Nitrogen gas (N_2), 99.999% (Lanna Chiang Mai, Thailand)
11) Sodium acetate 3-hydrate ($CH_3COONa \bullet 3H_2O$) (BDH, Poole, England)
12) Sodium perchlorate ($NaClO_4$) (Sigma-Aldrich, Steinheim, Germany)
13) Sufuric acid (H_2SO_4), 98% v/v (Merck, Darmstadt, Germany)

Preparation of Reagents

All reagents and standard solutions were prepared using ultrapure water (MilliQ system).

Acetate buffer solutions (1 mol/l and 0.6 mol/l, pH 4.6)

Sodium acetate 3-hydrate (136.08 g or 81.65 g) was dissolved with water. Acetic acid (58.8 ml or 34.1 ml) was added into the sodium acetate solution. Then the solution was made up to 1 l. The pH of the solution was measured.

Artificial rainwater (pH 4)

The artificial rainwater *(96)* contained $2x10^{-5}$ mol/l HNO_3, $3x10^{-5}$ mol/l H_2SO_4, $1x10^{-5}$ mol/l HCl and $2x10^{-5}$ mol/l NH_3. Ultrapure water was used as a solvent for this.

Humic acid solution (10 mg/l, pH 7)

The purchased humic acid (0.01g), without any pretreatment, was dissolved in 0.001 mol/l sodium perchlorate solution and the solution was made up to 1 l with 0.001 mol/l sodium perchlorate solution.

Iron(III) solution (10^{-3} mol/l , pH 2)

A stock Fe(III) solution was prepared by dissolving $FeCl_3$ in ultrapure water. The pH of the solution was adjusted by adding HCl.

Mercury (II) solution (500 mg/l Hg(II))

Mercury (II) nitrate 2-hydrate (0.25 g) was dissolved in 0.1 mol/l nitric acid and the volume was made up to 500 ml with 0.1 mol/l nitric acid.

Metal standard solutions (100 and 10 mg/l stock solutions)

Stock metal standard solutions for Zn(II), Cd(II), Pb(II) and Cu(II) were prepared by diluting 1000 mg/l standard solutions (atomic absorption spectrometric grade) with ultrapure water.

2.2 Equipment/Apparatus

1) Asymmetric flow field-flow fractionation (AF^4) system with a UV/VIS spectrophotometer (PN3240), HRFFF 10.000 Series, Postnova Analytics, Germany
2) Autotitrator, 665 Dosimat and 765 Dosimat, Metrohm, Switzerland
3) Ion chromatography (IC), DX300 DIONEX, USA

4) Inductively coupled plasma-mass spectrometer (ICP-MS), ELAN6000, Perkin Elmer, Germany
5) Microwave digestion unit, Milestone Microwave Laboratory System, USA
6) Peristaltic pump, MP3, EYELA, Japan
7) pH meter, PHM61 Laboratory pH meter, Radiometer Copenhagen, Denmark
8) Photon correlation spectroscopy, BI-90, Brookhaven Instruments, USA
9) Rotator, REX20, Heidelph, Germany
10) Scanning electron microscopy (SEM), CamScan CS44 FE, England
11) Shaker, SK-101, HL instruments, Thailand
12) Six-port injection valve, UpChurch Scientific, USA
13) Solenoid valve (3-port), 625E BUNKER CT.VERNON HILLS IL 60061, Cole-Palmer Instrument, USA
14) Total organic carbon (TOC) analyzer, TOC-5000, Shimadzu, Japan
15) Ultrasonic cleaner, 8891E-DTH, Cole-Palmer Instrument, USA
16) Voltammographs, Bioanalytical System (BAS), USA and 757 VA Computrace, Metrohm, Switzerland
17) X-ray diffraction (XRD) spectrometer using Cu-anode, Diffractometer XRD3000, SEIFERT, Germany
18) X-ray diffraction (XRD) spectrometer using Mo-anode, powder Diffractometer D5000, SIEMENS, Germany
19) X-ray fluorescence (XRF) spectrometer, SIEMENS SRS 3000, Germany

2.3 Instrumentation for On-line Systems

2.3.1 Asymmetric Flow Field-Flow Fractionation-Inductively Coupled Plasma-Mass Spectrometry

An asymmetric flow field-flow fractionation (AF^4) system was used to characterize colloids in different samples. The AF^4 system (**Figure 2.1a**) consists of a separation channel, an AF^4 module, a laminar pump, an injection pump, a 6-port injection valve, a cross-flow pump, a PC with software. The detector was a UV/Vis spectrometer. The separation channel (**Figure 1.1**) is equipped with a spacer, a regenerated cellulose membrane (molecular weight cutoff of 5 kD, Schleicher and Schuell, Germany), a ceramic frit and two polymethylmetacrylate (PMMA) channel blocks. The width or thickness of the channel was approximately 500 μm. The laminar and injection flows were provided by piston pumps while the cross-flow pump was delivered by a syringe pump.

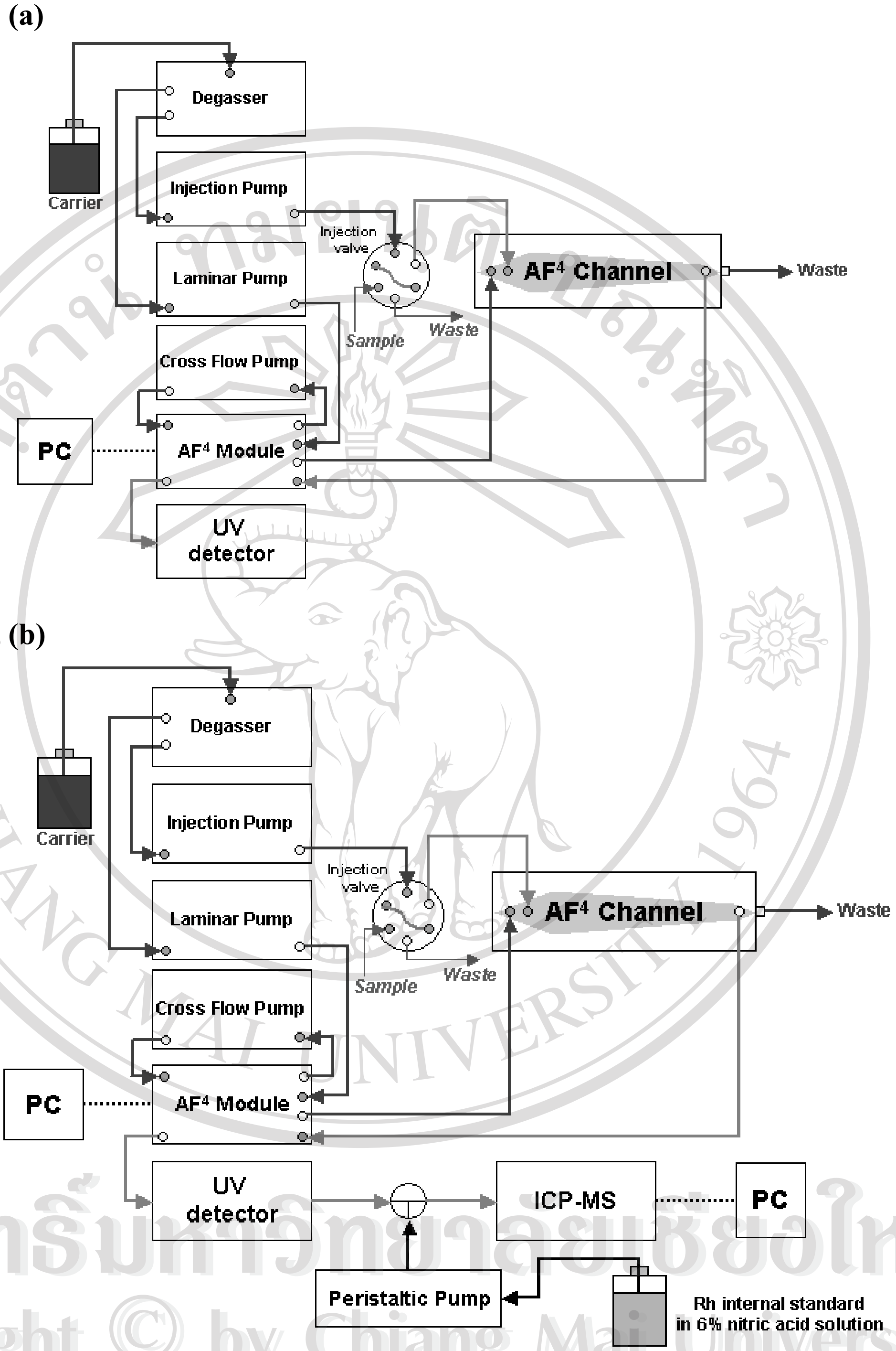


Figure 2.1 **(a)** the asymmetric flow field-flow fractionation (AF^4) system and **(b)** the asymmetric flow field-flow fractionation-inductively coupled plasma-mass spectrometric (AF^4-ICP-MS) system

To operate the system, firstly the carrier solution was pumped at the flow rate of 1 ml/min by the laminar pump through the channel and detectors. The base line was then established. A sample suspension was loaded via the 6-port injection valve (0.5 ml sample volume) and injected into the separation channel. Then it was focused (at the flow rate of 2 ml/min) by controlling the laminar in and out flows for 180 s. Then the carrier was pumped through the channel as the channel flow while the cross flow (0.7 ml/min) was also applied along the channel. The particles in the sample were separated according to their sizes, and were fractionated along the channel and further continuously directed to the UV/Vis detector which was set at 225 nm to monitor the absorbance of humic acid. The inductively coupled plasma-mass spectrometer (ICP-MS) was connected to the AF^4-UV/Vis flow-through cell via a 3-way valve (UpChurch, USA) to continuously analyze metal ions in a simultaneous mode. At the 3-way valve, the stream eluted from the AF^4 system was mixed with a stream of a mixture of the 6% HNO_3 and Rh internal standard (100 μg/l). The ICP-MS was set to the real time analysis mode. The ICP-MS operating parameters are shown in **Table 2.1**. The AF^4 and ICP-MS were controlled by 2 separated PCs with NOVAFFF System-Manager and ELAN NT softwares, respectively. The diagram of the AF^4-ICP-MS system is shown in **Figure 2.1b.** Latex standards (20, 50 and 100 nm) were used for size calibration. Water samples from mining areas were collected and analyzed by the AF^4-ICP-MS system for characterizing the colloids.

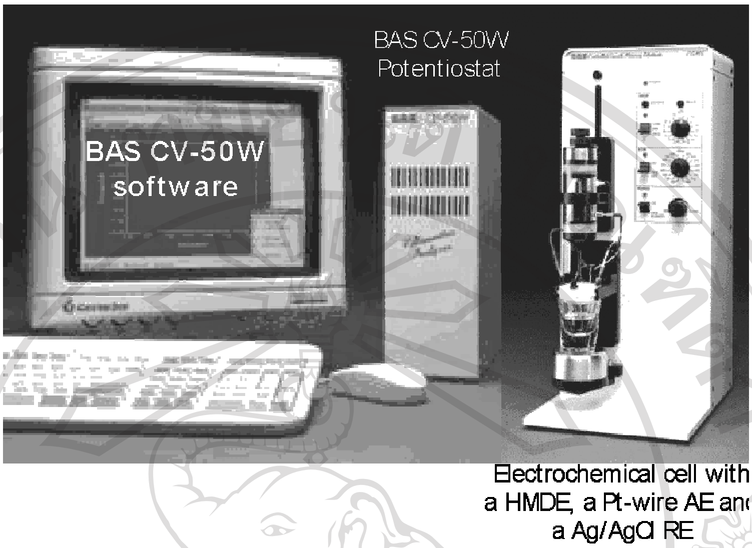
Table 2.1 ICP-MS parameters

Parameter	**Value**
RF generator frequency	40 MHz
RF forward power	1.6 kW
Torch	Standard type
Spray chamber	RYTON^{TM}
Nebulizer	Cross-flow (RYTON^{TM})
Nebulizer gas flow rate	0.9 l/min
Auxiliary gas flow rate	1.2 l/min
Outlet gas flow rate	15 l/min

2.3.2 Anodic Stripping Voltammetry (ASV)

Voltammographs used in this work are depicted in **Figure 2.2(a) and (b)**. Basically, a voltammograph consists of an electrochemical cell equipped with a working electrode (WE), a reference electrode (RE) and an auxiliary electrode (AE), a potentiostat and a computer with controlling software. Hanging mercury drop electrode (HMDE), silver/silver chloride (Ag/AgCl in 3 mol/l KCl) electrode, and a platinum wire (Pt-wire) were used as the WE, RE and AE, respectively. A mixture of sample/standard and buffer solutions (total volume of 10 ml) was placed in the electrochemical cell.

(a)



(b)

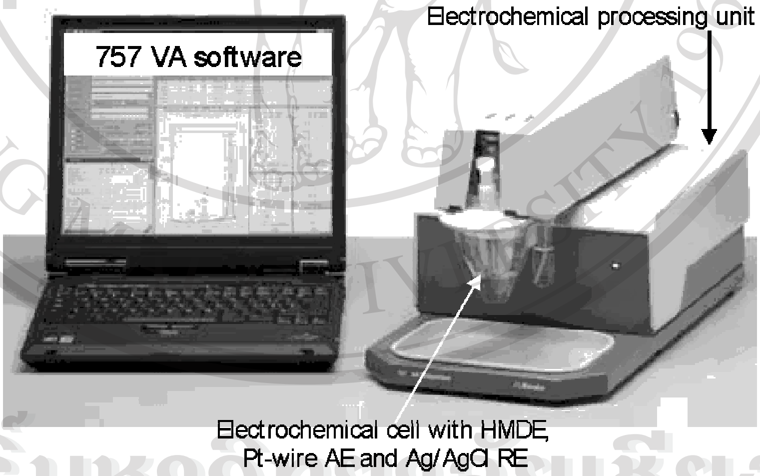


Figure 2.2 Voltammographs; **(a)** Bioanalytical System, **(b)** Metrohm

In the batch system, the sample solution was prepared by adding 2 ml of 1 mol/l acetate buffer solution to 100 μl sample and adjusting the volume by water. The sample cell was then placed in the voltammograph. The analysis steps are summarized in **Table 2.2**. Voltammetric parameters are represented in **Table 2.3**.

Table 2.2 Operating steps of batch analysis with standard addition method by the voltammetric procedure

Step	**Procedure**	**Time (s)**
1	Stir and purge oxygen by blowing nitrogen gas	300
2	Deposit metal ions to HMDE at the potential of –1.1 V	30
3	Stop stirring	5
4	Scan potential positively from –1.1 V to 0.2 V using square-wave scan mode	3
5	Repeat steps 2-3 using a new drop of mercury (2 times)	75
6	Add standard solution into the sample cell	
7	Stir and purge oxygen by blowing nitrogen gas	30
8	Repeat steps 2-3 using a new drop of mercury (3 times)	113

Table 2.3 Voltammetric parameters

Parameter	**Value**
Deposition potential	-1.1 V
Deposition time	30 sec
Initial potential	-1.1 V
Final potential	0.2 V
Scan mode	Square-wave (SW)
Step potential	0.01 V
SW amplitude	0.04 V
SW frequency	50 Hz or 15 Hz

2.3.3 Flow Injection Analysis-Anodic Striping Voltammetry (FIA-ASV)

The FIA-ASV system was developed for simultaneously determining some metal ions (Zn(II), Cd(II), Pb(II) and Cu(II)). The manifold of the FIA-ASV system is shown in **Figure 2.3**. The system consists of a peristaltic pump, a 6-port injection valve, a mixing coil (PTFE, 0.8 mm i.d.) and a voltammograph (BAS CV-50W) with an electrochemical flow-cell. Tygon tubing was used with the peristaltic pump for pumping the carrier solution through the system. PTFE tubing was used for delivering the solution in the flow system. The 6-port injection valve was used to introduce the metal standard solution or sample into the carrier stream. The flow-cell (**Figure 2.4**) is composed of a mercury film coated on a glassy carbon-disc-working electrode

(MF-GCE), a Ag/AgCl reference electrode (RE) and a stainless steel auxiliary electrode (AE). The volume of the electrochemical flow cell was approximately 20 μl.

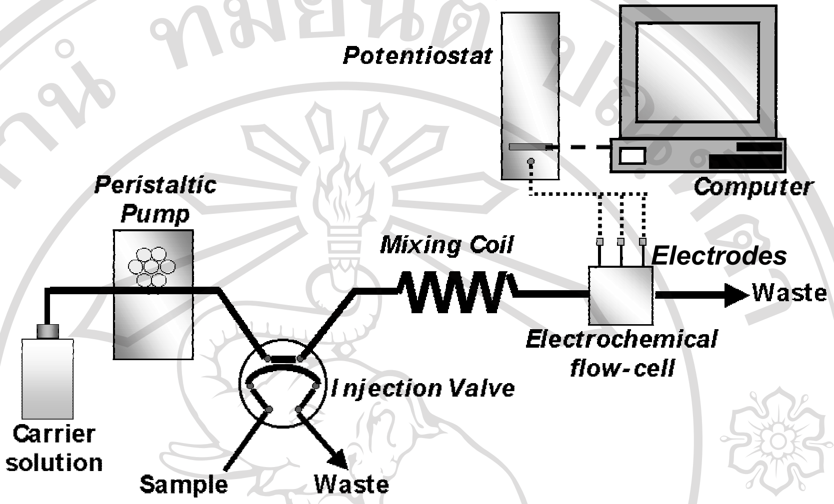


Figure 2.3 Schematic diagram of the FIA-ASV system

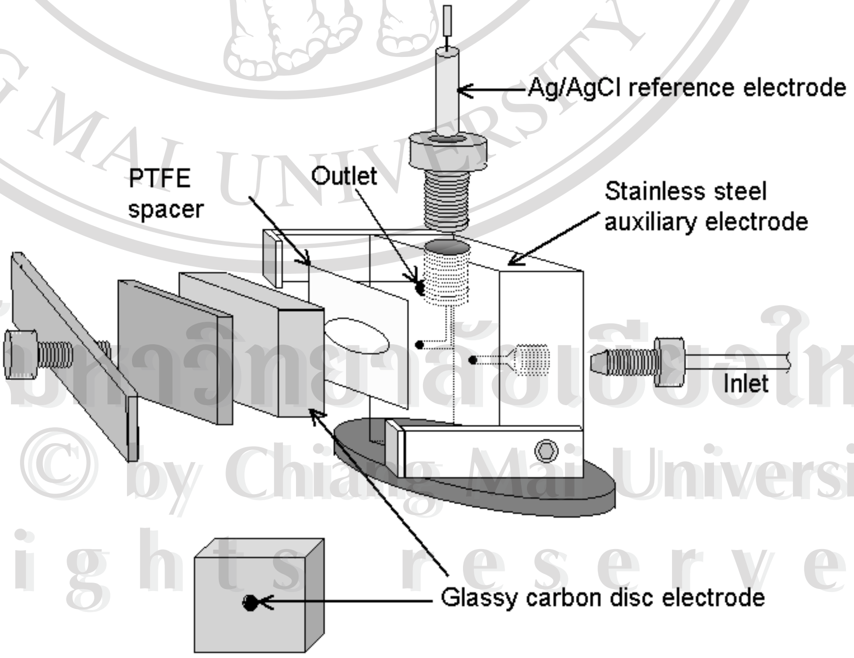


Figure 2.4 Components of the electrochemical flow-cell used in the FIA-ASV system

On-line preplated mercury film preparation

Hg(II) solution (500mg/l Hg(II) in 0.1 mol/l HNO_3) was aspirated at a flow rate of 0.5 ml/min through the electrochemical flow-cell. A potential of –0.8 V vs Ag/AgCl RE was applied to the GCE for 10 min. The MF was then obtained on the GCE.

Operation of the FIA-ASV system

The deoxygenated acetate buffer solution was pumped at a flow rate of 0.5 ml/min through the mixing coil into the flow-cell. A de-aerated standard solution or sample was injected into the carrier stream by the injection valve. When the sample zone reached the flow-cell (1 min after injection), the potential of –1.1 V vs Ag/AgCl RE was applied to the MF-GCE for 30 s for depositing metal ions onto the MFE. After the deposition step, the flow was stopped to strip the metal ions out off the MFE by anodically scanning the potential from –1.1 V to 0.2 V. A voltammogram was then obtained.

2.3.4 Sequential Injection Analysis-Anodic Stripping Voltammetry (SIA-ASV)

Two SIA-ASV systems, as depicted in **Figure 2.5** and **Figure 2.6**, were used in this study. The first system was set up for preliminary study and testing the performance of the SIA-ASV system using the experience from the development of the FIA-ASV system. The second SIA-ASV system (**Figure 2.6**) was developed to solve the problems found in using the first SIA-ASV system (**Figure 2.5**).

The SIA-ASV system I

The diagram of the SIA-ASV system *I* is shown in **Figure 2.5**. The SIA-ASV system *I* consists of a 665 Dosimat autotitrator, a holding coil (HC) (Tygon® tubing, 0.5 mm i.d., 4.5 m, 5.7 ml), a 10-port selection valve (SV), a mixing coil (PTFE tubing, 0.11 mm i.d., 23 cm, 438 µl) and a BAS voltammograph equipped with an electrochemical cross-flow cell (EFC). The 665 Dosimat autotitrator was used as a liquid delivery system. The 665 Dosimat autotitrator and the 10-port selection valve were automatically controlled by an in-house created program *I* (based on LabVIEW® software (National Instruments Co., USA)). The experiences from using the FIA-ASV system were applied with the SIA-ASV system *I*. Acetate buffer solution (0.6 mol/l, pH 4.6) was used as a carrier. Standard solutions and samples were connected to the SV. All solutions were purged with N_2 gas before use.

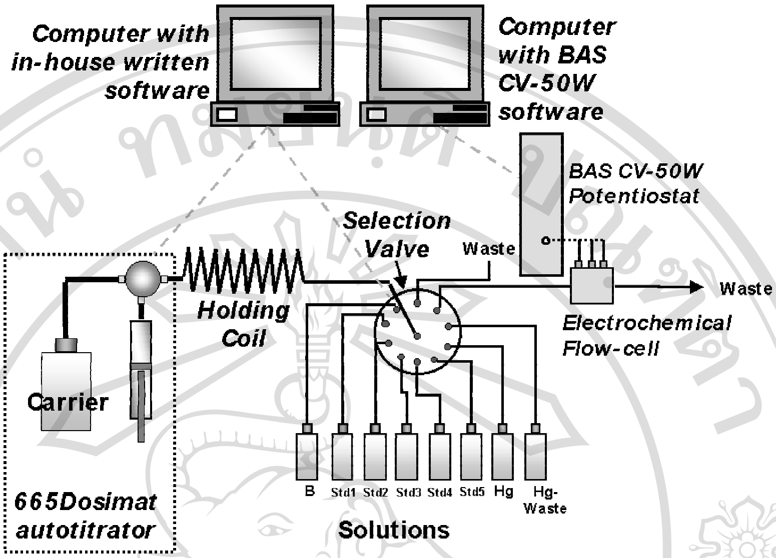


Figure 2.5 Diagram of the SIA-ASV system I for preliminary study

To operate the system, firstly, the buffer solution was aspirated through the HC, SV, MC and EFC. A sample or standard solution (130 μl) was aspirated to the HC and then was propelled to the MC through the EFC. A deposition potential of –1.1 V vs Ag/AgCl RE was applied to the MF-GCE while the sample zone was passing the EFC for 30 s. After that, the flow was stopped and a potential scan of –1.1 – 0.2 V was applied to the MF-GCE at the same time as the current of metal ion was measured. A voltammogram was then obtained. Calibration graphs of the metal standards were constructed by plotting the peak current (i_p) with the concentration of the metals. Precisions and detection limits were obtained. The SIA-ASV system *I* was applied to determine some metals in drinking water samples.

The SIA-ASV system II

The diagram of the SIA-ASV system *II* is depicted in **Figure 2.6**. The SIA-ASV system II setup is similar to the system *I*, except the modified autotitrator (765 Dosimat), the voltammograph (757 VA computrace) and some configurations, e.g. tubings. The diagram of the electrochemical flow-cell and the configuration of the flow channel are illustrated in **Figure 2.7**. The electrochemical flow-cell composes of a preplated MF-GCE, a Ag/AgCl RE and a carbon disc auxiliary electrode. The modified autotitrator and the selection valve were controlled by the in-house created program *II* (based on LabVIEW® software) (see **Appendix A**). The on-line preplated MFE was used as the working electrode (WE). On-line standard addition was performed by the SIA-ASV system *II*. Effect of sample volume was studied. The characterization of the SIA-ASV system *II* such as dynamic ranges of metals, precisions, and detection limits, was studied. The performance of the developed SIA-ASV system *II* was tested for the determination of metal ions in the model samples.

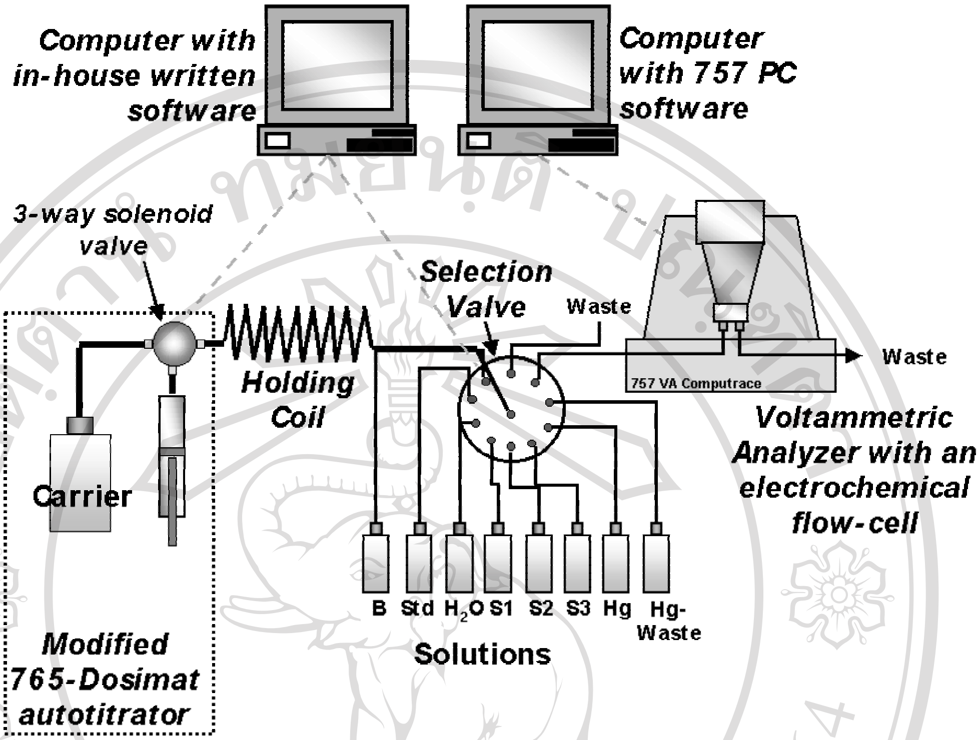


Figure 2.6 The diagram of the developed SIA-ASV system II

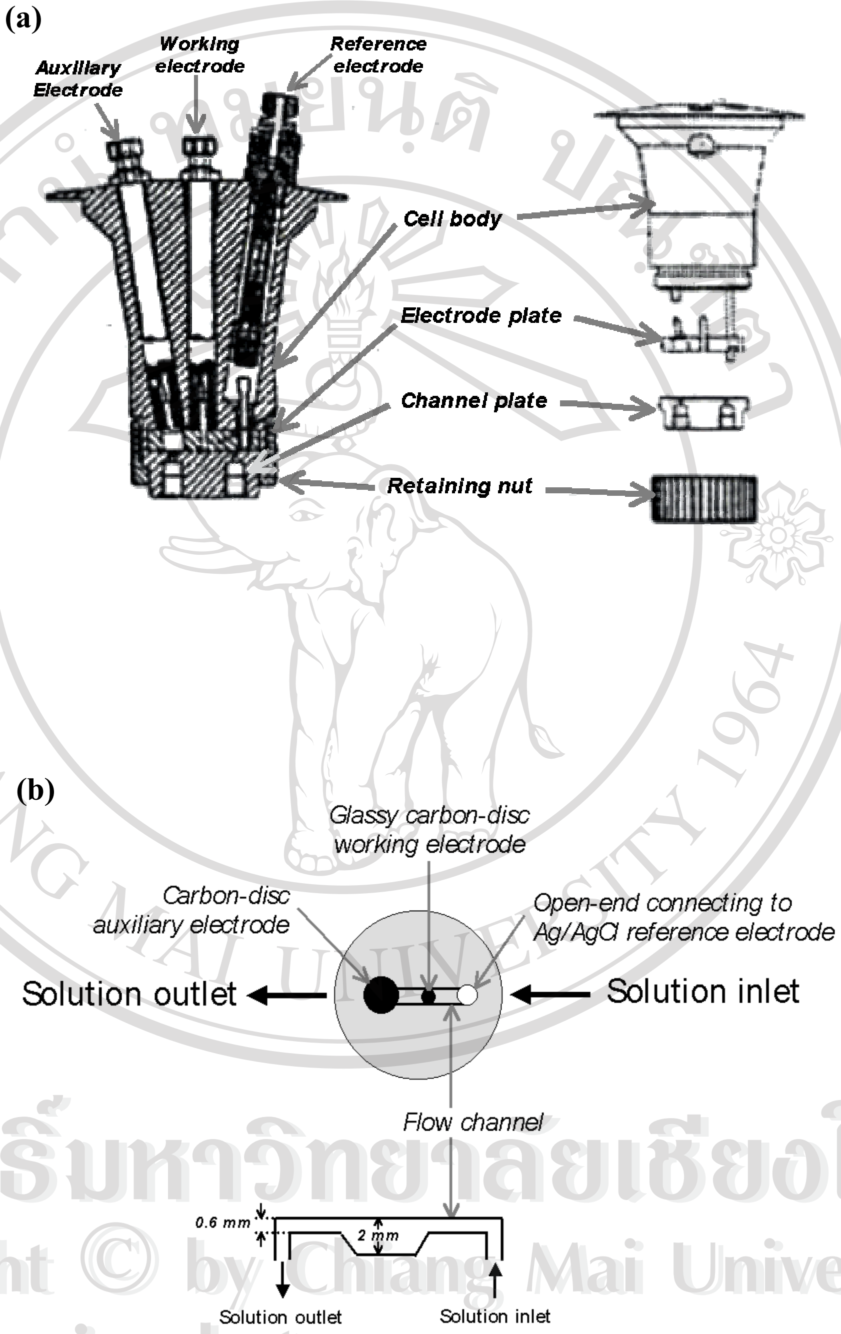


Figure 2.7 **(a)** Electrochemical flow-cell for the SIA-ASV system and **(b)** the flow channel

2.3.5 Homemade UV Digestion Unit

To analyze the dissolved metal forms in water samples, organic matter can interfere the analysis. Therefore, a prior sample digestion was needed. A homemade UV digester was used to remove organic compounds in the samples before analysis by ASV. The homemade UV digester (**Figure 2.8**) consists of 2 UV-lamps (180-280 nm, 15 W each). To prevent the UV irradiation of the operator, aluminium foil was used for covering the inside of the digester house. Sample trays were made by cutting brown-colored glass bottles in to halves. The irradiation area of the sample is thus increased. The trays were stored in nitric acid and rinsed with ultrapure water before use. H_2O_2 solution (0.1 ml), as an oxidant, was added to 10 ml of sample in the tray. The sample trays were then put in the UV digester for 2 hours.

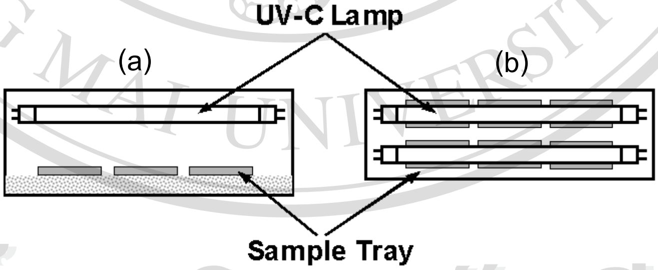


Figure 2.8 The homemade UV digestion unit: **(a)** side view, and **(b)** top view.

2.4 Characterization of Coal-mining Disposal Soil Samples

The contaminated soil samples (BP-1, BP-2 and LN-1) were collected from coal-mining disposal area in northern Thailand. The soil samples were characterized as described below.

2.4.1 Size Distribution of the Soil Samples by Sieving

The stainless steel sieves of 1 mm and 125 μm pore sizes were used to separate the soil particles. The soil particles of the smaller (>1 mm), medium (1 mm-0.125 mm) and larger (<0.125 mm) sizes were obtained and weighed. Percentage of each particle size group was calculated.

2.4.2 Mineralogical Characterisation

For mineralogical characterisation, the medium and smaller sizes of each sample were re-mixed into the original ratio and then analysed by XRD using Cu-anode and Mo-anode, and SEM with the electron back scatter technique.

2.4.3 Elemental Analysis

Analysis of elements in the soil samples was performed by direct analysis of the soil by XRF and by total analysis of the digested soil samples. In the first case, the larger sized particles of the samples were ground to a grain size of less than 0.125

mm. Each size group of each soil sample (6 g) was weighed and mixed with 1.5 g of wax. The mixture was pressed to make a tablet for XRF analysis.

For total analysis using microwave-assisted digestion, 120 mg of each soil sample was weighed in a PTFE vessel. Nitric acid (60% v/v ultrapure) and hydrofluoric acid (48% v/v ultrapure) were added stepwise to the sample into the vessel as shown in the digestion program (**Appendix B**). After the digestion and evaporation of the acid, the residue was dissolved by 2 ml of HNO_3 and transferred to a 25 ml volumetric flask. The vessel was rinsed with MilliQ water. The rinsing solution was used to fill up the volumetric flask. The digested sample solutions were diluted with MilliQ water and analyzed by ICP-MS.

2.4.4 Total Carbon Analysis

Organic and inorganic carbon contents in the soil samples were analyzed by the TOC analyzer. The soil sample was weighed in a sample boat, which has been previously treated in the heating furnace to remove carbon. Then the sample boat was put in the TOC analyzer. Oxalic acid 2-hydrate was used as the standard.

2.5 Leaching of Contaminated Soil

Batch leaching test, using artificial rainwater (pH 4) and 10 mg/l Aldrich humic acid (pH 7) as the leaching solutions, was performed to follow the leaching behavior of some metals in the contaminated soil by rainwater and surface water. 50 ml of a leaching solution, artificial rainwater or humic acid solution, was added to 2.5

g of a soil sample. The mixture was rotated vertically at the rate of 1 rpm for 10 days. After the leaching period, the mixture was centrifuged, and the leachate was separated and collected into a container. Metal ions and anions in the leachates were analyzed by ICP-MS and IC, respectively.

2.6 Formation of Metal-Humic Acid Colloids

In order to investigate the colloid formation of the released metals from coal-mining spoils with organic colloids in natural water, Fe(III) solutions and the leachate solutions (collected from the leaching experiment) were mixed with humic acid solution, used as the most relevant representative of organic colloids in surface water. The metal-colloidal phases were analyzed by the on-line asymmetric flow field-flow fractionation-inductively coupled plasma-mass spectrometry (AF^4-ICP-MS) system.

2.6.1 Model System

A solution containing Fe(III), as the main component of the leachate solutions, was used to study the formation of metal ion-humic acid colloids. The Fe(III) solution was diluted with 10 mg/l humic acid (pH 7) to obtain distinct molar ratios of Fe(III) and humic acid (1:1, 1:2, 1:3, 1:5 and 1:10). The equivalent concentration of COO^- groups is taken as the humic acid concentration, which has been previously determined by potentiometric titration. COO^- groups are assumed to represent the most important functional groups responsible for the complexation of metal ions. Fe(III) is believed to interact with 3 carboxylate functional groups. The following

Fe(III) to COO^- equivalent ratios were established. The effect of pH (pH 2, 4, 6 and 7) was studied additionally to the ratios of 1:2, 1:3, 1:4 and 1:5. The solutions were kept for a week and the colloids were observed by photon correlation spectroscopy (PCS). The concentrations of Fe in the centrifuged solutions (3000 rpm, 15 min) were determined by ICP-MS. The dissolved organic carbon (DOC) concentration in the solutions after centrifugation (3000 rpm, 15 min) was determined by TOC analyzer and used to indicate the amount of dissolved humic acid. Characterization of Fe contents in the colloids in the filtered solution (filter pore size of 450 nm) was performed by AF^4-ICP-MS.

2.6.2 Artificial System

In order to follow the interaction of metal ions, released from the contaminated soil, with the organic colloids in surface water, the leachate solution from the batch leaching experiment was mixed with 10 mg/l humic acid solution (pH 7). The mixing ratios studied in the present work were 1:100, 1:200, 1:300, 1:500 and 1:1000. The effect of pH (pH 5, 7 and 9) was also studied. The mixture solutions were stored up to a week. The metal contents in the mixture were analyzed by ICP-MS. The metal-humic acid colloids (<450 nm) were analyzed by the AF^4-ICP-MS system.